# Supporting Information

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## SI Text

**Biochemical Measurement of Surface Receptors.** For assays with dissociated neurons, cortical cultures (14–18 DIV) were prepared and infected with HSV vectors (1–2 multiplicity of infection, MOI) 20–24 h before biotinylation experiments. Cultures were rinsed with warm DMEM and treated with NMDA (20 $\mu$M, 3 min). After brief rinses with DMEM, conditioned medium was added back, and plates were incubated at 37 °C with 5% $CO_2$ for 30 min. Surface receptors were biotinylated with 1.5 mg/mL biotin (Sulfo-NHS-SS-biotin; Pierce) in ACSF (in mM: 124 NaCl, 5 KCl, 1.23 $NaH_2PO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, and 10 D-glucose, saturated with 95% $O_2$/5% $CO_2$) (20 min) on ice. Cultures were rinsed 3 times (1× with TBS and 2× with ACSF) and solubilized with RIPA buffer (1% Triton X-100, 0.5% SDS, 0.5% deoxycholic acid, 50 mM NaPO4, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 mM PMSF, and 1 mg/mL leupeptin). Homogenates were centrifuged (14,000 × $g$) for 15 min at 4 °C. Supernatants were collected, and protein concentration was measured by using a DC protein assay kit (Bio-Rad Laboratories). Extracts containing 50 $\mu$g of protein were incubated with Immobilized Neutravidin agarose (80 $\mu$L; Pierce) overnight at 4 °C, washed (3×; RIPA buffer), then resuspended in SDS sample buffer (40 $\mu$L) and boiled.

Quantitative immunoblotting was performed on biotinylated surface proteins by using primary antibodies to TfR (Chemicon) and to Glur1 (Upstate). An antibody to the GluR1 subunit was used in these experiments because of the limited amounts of sample, making it necessary to strip and reprobe immunoblots after visualization of TfR protein levels. It should be noted that previous research has shown that activity-dependent changes in GluR2/3 and GluR1 subunit levels within the visual cortex mirror one another and are highly correlated (1).

TfR internalization assays were performed as described in ref. 2 with minor changes. Briefly, cultures were preincubated with leupeptin (100 $\mu$g/mL, 30 min) before biotinylation. After 3 rinses with D-PBS, cells were incubated with 1 mg/mL Sulfo-NHS-SS-biotin in D-PBS on ice (20 min). Remaining biotin was quenched with 50 mM glycine (3 × 5 min). Whereas control samples remained on ice, experimental samples were treated with 40 $\mu$g/mL transferrin in D-PBS (30 min, 37 °C). Biotinylated receptors remaining or recycled back to the surface were stripped with glutathione buffer (100 mM glutathione, 75 mM NaCl, 10 mM EDTA, 1% BSA, 150 mM NaOH to pH 8.6) for 15 min (2×) and quenched with iodoacetamide (5 mg/mL; 3 × 5 min). Cells were lysed with RIPA buffer, and biotinylated proteins were purified with Neutravidin agarose. To control for nonspecific binding and entrance of biotin into cells, data from transferrin-treated samples were normalized to control samples maintained at 4 °C.

For biochemical assays using cortical slices, viral vectors were unilaterally injected into visual cortex 2 days before biochemical assay. For all HSV infections, a small volume of virus (1–1.5 $\mu$L total) was injected into the binocular visual cortex 450 $\mu$m below dural surface at a rate of 0.1 $\mu$L/min. Visual cortical slices were prepared from both hemispheres, as described in ref. 1, 2 days postinfection. After recovery (1–2 h) in ACSF, slices were transferred to a NMDA (100 $\mu$M) containing chamber and incubated (15 min). Slices were transferred back to a recovery chamber and incubated for 20 min. HSV-infected and corresponding control areas from the opposing hemisphere were microdissected, homogenized in RIPA buffer, and processed as described above by using antibodies to the GluR1 and GluR2/3 subunits of AMPARs (Upstate).

