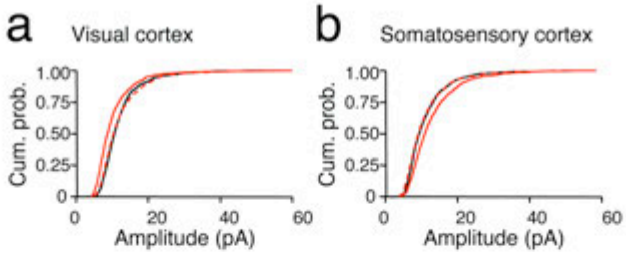
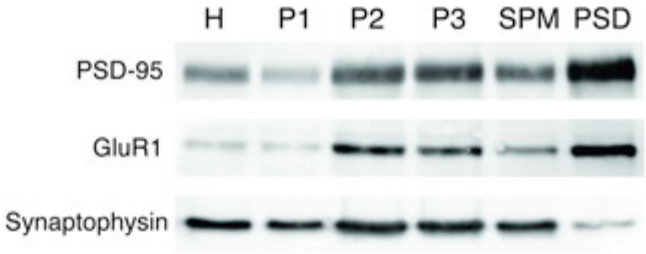
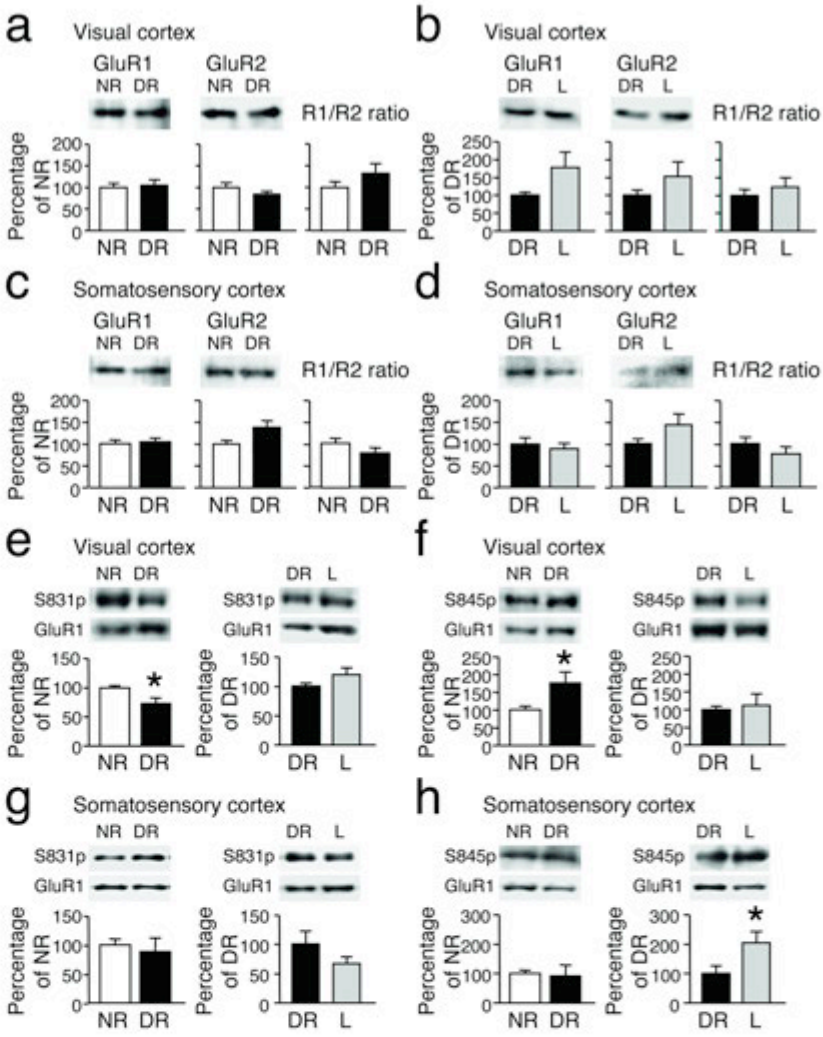


