Supporting Information

Beier et al. 10.1073/pnas.1110854108

SI Materials and Methods

Virus Construction and Production. All VSV constructs were created by using the VSVΔG backbone (1). mCherry or YFP were cloned into the first (GFP) position by using XhoI and MscI sites, and VSV-G, RABV-G (a gift from Ed Callaway, Salk Institute) and LCMV-G were cloned into the fifth (G) position by using the MluI restriction site. All plasmids and their respective maps will be deposited at the Addgene Repository (http://www.addgene.org/pgvec1). The M51R mutant (2), which was used for physiological analyses of connectivity, was engineered by using mutagenesis PCR (Quikchange) into the VSVΔG backbone.

Viruses were rescued as described (3). For replication-competent viruses, eight 10-cm plates of BSR cells at 95% confluency were infected at an MOI of 0.01. Viral supernatants were collected at 24-h time intervals for 4 d, combined, ultracentrifuged at 80,000 × *g* by using a SW28 rotor, and resuspended in 0.2% of the original volume by using culture medium (DMEM + 10% FBS). For titering, concentrated viral stocks were applied in a dilution series to 100% confluent BSR cells, and plates were examined at 12 hpi for fluorescent foci. Concentrated viral titers reached as high as 2×10^{12} focus forming units (ffu)/mL The range of titers used was from 3×10^{10} (LGN/eye injections) to 3×10^{7} ffu/mL (CP injections). Appropriate dilutions were made in DMEM + 10% FBS. Viral stocks were stored at −80 °C. All work with viruses was conducted under BL2 conditions. Further information about recombinant VSV vectors can be found elsewhere (3).

For glycoprotein-deleted viruses, 293T cells were transfected at 70% confluency on 10-cm dishes with 5 μg of pCAG-LCMV-G (from clone 13, made by S.P.J.W.; gift of virus from A. Zajac, University of Alabama, Birmingham) or pCMMP EnvARGCD IRES GFP (4). At 24 h after transfection, the cells were infected at an MOI of 0.01 with VSVΔG. Viral supernatants were collected for the subsequent 4 d at 24-h intervals, combined, and concentrated and titered as above. Concentrated titers reached as high as 2×10^{10} ffu/mL. The range of titers used was from 1×10^{8} (CP injections) to 5×10^{5} ffu/mL (slice cultures).

Mouse Injections. Eight-week-old CD-1 mice were injected by using pulled capillary microdispensers (Drummond Scientific), using coordinates from *The Mouse Brain in Stereotaxic Coordinates* (5). Injection coordinates (in mm) used were: LGN, A/P −2.46 from bregma, L/M 2, D/V −2.75; CP, A/P 1 from bregma, L/M 1.8, D/V −2.5.

For CP injections, 100 nL of virus at 3×10^{7} ffu/mL was injected at a rate of 100 nL/min. For VSVΔG(LCMV-G), 100 nL of 1×10^{8} ffu/mL was injected. For retinal injections, 0.5 μL of 3×10^{10} ffu/mL VSV, VSV(RABV-G), or VSV(LCMV-G) was injected intravitreally 1 wk after 0.5 μL of 1 μg/mL plasmin (EMD4 Biosciences/Novagen) dissolved in PBS was injected intravitreally. For LGN injections, 1 μL of 3×10^{10} ffu/mL virus was injected. For analysis of brain labeling from an LGN injection, 100 nL of 3×10^{10} ffu/mL was injected. For olfactory epithelial infections, 5 μL of 3×10^{8} ffu/mL VSV(LCMV-G) was injected into each nostril.

Hippocampal Slice Cultures. Monosynaptic tracing studies were carried out in organotypic hippocampal slice cultures prepared from postnatal day 5–7 Sprague–Dawley rats as described (6). Slices were biolistically transfected at 7 d in vitro (DIV) and imaged or recorded at 8–10 DIV. Bullets were prepared by using 12.5 mg of 1.6-mm gold particles and 75 μg of total DNA for triple transfection (25 μg of each plasmid). At 1 d posttransfection, slices were infected with 100 μL of 5×10^{5} ffu/mL VSVΔG (ASLV-A/RABV-G), and slices were fixed 18 hpi. For electrophysiological analyses, recordings were initiated 8 hpi.

Electrophysiological Recordings from Hippocampal Cultures. Whole-cell recordings were obtained from cultured neurons by using video-IR/DIC. Neurons were identified as upstream (transfected^{+}/infected^{+}), downstream (transfected^{-}/infected^{+}), or control (transfected^{-}/infected^{-}) by using epifluorescence illumination. For current clamp recordings of upstream cells, glass electrodes (2–4 MΩ) were filled with K^{+}-based internal solution containing (in mM): 135 KMeSO4, 5 KCl, 5 Hepes, 4 MgATP, 0.3 NaGTP, 1 mM EGTA, and 10 μM Alexa Fluor 594 (to image neuronal morphology) adjusted to pH 7.4 with KOH. For voltage clamp recordings of downstream and control neurons, the same glass electrodes were filled with a Cs^{+}-based internal solution containing (in mM): 120 CsMeSO4, 15 CsCl, 10 Hepes, 8 NaCl, 10 TEACl, 4 ATPMg, 0.3 GTPNa, 2 QX314Cl, 1 mM EGTA, and 10 μM Alexa Fluor 594 adjusted to pH 7.3 with CsOH. Current and voltage recordings were made at room temperature by using a Multiclamp 700B amplifier. Data were filtered at 5 kHz and digitized at 10 kHz. ChR2 stimulation was achieved by using a 2-ms collimated pulse of 7 or 20 mW of 473-nm laser light (Opto Engine LLC).