**In Vitro Slice Electrophysiology.** Mice were injected in the visual cortex unilaterally with HSV-G2CT as described above, and acute slices were prepared 48–72 h later as described in ref. 3. Slices recovered in ACSF containing (in mM): 124 NaCl, 5 KCl, 1.23 $NaH_2PO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, and 10 D-glucose (saturated with 95% $O_2$/5% $CO_2$) for 0.5 h at 32 °C, and transferred to room temperature for an additional 0.5 h before recording. Recordings were made in ACSF at 30 °C. Layer 4 pyramidal neurons were identified under IR-DIC and examined for GFP fluorescence. GFP-positive cells and neurons from the control noninfected hemisphere were patched under IR-DIC. Somatic whole cell recordings were made by using 4–6 MΩ pipets filled with (in mM): 103 D-gluconic acid, 103 CsOH, 20 Hepes, 5 TEA-Cl, 2.8 NaCl, 0.2 EGTA, 4 MgATP, 0.3 NaGTP, 10 Na-phosphocreatine, 5 QX-314, and 0.2% biocytin (290–300 mOsM, pH adjusted to 7.2 with CsOH). A liquid junction potential (approximately −14 mV) was corrected, and series resistance ($R_s$) was monitored throughout. Experiments showing a >20% change in $R_s$ were excluded, as were cells with a resting membrane potential less negative than −50 mV. When possible, attempts were made to record from control and G2CT expressing neurons in slices prepared from the same animal on the same day. Baseline EPSCs were evoked (0.05 Hz) under voltage clamp (−65 mV) with a bipolar stimulating electrode (FHC) placed in the white matter. Following a stable baseline, input-output curves were recorded, and a stimulation intensity yielding a half-maximal response was chosen for further study. For LTD experiments, a stable baseline was recorded for 10 min, and LTD was induced by pairing 600 pulses at 1 Hz with depolarization of the postsynaptic neuron to −40 mV (3).

AMPA/NMDA ratios were determined essentially as described in ref. 4 (Fig. S2). AMPA-mediated EPSCs were recorded at −90 mV in ACSF supplemented to (in mM) 4 $CaCl_2$, 4 $MgCl_2$, and 25 D-glucose. Mixed AMPA and NMDA EPSCs were then recorded at +40 mV. A 1-ms time window, set flanking the peak of the AMPA-mediated response, was used to determine the AMPA component of the mixed EPSC. The NMDA component of the mixed EPSC was determined by using a 1-ms time window set 45 ms following the stimulus artifact, a period at which the AMPA-mediated response at −90 mV has decayed to baseline. At each holding potential, 4–6 responses were recorded at 0.05 Hz, responses showing polysynaptic activity were excluded from analysis. The ratio of AMPA to NMDA amplitude was calculated for HSV-G2CT-infected neurons and normalized to the ratio obtained in control neurons.

After recording, slices were fixed in 4% paraformaldehyde and stained by using Alexa-555-conjugated streptavadin (Molecular Probes) and deep-red Nissl stain (NeuroTrace 640/660; Molecular Probes). All recorded neurons were spiny, and located within layer 4.

1. Heynen AJ, et al. (2003) Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation. *Nat Neurosci* 6:854–862.

2. Ehlers MD (2000) Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* 28:511–525.

3. Crozier RA, Wang Y, Liu CH, Bear MF (2007) Deprivation-induced synaptic depression by distinct mechanisms in different layers of mouse visual cortex. *Proc Natl Acad Sci USA* 104:1383–1388.
4. Myme CI, Sugino K, Turrigiano GG, Nelson SB (2003) The NMDA-to-AMPA ratio at synapses onto layer 2/3 pyramidal neurons is conserved across prefrontal and visual cortices. *J Neurophysiol* 90:771–779.
5. Neve RL, Neve KA, Nestler EJ, Carlezon WA, Jr (2005) Use of herpes virus amplicon vectors to study brain disorders. *Biotechniques* 39:381–391.

