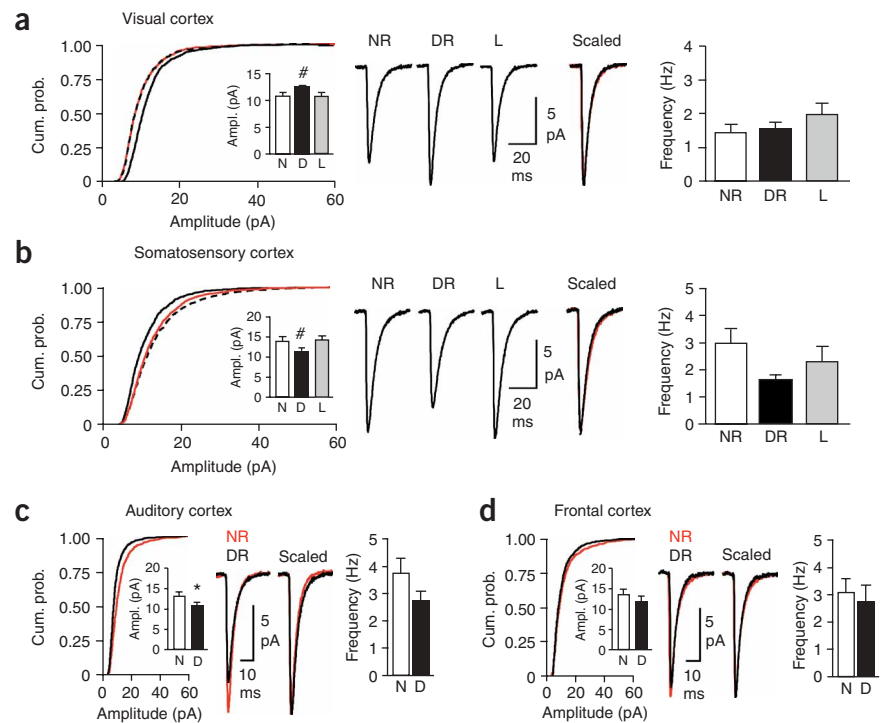


Cross-modal regulation of synaptic AMPA receptors in primary sensory cortices by visual experience

Anubhuthi Goel^{1,2,5}, Bin Jiang³⁻⁵, Linda W Xu¹, Lihua Song¹, Alfredo Kirkwood³ & Hey-Kyoung Lee^{1,2}

Lack of a sensory input not only alters the cortical circuitry subserving the deprived sense, but also produces compensatory changes in the functionality of other sensory modalities. Here we report that visual deprivation produces opposite changes in synaptic function in primary visual and somatosensory cortices in rats, which are rapidly reversed by visual experience. This type of bidirectional cross-modal plasticity is associated with changes in synaptic AMPA receptor subunit composition.

Figure 1 Cross-modal changes in AMPAR-mediated synaptic transmission by visual experience. **(a–d)** Left, cumulative probability of AMPAR-mediated mEPSC amplitudes. Middle, average mEPSC traces. Right, change in mEPSC frequency with visual experience. NR, rats reared normally (red solid line); DR, rats reared in the dark for 1 week (black solid line); L, rats reared in the dark for 1 week and then re-exposed to light for 2 d (black dotted line). Insets in cumulative probability graphs, average mEPSC amplitudes from NR (N), DR (D) and L groups. Results are from **(a)** visual, **(b)** somatosensory, **(c)** auditory and **(d)** frontal cortex. In visual, somatosensory and auditory cortex, mEPSC of the DR group was significantly different from that of the NR and L groups (Kolmogorov-Smirnov test: $P < 0.001$). In visual, somatosensory and frontal cortex, there was no change in mEPSC kinetics across groups. In auditory cortex, there was a significant increase in decay time constant (τ) in the DR group (NR: 4.0 ± 0.25 ms, $n = 17$; DR: 4.9 ± 0.36 ms, $n = 18$; t -test: $P < 0.04$). In all four cortices, there was no significant change in mEPSC frequency with visual experience. # $P < 0.03$ by ANOVA, Fisher's protected least significant difference (PLSD) *post-hoc* test. * $P < 0.04$ by t -test. Error bars represent s.e.m. All experiments were approved by the Institutional Animal Care and Use Committees (IACUCs) of the University of Maryland and Johns Hopkins University.