1. Chandran K, Sullivan N-J, Felbor U, Whelan S-P, Cunningham J-M (2005) Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. *Science* 308: 1643–1645.
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4. Wickersham IR, et al. (2007) Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. *Neuron* 53:639–647.
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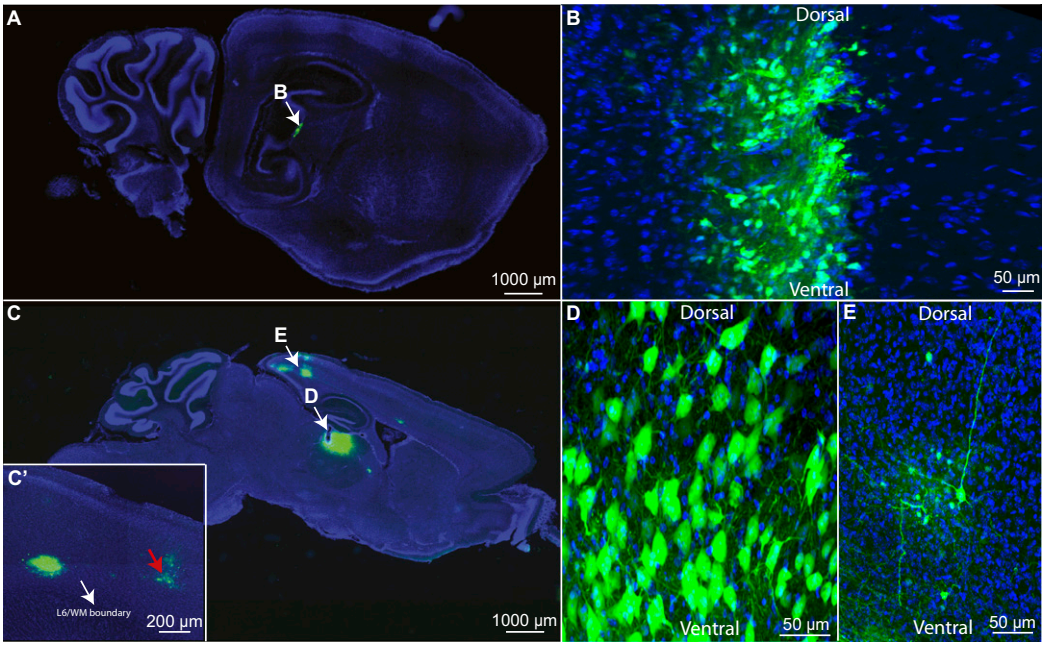

Fig. S1. Parasaggital sections from brains after VSV(LCMV-G) injection into either the eye (*A* and *B*) or LGN (*C*–*E*). (*A*) From a vitreal injection into the eye, labeling could be observed in the LGN by 3 dpi (high magnification in *B*). (*C*) When VSV(LCMV-G) was injected into the LGN, the LGN (*D*) and visual cortex (*E*) were labeled, but not the SC. Cortical labeling was observed to initiate in layer 4 (*C'*, red arrow, from an adjacent section. White arrow indicates layer 6/white matter boundary).

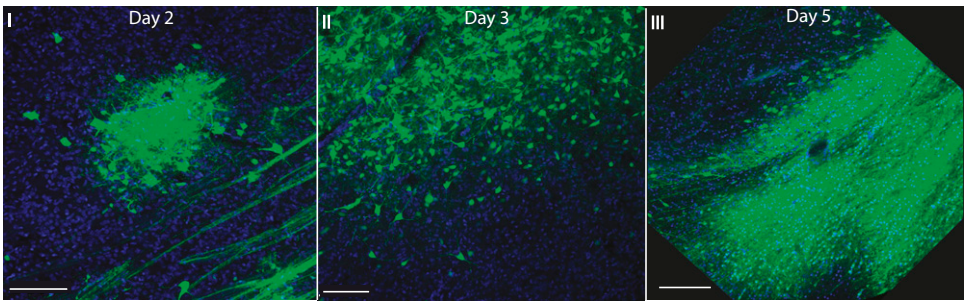

Fig. S2. High magnification images from Fig. 3. *I*, Fig. 3*D*, CP; *II*, Fig. 3*E*, GP; *III*, Fig. 3*G*, STn. (Scale bars: 100 μm.)

Table S1. Visual system quantification with VSV-LCMV-G

dpi	SCN	LGN	Superior colliculus
3	478	29	100
3	389	4	8
4	406	25	13
4	134	12	3
5	1,445	271	2
6	405	2,249	2,986
6	115	3,662	2,729
6	19	1,565	323
7	11	87	3,292
8	199	1,953	3,280