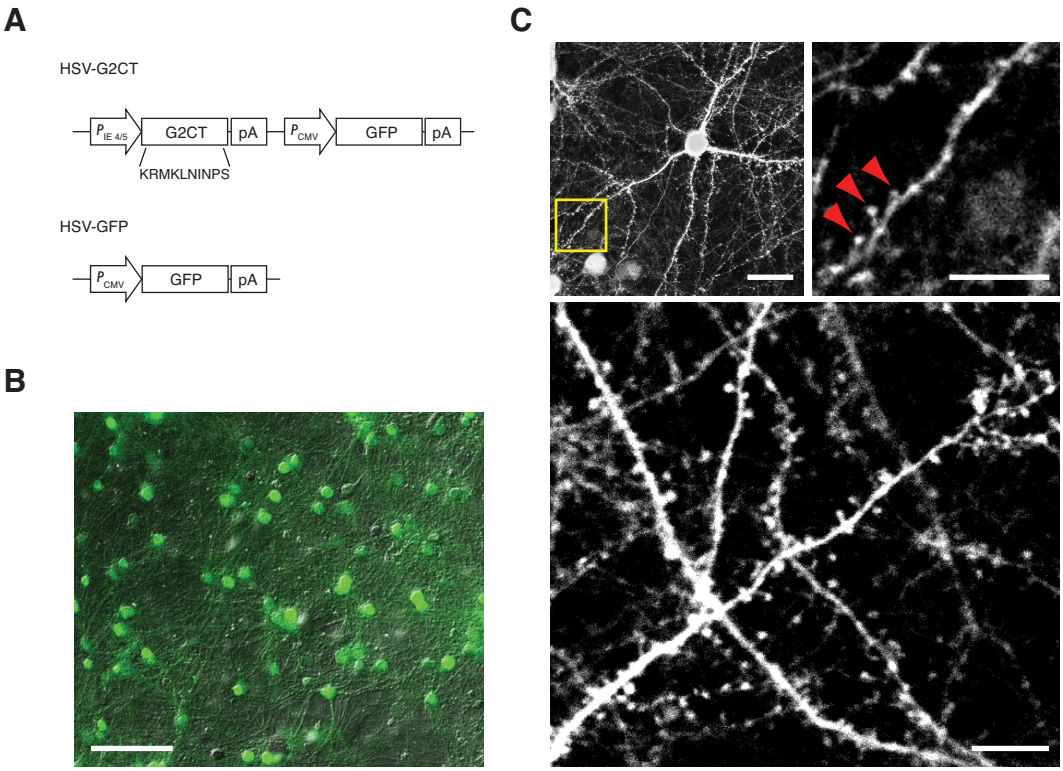


**Fig. S1.** HSV expression vector construction and in vitro infection efficiency. (*A*) Schematic diagram of HSV-G2CT and HSV-GFP expression vectors. The p1005+ HSV-amplicon bicistronic plasmid was used as a backbone to generate both viral vectors. The first cistron is composed of the native IE4/5 promoter, a multiple cloning site (MCS), and an SV40 polyA signal, whereas the second cistron is composed of a complete CMV-GFP expression cassette. A 50-bp fragment encoding the G2CT peptide (KRMKLNINPS) was amplified by using PCR and 2 overlapping primers (5-ctcgaggcatgaaacgaatgaagctgaatattaaccc-3′, 5′-accggtctaagatgggttaatattcagc-3′). The resultant PCR product was then cloned into the MCS, downstream of the HSV IE 4/5 promoter. HSV-GFP was generated by using the unaltered p1005+ plasmid. Viral packaging was carried out as described in ref. 5. (*B*) HSV-G2CT readily infects cultured visual cortical neurons. The photomicrograph of cultured visual cortical neurons demonstrates that >90% of the neurons expressed GFP within 24 h after incubation of virus at a multiplicity of infection of approximately 2 (gray-scale DIC image overlayed with the GFP channel in green) (Scale bar, 50 $\mu$m.) (*C*) HSV-G2CT-infected neurons exhibit normal cell morphology. (*Top Left*) Deconvolved maximum-intensity projection (15 $\mu$m total using a 1.0 $\mu$m Z-step) of an isolated neuron with spiny dendrites. The yellow box indicates region with spines visible on distal dendrites. (*Top Right*) Close-up, single XY-scan image of the highlighted field in *Top Left* showing spines located on a distal dendritic segment (red arrowheads). (*Bottom*) Single XY-scan image from a different area of the same culture showing a dense field of distal dendritic segments with numerous spines (Scale bars, *Top Right*, 25 $\mu$m; *Top Left* and *Bottom*, 10 $\mu$m.) All images in *C* were acquired by using a 2-photon laser-scanning microscope (Ultima; Prairie Technologies) with the excitation wavelength set to 930 nm and with a 60×-0.90NA water-immersion objective.