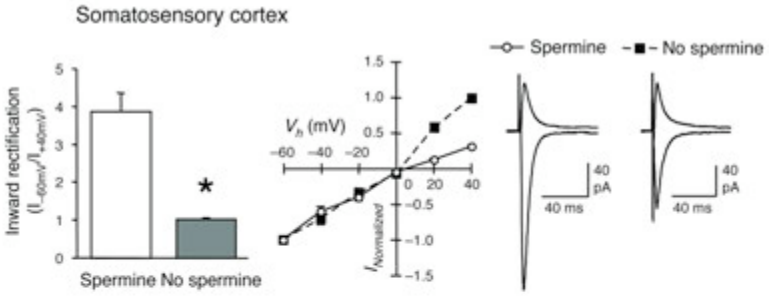
Supplementary Figure 1. Changes in mEPSC amplitude by visual experience follow the rules of a form of homeostatic synaptic plasticity termed "synaptic scaling".
(a) Normalized cumulative probability of mEPSC amplitudes in visual cortex neurons from normal-reared (NR: red solid line) and rats dark-reared for 1 week (DR: black solid line). Superimposed on the graph is a cumulative probability of mEPSC amplitudes from NR that are multiplied by a factor (1.2) to match the average mEPSC amplitude to that from DR (NR_{scaled}: red dotted line). There was no statistically significant difference between cumulative probability of DR and that of NR_{scaled} (Kolmogorov-Smirnov test: $P > 0.1$). This is consistent with a multiplicative scaling of synaptic strength as proposed by Turrigiano *et al.* (1998).
(b) Normalized cumulative probability of mEPSC amplitudes in somatosensory cortex neurons from NR (red solid line) and 1 week DR (black solid line). Superimposed on the graph is a cumulative probability of mEPSC amplitudes from NR that are multiplied by a factor (0.8) to match the average mEPSC amplitude to that from DR (NR_{scaled}: red dotted line). There was no statistically significant difference in the cumulative probability of DR and that of NR_{scaled} (Kolmogorov-Smirnov test: $P > 0.1$).



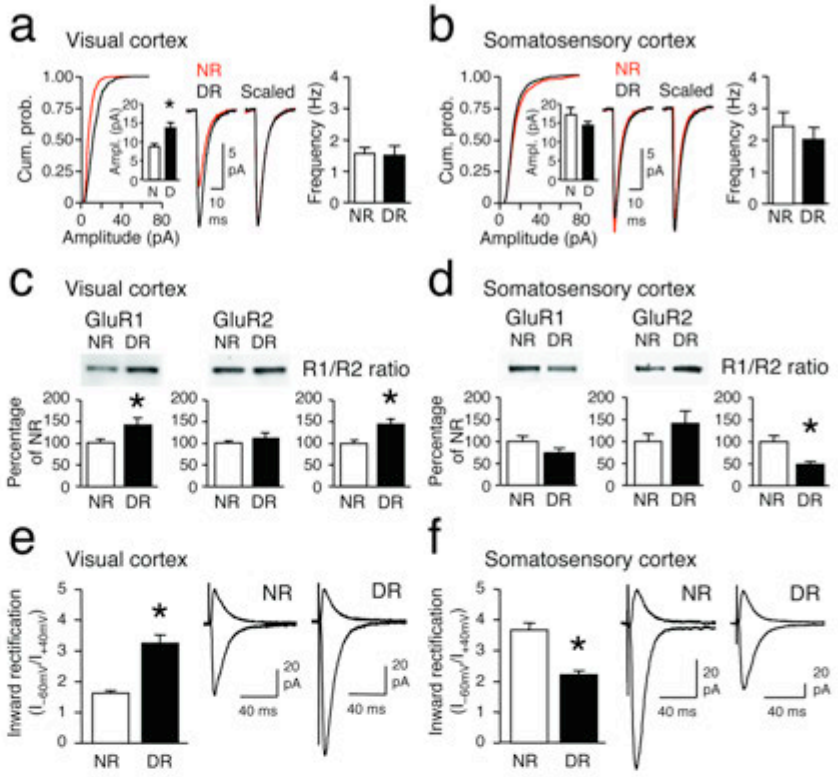
Supplementary Figure 2. Biochemical isolation of postsynaptic density (PSD) fractions.
Equal amount of protein samples taken from each preparation step (H: homogenate, P1: pellet 1, P2: pellet 2, P3: pellet 3, SPM: synaptic plasma membrane, PSD: postsynaptic density: see Supplementary Methods for detail) were loaded on each lane and probed with PSD-95 antibody, GluR1-C terminal antibody, and synaptophysin antibody. Note an enrichment of postsynaptic proteins PSD-95 and GluR1 in the PSD fraction, while a loss of a presynaptic marker synaptophysin.