Loss of vision is usually accompanied by the increased functionality of other sensory modalities^{1,2}. Systems-level analyses of cross-modal plasticity have revealed anatomical and functional rewiring of cortical circuits³. However, little is known about the cellular and molecular mechanisms underlying this type of plasticity. Here we examined whether manipulation of visual experience can induce bidirectional cross-modal plasticity of synaptic function in primary sensory cortices, and investigated the molecular mechanisms underlying this form of plasticity.

To study cross-modal changes in synaptic function by visual deprivation, we dark-reared 4-week-old Long-Evans rats for a period of 1 week and then measured AMPA receptor (AMPA)-mediated miniature excitatory postsynaptic currents (mEPSCs) in layer 2/3 pyramidal neurons in slices from primary visual, somatosensory and auditory cortex (**Supplementary Methods** online). In visual cortex, dark rearing produced an increase in mEPSC amplitude that was reversed by re-exposing the rats to lighted conditions for 2 d (normal-reared (NR): 10.7 ± 0.6 pA, $n = 8$; dark-reared (DR): 12.4 ± 0.4 pA, $n = 16$; re-exposure to light (L): 10.7 ± 0.4 pA, $n = 13$; analysis of

¹Department of Biology, University of Maryland, College Park, Maryland 20742, USA. ²Neuroscience and Cognitive Science (NACS) Program, University of Maryland, College Park, Maryland 20742, USA. ³Department of Neuroscience, The Mind/Brain Institute, Johns Hopkins University, Baltimore, Maryland 21218, USA. ⁴Current address: Brain Science Institute, Riken, Wako City, Saitama, Japan. ⁵These authors contributed equally to this work. Correspondence should be addressed to H.-K.L. (hlee21@umd.edu).