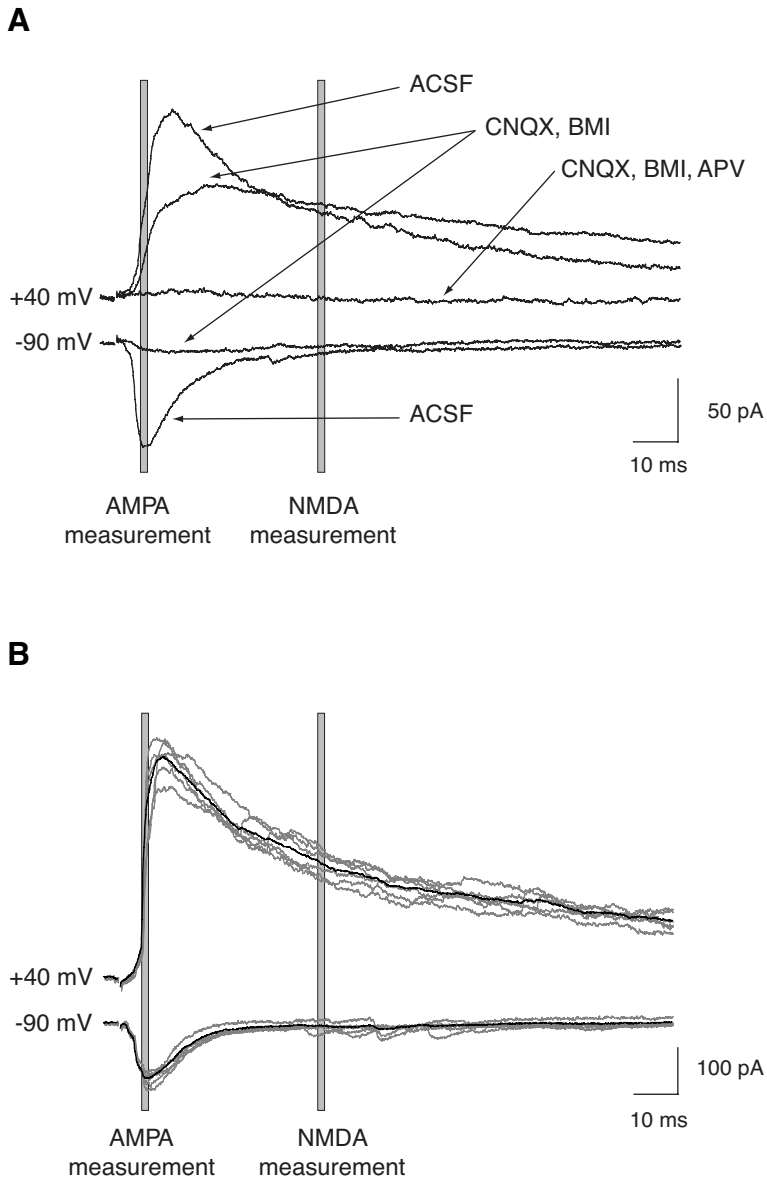


**Fig. S2.** AMPA/NMDA ratio analysis. (*A*) Pharmacological isolation of AMPA- and NMDA-mediated EPSCs. Rectangles indicate time windows used in *B*. The application of CNQX (20 $\mu$M) and BMI (10 $\mu$M) completely abolished the AMPA-mediated response recorded at −90 mV and reduced the fast component of the response at +40 mV, revealing a NMDA-mediated EPSC. This response was completely blocked by addition of 100 $\mu$M DL-APV. (*B*) Representative examples of responses recorded in layer 4 neurons evoked by white matter stimulation. AMPA-mediated EPSCs were recorded at −90 mV in ACSF supplemented to (in mM) 4 $CaCl_2$, 4 $MgCl_2$, and 25 D-glucose. Mixed AMPA and NMDA EPSCs were then recorded at +40 mV. Gray traces are responses to individual stimuli, and the average trace is shown in black. The peak of the response at −90 mV, which is presumably mediated entirely by AMPA currents, was used to establish a 1-ms time window (indicated by gray rectangle) that was then used to determine the AMPA component of the mixed EPSC recorded at +40 mV. A second 1-ms time window, set 45 ms after the stimulus artifact was used to measure the NMDA component of the mixed EPSC. This second window is set at a point after complete decay of the AMPA-mediated current. This method is based on Myme et al. (4).

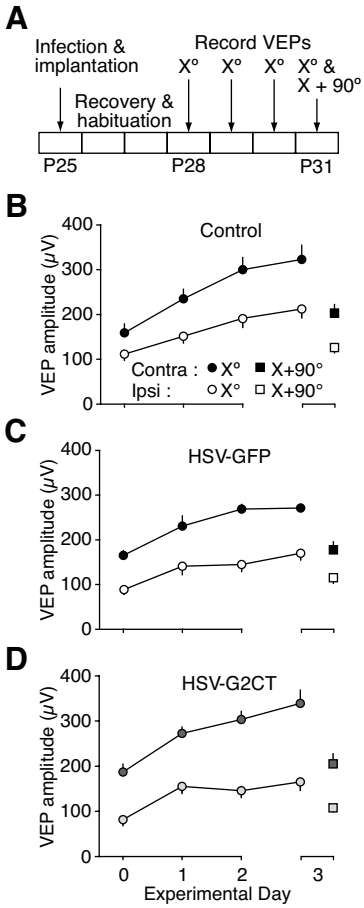


**Fig. S3.** Stimulus-selective response potentiation is unaffected by HSV-G2CT. (*A*) Experimental design for SRP experiments. VEPs were recorded in response to stimuli of a single orientation (X°) for 4 days, then in response to the orthogonal orientation (X + 90°). (*B–D*) Significant potentiation of responses evoked by the trained orientation was observed in noninfected control (*B*), HSV-GFP (C), and HSV-G2CT (*D*) infected animals (day 0 vs day 3, paired *t* test, $P < 0.01$ in all cases). Levels of SRP expression in all 3 groups are comparable (repeated measures ANOVA, $P > 0.5$).