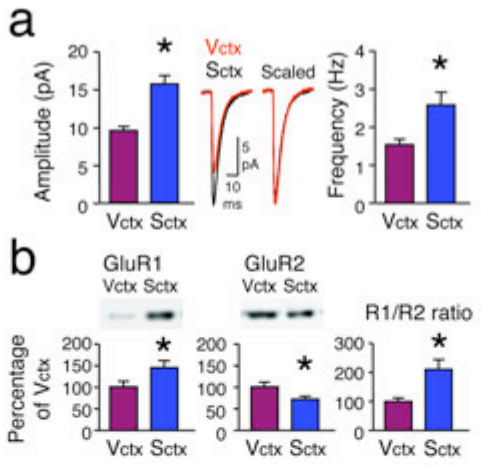
Supplementary Figure 3. Comparison of GluR1/GluR2 ratio in total brain homogenate and alterations in GluR1 phosphorylation sites by visual experience. **(a)** No significant changes in GluR1 (NR = 100 ± 9.3% of NR, n =8; DR = 105 ± 12.5% of NR, n = 9; t-test: $P > 0.7$), GluR2 (NR = 100 ± 10.5% of NR; DR = 84 ± 6.2% of NR; t-test: $P > 0.2$), or GluR1/GluR2 ratio (NR = 100 ± 12.4% of NR; DR = 132 ± 22.6% of NR; t-test: $P > 0.2$) in total homogenate of visual cortex following 1 week of DR. **(b)** Re-exposing 1 week DR rats to 2 days of light (L) increased both GluR1 (DR = 100 ± 7.2% of DR, n = 15; L = 178 ± 42.4% of DR, n = 14; t-test: $P > 0.09$) and GluR2 (DR = 100 ± 14.2%; L = 151 ± 41.2%; t-test: $P > 0.2$) in visual cortex homogenate, which did not reach statistical significance. There was no significant change in R1/R2 ratio in the homogenate of visual cortex (DR = 100 ± 16.9%; L = 125 ± 25.9%; t-test: $P > 0.4$). **(c)** DR for 1 week did not significantly alter GluR1 (NR: 100 ± 6.8%, n = 9; DR: 103 ± 7.4%, n = 8; t-test: $P > 0.9$), GluR2 (NR: 100 ± 6.7%; DR: 138 ± 14.2%; t-test: $P > 0.2$), or R1/R2 ratio (NR: 100 ± 10.2%; DR: 78 ± 10.6%; t-test: $P > 0.4$) in total homogenate of somatosensory cortex. **(d)** Re-exposing DR animals to 2 days of light (L) did not produce statistically significant changes in GluR1 (DR: 100 ± 12.8%, n = 9; L: 88 ± 11.4%, n = 9; t-test: $P > 0.4$), GluR2 (DR: 100 ± 10.2%; L: 143 ± 24.7%; t-test: $P > 0.1$), or R1/R2 ratio (DR: 100 ± 14.8%; L: 76 ± 15.9%; t-test: $P > 0.2$) in the total homogenate of somatosensory cortex. **(e)** DR for 1 week decreased GluR1 phosphorylation on serine 831 (S831p) in visual cortex (Left panel: NR = 100 ± 3.9% of NR, n = 9; DR = 73 ± 9.3% of NR, n = 9; t-test: $P < 0.02$), which was not reversed by 2 days of re-exposure to light (Right panel: DR = 100 ± 4.6% of DR, n = 9; L = 120 ± 10.3% of DR, n = 9; t-test: $P > 0.1$). **(f)** DR for 1 week increased GluR1 phosphorylation on serine 845 (S845p) in visual cortex (Left panel: NR = 100 ± 9.1% of NR, n = 8; DR = 174 ± 30.5% of NR n = 9; t-test: $P < 0.05$). Re-exposing DR animals to light for 2 days (L) did not produce significant changes in GluR1 S845 phosphorylation (Right panel: DR = 100 ± 8.1% of DR, n = 9; L = 112 ± 12.8% of DR, n = 9; t-test: $P > 0.6$). **(g)** GluR1 S831 phosphorylation in somatosensory cortex did not change significantly following 1 week of DR (Left panel: NR = 100 ± 9.0% of NR, n = 9; DR = 88 ± 23.0% of NR, n = 8; t-test: $P > 0.6$) or re-exposing dark-rearing animals to 2 days of light (Right panel: DR = 100 ± 21.5% of DR, n = 8; L = 67 ± 11.4% of DR, n = 9; t-test: $P > 0.1$). **(h)** GluR1 S845 phosphorylation in somatosensory cortex did not change with 1 week of DR (Left panel: NR = 100 ± 10.1%, n = 9; DR = 90 ± 36.9%, n = 6; t-test: $P > 0.8$), but significantly increased when DR animals were re-exposed to light for 2 days (Right panel: DR = 100 ± 24.8%, n = 6; L = 203 ± 36.5%, n = 9; t-test: $P < 0.04$).