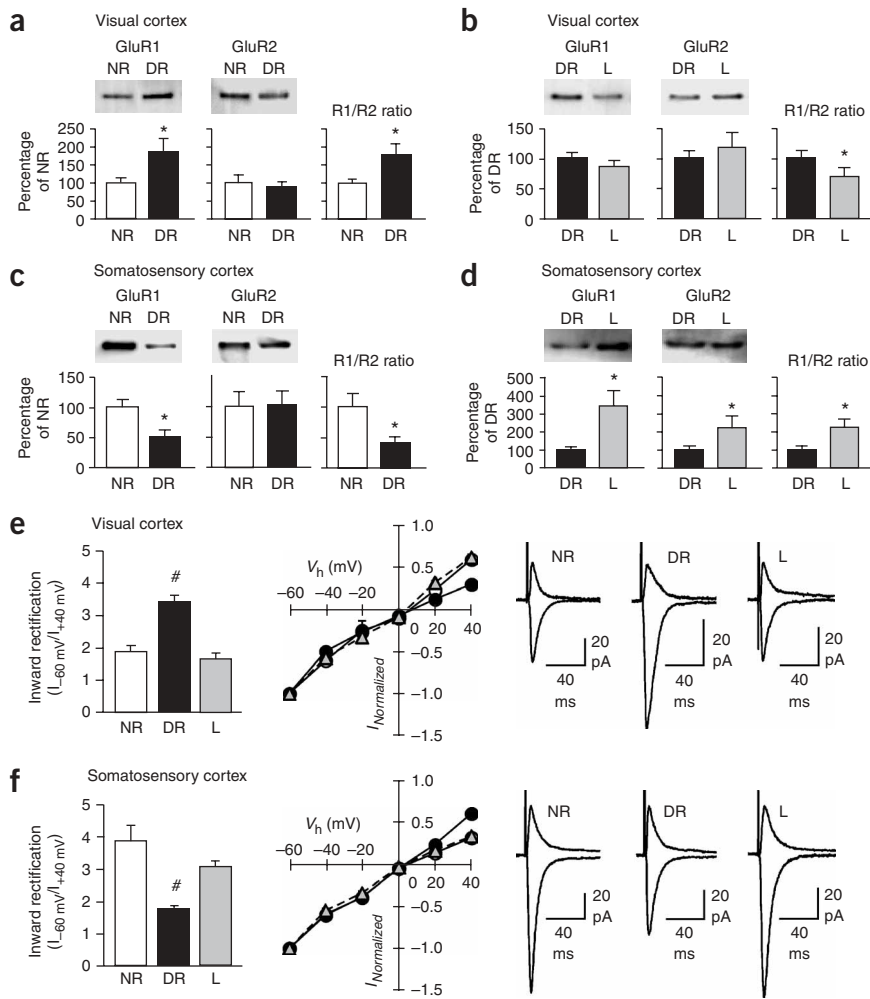


Figure 2 Cross-modal changes in synaptic AMPAR subunit composition by visual experience. **(a)** Dark rearing for 1 week (DR) increased the GluR1/GluR2 (R1/R2) ratio in PSDs of visual cortex, by increasing GluR1 over that in the NR group. **(b)** Re-exposing DR rats to light for 2 d (L) decreased the GluR1/GluR2 ratio in the PSD of visual cortex. **(c)** Dark rearing decreased GluR1/GluR2 ratio in somatosensory cortical synapses by decreasing GluR1 content. **(d)** Re-exposure to light for 2 d increased the GluR1/GluR2 ratio as compared to that in the DR group. Both GluR1 and GluR2 content were significantly increased in the L group. **(e, f)** Left, inward rectification of current through synaptic AMPARs. Middle, AMPAR I - V curves from NR (white circles), DR (black circles) and L (gray triangles). Right, representative AMPAR-mediated current traces at -60 mV and $+40$ mV. In visual cortex **(e)**, dark rearing increased the inward rectification of current through AMPARs, whereas in somatosensory cortex **(f)**, it resulted in a more linear current. Both were reversed by 2 d of light exposure. * $P < 0.05$ by t -test. # $P < 0.01$ by ANOVA, Fisher's PLSD *post-hoc* test. Error bars represent s.e.m.

variance (ANOVA): $F_{2,34} = 5.968$, $P < 0.01$; **Fig. 1a**). Notably, we observed the opposite changes in somatosensory cortex, where 1 week of dark rearing decreased the amplitude of mEPSCs and 2 d of light exposure reversed this effect (NR: 13.8 ± 0.8 pA, $n = 12$; DR: 11.3 ± 0.7 pA, $n = 16$; L: 14.1 ± 0.9 pA, $n = 16$; ANOVA: $F_{2,40} = 3.830$, $P < 0.04$; **Fig. 1b**). Changes in synaptic transmission by dark rearing seems to be general for primary sensory cortices, as dark rearing also reduced mEPSC amplitudes in auditory cortex (NR: 13.0 ± 0.9 pA, $n = 17$; DR: 10.7 ± 0.5 pA, $n = 18$; t -test: $P < 0.04$; **Fig. 1c**), but not in frontal cortex (NR: 13.0 ± 1.2 pA, $n = 11$; DR: 11.7 ± 1.2 pA, $n = 9$; t -test: $P > 0.3$; **Fig. 1d**). There was no significant change in mEPSC frequency across groups in any of the cortical areas (**Fig. 1a–d**): visual cortex: NR = 1.4 ± 0.2 Hz, DR = 1.5 ± 0.2 Hz, L = 2.0 ± 0.3 Hz, (ANOVA: $F_{2,34} = 1.222$,

$P > 0.3$); somatosensory cortex: NR = 2.9 ± 0.5 Hz, DR = 1.6 ± 0.1 Hz, L = 2.3 ± 0.6 Hz (ANOVA: $F_{2,40} = 1.755$, $P > 0.1$); auditory cortex: NR = 3.8 ± 0.5 Hz, DR = 2.8 ± 0.3 Hz (t -test: $P > 0.1$); frontal cortex: NR = 3.1 ± 0.5 Hz, DR = 2.8 ± 0.6 Hz (t -test: $P > 0.6$). The observed increase in mEPSC amplitude in visual cortex of dark-reared rats is consistent with homeostatic plasticity reported previously⁴ (**Supplementary Fig. 1** online). Moreover, visual deprivation decreased mEPSC amplitude in both primary somatosensory and auditory cortices. In addition, our results suggest that visual experience can bidirectionally modify synapses in the visual cortex and can produce the opposite changes in somatosensory cortex.