Supplementary Figure 4. Inward rectification is dependent on intracellular spermine.
Left: Inward rectification (IR: I_{-60mV}/I_{+40mV}) of AMPA receptor currents observed in normal reared (NR) somatosensory cortical cells was abolished when using intracellular solution without spermine (Spermine: IR = 3.87 ± 0.46, n = 10 cells; No spermine: IR = 1.02 ± 0.04, n = 4 cells). Asterisk indicates statistically significant difference (Student's t-test: $P < 0.001$). Middle: Comparison of AMPAR I-V curves between NR somatosensory cortical cells recorded with (white circles) and without spermine (black squares). Right: Superimposed representative traces of evoked AMPA receptor-mediated currents measured at –60 mV and +40 mV from NR somatosensory cortical neurons recorded with or without spermine inside the recording pipette.



Supplementary Figure 5. Changes in synaptic AMPA receptors in visual and somatosensory cortices by 5 weeks of dark-rearing from birth. **(a)** Left: Cumulative probability of AMPAR-mediated mEPSC amplitudes in visual cortex neurons from NR (red line) and rats DR for 5 weeks (black line). There was a significant difference between cumulative probability of NR and DR (Kolmogorov-Smirnov test: $P < 0.001$). Inset: Average mESPC amplitude from cells of NR (N: 8.8 ± 0.6 pA, $n = 12$) and 5 week DR (D: 13.7 ± 1.5 pA, $n = 9$). *: t-test, $P < 0.01$. Middle: Average mEPSC traces (NR: red, DR: black, Scaled: average NR trace scaled (red) and superimposed on the average DR trace (black)). Right: No significant change in mEPSC frequency with 5 weeks of DR (NR: 1.6 ± 0.2 Hz, $n = 12$; DR: 1.5 ± 0.3 Hz, $n = 9$; t-test: $P > 0.7$). **(b)** Left: Cumulative probability of mEPSC amplitudes in somatosensory cortex pyramidal neurons from NR (red line) and 5 weeks DR (black line). There was a significant difference between cumulative probability of mEPSC amplitudes from NR and that from DR (Kolmogorov-Smirnov test: $P < 0.001$). Inset: Comparison of average mESPC amplitude from cells from NR (N: 17.1 ± 1.8 pA, $n = 15$) and 5 week DR (D: 14.3 ± 1.0 pA, $n = 19$). This difference did not reach statistical significance (t-test, $P = 0.19$). Middle: Average mEPSC traces (NR: red, DR: black, Scaled: average DR trace was scaled (black) and superimposed on the average NR trace (red)). There was no significant difference in mEPSC kinetics. Right: No significant change in mEPSC frequency with 5 weeks of DR (NR: 2.4 ± 0.4 Hz, $n = 15$; DR: 2.0 ± 0.4 Hz, $n = 19$; t-test: $P > 0.4$). **(c)** GluR1 content in PSDs from 5 weeks DR rat visual cortex was greater compared to that from NR controls (NR: 100 ± 7% of average NR, $n = 9$; DR: 140 ± 16% of average NR, $n = 13$; t-test: $P < 0.04$), which occurred without changes in GluR2 (NR: 100 ± 4% of average NR; DR: 111 ± 12% of average NR; t-test: $P > 0.3$). This led to a significant increase in GluR1/GluR2 (R1/R2) ratio in 5 weeks DR (NR: 100 ± 6% of average NR; DR: 137 ± 13% of average NR; t-test: $P < 0.02$). **(d)** A significant decrease in GluR1/GluR2 ratio in PSDs from somatosensory cortex of 5 week DR compared to NR controls (NR: 100 ± 14% of average NR, $n = 8$; DR: 49 ± 7% of average NR, $n = 9$; t-test: $P < 0.01$). This occurred without statistically significant changes in GluR1 or GluR2 content in the PSD (GluR1: NR = 100 ± 12% of average NR, DR = 74 ± 10% of average NR, t-test: $P > 0.1$; GluR2: NR: 100 ± 17% of average NR, DR = 141 ± 27% of average NR, t-test: $P > 0.2$). **(e)** Inward rectification of current through synaptic AMPA receptors was larger in visual cortical neurons in 5 weeks DR rats when compared to age-matched NR controls (inward rectification (I_{-60mV}/I_{+40mV}): NR = 1.63 ± 0.07, $n = 11$; DR = 3.25 ± 0.26, $n = 14$; t-test: $P < 0.0001$). **(f)** In the somatosensory cortex, there was less inward rectification of AMPA receptor currents in neurons from 5 weeks DR rats compared to NR controls (inward rectification (I_{-60mV}/I_{+40mV}): NR = 3.65 ± 0.22, $n = 14$; DR = 2.20 ± 0.13, $n = 18$; t-test: $P < 0.0001$).



Supplementary Figure 6. Differences in synaptic AMPA receptor function and subunit composition in visual and somatosensory cortices of normal-reared rats.
(a) Left: In 5 weeks old NR rats, average mEPSC amplitude in somatosensory cortex (Sctx) was greater than in visual cortex (Vctx) (Vctx: 9.5 ± 0.5 pA, $n = 20$; Sctx: 15.6 ± 1.1 pA, $n = 27$; t-test: $P < 0.0001$). Middle: Average mEPSC traces (Vctx: red, Sctx: black, Scaled: average mEPSC trace from visual cortex was scaled (red) and superimposed on average mEPSC trace from somatosensory cortex (black)). There was no difference in mEPSC kinetics. Right: Average mEPSC frequency was higher in cells from somatosensory cortex (Vctx: 1.5 ± 0.1 Hz, $n = 20$; Sctx: 2.6 ± 0.3 Hz, $n = 27$; t-test: $P < 0.001$).
(b) Somatosensory cortex has more GluR1 (Vctx: 100 ± 13% of average Vctx, $n = 7$; Sctx: 145 ± 15% of average Vctx, $n = 6$; t-test: $P < 0.05$), while less GluR2 (Vctx: 100 ± 11% of average Vctx; Sctx: 72 ± 7% of average Vctx; t-test: $P < 0.05$) in the PSD than visual cortex. This resulted in a larger GluR1/GluR2 ratio at synapses of somatosensory cortex (Vctx: 100 ± 11% of average Vctx; Sctx: 210 ± 34% of average Vctx; t-test: $P < 0.03$).

Supplementary methods

Dark-rearing animals

Long-Evans rats (Charles River) were raised under normal lighted environment (12 hr light/12 hr dark cycle) until 4 weeks of age. Dark-rearing was initiated at 4 weeks of age for a duration of 1 week, while control (normal-reared) animals were continuously raised in the normal lighted condition for the same duration. The animals in the dark were cared for using infrared vision goggles under dim infrared light. After 1 week of dark-rearing, some of the rats were taken out to the lighted environment for 2 days to study the effect of re-exposure to light. In a parallel set of experiments, Long-Evans rats were raised from birth in a light-tight dark room for 5 weeks (dark-reared from birth), while control animals were raised in the normal lighted environment for the same duration.

Preparation of visual cortical slices

Each animal was deeply anesthetized by placing it in a chamber with halothane. The brain was rapidly removed and immersed in ice-cold dissection buffer (212.7 mM sucrose, 5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose, 3 mM $MgCl_2$, 1 mM $CaCl_2$) bubbled with 95% O_2/5% CO_2 mixture. Blocks of primary visual cortex, primary somatosensory cortex, primary auditory cortex, or frontal cortex were rapidly dissected, and sectioned in the coronal plane into 300 m thick slices using a Vibratome 3000 plus^{TM} microslicer (Ted Pella). The slices were collected in ice-cold dissection buffer and gently transferred to a submersion holding chamber with artificial cerebral spinal fluid (ACSF: 124 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose, 1 mM $MgCl_2$, 2 mM $CaCl_2$) saturated with 95% O_2/5% CO_2. The slices were recovered for ~1 hour at room temperature prior to the recording.