To examine whether bidirectional changes in mEPSC amplitude by visual experience are due to the regulation of postsynaptic AMPARs, we biochemically isolated postsynaptic density (PSD) fractions from both visual and somatosensory cortices (**Supplementary Methods** and **Supplementary Fig. 2** online). In visual cortex, 1 week of dark rearing increased GluR1 (NR: $100 \pm 12\%$ of average NR, $n = 9$; DR: $190 \pm 34\%$ of NR, $n = 9$; t -test: $P < 0.04$) but not GluR2 (NR: $100 \pm 21\%$ of NR, $n = 9$; DR: $92 \pm 11\%$ of NR, $n = 9$; t -test: $P > 0.7$) (**Fig. 2a**), resulting in a significant increase in the ratio of GluR1 to GluR2 (GluR1/GluR2 ratio; NR: $100 \pm 10\%$ of NR, $n = 9$; DR: $180 \pm 29\%$ of NR, $n = 9$; t -test: $P < 0.03$). Re-exposing dark-reared rats to light for 2 d reversed the increase in the GluR1/GluR2 ratio (DR: $100 \pm 11\%$ of average DR, $n = 14$; L: $69 \pm 14\%$ of DR, $n = 14$; t -test: $P < 0.05$; **Fig. 2b**). In contrast, in somatosensory cortex, dark rearing decreased GluR1 (NR: $100 \pm 11\%$ of NR, $n = 12$; DR: $51 \pm 11\%$ of NR, $n = 12$; t -test: $P < 0.005$) without changing GluR2 (NR: $100 \pm 23\%$ of NR, $n = 12$; DR: $101 \pm 24\%$ of NR, $n = 12$; t -test: $P > 0.9$), resulting in a significant decrease in the GluR1/GluR2 ratio (NR: $100 \pm 22\%$ of NR, $n = 12$; DR: $41 \pm 10\%$ of NR, $n = 12$; t -test: $P < 0.03$; **Fig. 2c**), which was reversed by 2 d of light

exposure (DR: $100 \pm 13\%$ of DR, $n = 12$; L: $224 \pm 42\%$ of DR, $n = 12$; t -test: $P < 0.02$; **Fig. 2d**). These changes at the PSD were not reflected in the total homogenate and were accompanied by changes in GluR1 phosphorylation (**Supplementary Fig. 3** online), suggesting that post-translational mechanisms may be involved. Notably, GluR1 serine 845 phosphorylation correlated with an increase in mEPSC amplitude in both visual and somatosensory cortex (**Supplementary Fig. 3**).

AMPA receptors lacking or having reduced copies of GluR2 display inward rectification of current^{5,6}. Therefore, we assessed the GluR1/GluR2 ratio electrophysiologically by determining an inward rectification index (IR = $(I_{-60 \text{ mV}})/(I_{+40 \text{ mV}})$; **Supplementary Methods**) of AMPAR synaptic responses evoked by layer 4 stimulation. Consistent with our biochemical data, in visual cortex, dark rearing produced an

increase in inward rectification that was reversed by re-exposure to light (NR = 1.88 ± 0.15 , $n = 10$; DR = 3.42 ± 0.20 , $n = 22$; L = 1.68 ± 0.14 , $n = 11$; ANOVA: $F_{2,40} = 25.929$, $P < 0.001$; **Fig. 2e**), whereas opposite changes were observed in somatosensory cortex (NR = 3.87 ± 0.46 , $n = 10$; DR = 1.76 ± 0.07 , $n = 18$; L = 3.07 ± 0.17 , $n = 9$; ANOVA: $F_{2,34} = 23.440$, $P < 0.001$; **Fig. 2f**). Inward rectification was dependent on intracellular spermine (**Supplementary Fig. 4** online). Incidentally, we noticed that neurons in somatosensory cortex from normal-reared rats showed larger inward rectification and mEPSC amplitudes than cells from the visual cortex of these rats (**Fig. 2** and **Supplementary Fig. 5** online), consistent with basal differences in AMPAR subunit composition between these two cortical areas (**Supplementary Fig. 6** online). Collectively, our biochemical and electrophysiological data suggest that cross-modal plasticity may be mediated by changes in the subunit composition of synaptic AMPARs.

Our results demonstrate that manipulation of visual experience not only bidirectionally regulates synaptic AMPARs in visual cortex, but also produces complementary changes in the somatosensory cortex. These changes are rapid, as dark rearing for only 1 week produced similar changes in AMPARs as dark rearing from birth (**Supplementary Fig. 5**). It remains to be determined whether the cross-modal plasticity induced in somatosensory cortex is due to the altered cortical processing of tactile inputs (that is, top-down) or to differences in tactile experience (that is, bottom-up). The former mechanism would engage cortico-cortical inputs, whereas the latter involves thalamocortical inputs for triggering homeostatic plasticity. In any case, similar changes in AMPAR function in auditory cortex suggest that these changes may occur globally across sensory cortices. The bidirectional synaptic changes we observed are consistent with a homeostatic plasticity mechanism, where chronic deprivation of inputs increases AMPAR function whereas a prolonged increase in activity decreases it⁷. *In vitro* studies suggest that homeostatic synaptic plasticity is associated with changes in the synaptic content^{8–10} and subunit composition^{11,12} of AMPARs. We not only provide evidence that these changes also occur *in vivo* by natural experience, but suggest that these mechanisms can be recruited cross-modally. Taken together with a recent study demonstrating synapse-specific regulation of AMPARs by sensory experience¹³, our results suggest that AMPAR regulation may be a

common downstream mechanism for both synapse-specific and global homeostatic plasticity *in vivo*.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

The authors would like to thank E.M. Quinlan for helpful discussions and comments on this manuscript. This work was supported by a US National Institutes of Health grant (R01-EY014882) and a Sloan Research Fellowship to H.-K.L., a US National Institutes of Health grant (R01-EY012124) to A.K. and a Howard Hughes Medical Institute Undergraduate Research Fellowship to L.W.X.

AUTHOR CONTRIBUTIONS

A.G. and B.J. conducted the electrophysiology experiments (mEPSC recordings and rectification measurements, respectively) and assisted in writing the manuscript; L.W.X. and L.S. performed the biochemistry experiments; A.K. oversaw the electrophysiology (rectification measurements), contributed to discussions on experimental designs and collaborated on manuscript writing; H.-K.L. designed the studies, oversaw experiments, contributed to the electrophysiology (mEPSC recordings) and biochemistry and wrote the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Published online at <http://www.nature.com/natureneuroscience>

Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions/>

1. Goldreich, D. & Kanics, I.M. *J. Neurosci.* **23**, 3439–3445 (2003).
2. Lessard, N., Pare, M., Lepore, F. & Lassonde, M. *Nature* **395**, 278–280 (1998).
3. Bavelier, D. & Neville, H.J. *Nat. Rev. Neurosci.* **3**, 443–452 (2002).
4. Desai, N.S., Cudmore, R.H., Nelson, S.B. & Turrigiano, G.G. *Nat. Neurosci.* **5**, 783–789 (2002).
5. Verdoorn, T.A., Burnashev, N., Monyer, H., Seeburg, P.H. & Sakmann, B. *Science* **252**, 1715–1718 (1991).
6. Washburn, M.S., Numberger, M., Zhang, S. & Dingledine, R. *J. Neurosci.* **17**, 9393–9406 (1997).
7. Turrigiano, G.G. & Nelson, S.B. *Nat. Rev. Neurosci.* **5**, 97–107 (2004).
8. Lissin, D.V. *et al. Proc. Natl. Acad. Sci. USA* **95**, 7097–7102 (1998).
9. O'Brien, R.J. *et al. Neuron* **21**, 1067–1078 (1998).
10. Turrigiano, G.G., Leslie, K.R., Desai, N.S., Rutherford, L.C. & Nelson, S.B. *Nature* **391**, 892–896 (1998).
11. Ju, W. *et al. Nat. Neurosci.* **7**, 244–253 (2004).
12. Thiagarajan, T.C., Lindskog, M. & Tsien, R.W. *Neuron* **47**, 725–737 (2005).
13. Clem, R.L. & Barth, A. *Neuron* **49**, 663–670 (2006).