Whole-cell recording of AMPA receptor-mediated mEPSCs and evoked AMPA receptor-mediated synaptic currents

Primary visual (V1), primary somatosensory (S1), primary auditory (A1), or frontal cortical slices were moved to a submersion recording chamber mounted on a stage of an upright microscope (E600 FN, Nikon) equipped with infrared differential interference contrast (IR-DIC). Layer 2/3 pyramidal cells were visually identified and patched using a whole-cell patch pipette (tip resistance: 2-5 M) filled with intracellular solution (130 mM

Cs-gluconate, 8 mM KCl, 1 mM EGTA, 10 mM HEPES, 4 mM ATP and 5 mM QX-314; pH 7.4; 285-295 mOsm). To isolate AMPA receptor-mediated mEPSCs, 1 M TTX, 20 M bicuculline, and 100 M D,L-APV were added to the ACSF (2 ml/min, 30 ± 1 C) continually bubbled with 95% O_2/5% CO_2. mEPSCs were recorded at a holding potential (Vh) of –80 mV using Axopatch-clamp amplifier (Axon Instruments), digitized at 2 kHz by a data acquisition board (National Instruments), and acquired using the Igor Pro™ software (Wave Metrics). Acquired mEPSCs were analyzed using the Mini Analysis Program™ (Synaptosoft). The threshold for detecting mEPSCs was set at 3 times the Root Mean Square (RMS) noise. There was no significant difference in RMS noise between the experimental groups (Vctx: NR: 1.6 ± 0.04, n = 8; 1 wk DR: 1.9 ± 0.07, n = 16; L: 1.7 ± 0.09, n = 13, ANOVA: $F_{2,34}$ = 3.086, P > 0.05; Vctx: NR: 1.5 ± 0.1, n = 12; 5 wk DR: 1.8 ± 0.15, n = 9; t-test: P > 0.1; Sctx: NR: 1.8 ± 0.06, n = 12; 1 wk DR: 1.9 ± 0.08, n = 16; L: 1.7 ± 0.07, n = 15, ANOVA: $F_{2,40}$ = 0.825, P > 0.4; Sctx: NR: 2.0 ± 0.09, n = 15; 5 wk DR: 1.9 ± 0.07, n = 19; t-test: P > 0.08; Actx: NR: 1.9 ± 0.03, n = 17; 1wk DR: 1.8 ± 0.05, n = 18; t-test: P > 0.1; Fctx: NR: 1.9 ± 0.06, n = 11; 1 wk DR: 2.0 ± 0.09, n = 9; t-test: P > 0.3). A possibility of dendritic filtering was assessed by plotting mEPSC amplitude against mEPSC rise time. Cells showing a negative correlation between mEPSC amplitude and rise time (i.e. dendritic filtering present) were excluded from analysis, as well as mEPSCs with greater than 3 msec rise time (measured between 10-90% of amplitude). Average mEPSC amplitude and frequency were calculated and compared across different experimental groups using one-factor ANOVA or unpaired Student's t-test.

Evoked AMPA receptor-mediated synaptic currents (EPSCs) were measured from layer 2/3 pyramidal cells of either visual or somatosensory cortices in response to stimulation through an electrode (concentric bipolar, FHC) placed in layer 4. To isolate the AMPA receptor component, 100 M D,L-APV and 100 M bicuculline were added to the bath solution. The concentration of $CaCl_2$ and $MgCl_2$ in the ACSF were changed to 4 mM and 2 mM, respectively, to prevent polysynaptic responses upon stimulation in the presence of bicuculline. Intracellular recording solution containing 200 M spermine (in 90 mM $CsMeSO_3H$, 5 mM $MgCl_2$, 8 mM NaCl, 10 mM EGTA, 20 mM HEPES, 1 mM QX-314, 0.5 mM Na_3GTP, and 2 mM Mg•ATP, pH 7.2, 250-270 mOsm) was used. For generating I-V curves for rectification measurements, cells were held at –60, –40, –20, 0, +20 and +40 mV. Inward Rectification (IR) was calculated by dividing the absolute amplitude of average EPSC measured at –60 mV by that at +40 mV. There were no significant differences in

calculated reversal potentials between groups (Vctx: NR = 2 ± 3.0 mV, n = 6; DR = 1 ± 1.1 mV, n = 9; L = 4 ± 1.4 mV, n = 5; ANOVA: $F_{2,17} = 0.457$, $P > 0.6$; Sctx: NR = 4 ± 2.2 mV, n = 6; DR = 4 ± 0.9 mV, n = 9; L = 2 ± 1.1 mV, n = 5; ANOVA: $F_{2,17} = 0.785$, $P > 0.4$). Reversal potentials were calculated using equations generated by fitting a linear regression curve to the current values collected at negative holding potentials. Only the cells and recording conditions that meet the following criteria were studied: Vm –65 mV, input R 100 M⏐, series R 25 M⏐. Cells were discarded if input R or series R changed more than 15%. Junction potentials were typically less than 5 mV, and were left uncompensated.

Postsynaptic density (PSD) preparation

Primary visual (V1) and primary somatosensory (S1) cortices from normal-reared and dark-reared rats were gently homogenized on ice in HEPES-buffered sucrose (0.32 M sucrose, 4 mM HEPES, pH 7.4) containing 2 mM EGTA, 50 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 M okadaic acid, and protease inhibitors (Protease Inhibitor Cocktail, Pierce). Primary visual or primary somatosensory cortices from two animals were pooled together for one data point. The homogenates (H) were centrifuged at 800 × g for 10 min (4 C) to remove pelleted nuclear fraction (P1), and the resulting supernatants (S1) were centrifuged at 10,000 × g for 15 min (4 C) to yield the crude membrane pellets (P2). P2 fractions were resuspended in HEPES-buffered sucrose with inhibitors and respun at 10,000 × g for 15 min (4 C) to yield the washed crude membrane fractions (P2'). P2' fractions were lysed by hypo-osmotic shock in ice-cold 4 mM HEPES (pH 7.4, with inhibitors), and centrifuged at 25,000 × g for 20 min to generate lysed synaptosomal membrane fractions (P3). P3 was subsequently resuspended in HEPES-buffered sucrose with inhibitors, and run on a discontinuous sucrose gradient (1.2 M, 1.0 M, and 0.8 M sucrose with inhibitors) at 150,000 × g for 2 hours (4 C). Synaptic plasma membrane (SPM) fractions were collected between 1.0 M and 1.2 M sucrose and diluted with 2.5 volumes of 4 mM HEPES with inhibitors. SPM was pelleted by centrifugation at 150,000 × g for 30 min (4 C), resuspended in 0.5% Triton X-100, HEPES-EDTA solution (50 mM HEPES, 2 mM EDTA, pH 7.4) with inhibitors, and rotated for 15 min at 4 C. Solubilized SPM was then centrifuged at 32,000 × g for 20 min to pellet the postsynaptic density fraction (PSD). PSD fractions were resuspended in gel

sample buffer and processed for SDS-PAGE (4 g of PSD proteins were loaded per lane) and immunoblot analysis.

Immunoblot analysis

SDS-PAGE gels were transferred to polyvinyl difluoride (PVDF) membranes (Immobilon^{TM}, Millipore). The PVDF membrane blots were blocked for ~1 hr in blocking buffer (1% bovine serum albumin and 0.1% Tween-20 in phosphate buffered saline (PBS), pH 7.4), and subsequently incubated for 1-2 hrs in primary antibodies diluted in blocking buffer to yield the effective concentration as tested prior to the experiments. After 5 times 5 min washes in blocking buffer, the blots were incubated for 1 hr in secondary antibody linked to alkaline phosphatase (AP) diluted 1:10,000 in blocking buffer. The blots were washed 5 times 5 min, and developed using enhanced chemifluorescence substrate (ECF substrate, Amersham). The ECF blots were scanned and quantified using the Versa Doc 3000^{TM} gel imaging system (Bio Rad). The signal of each sample on a blot was normalized to the average signal from normal-reared (NR) or dark-reared (DR) samples respectively to obtain the % of average NR or % of average DR values, which were compared across different experimental groups using one-factor ANOVA or unpaired Student's t-test.