Two distinct mechanisms for experience-dependent homeostasis

Michelle C. D. Bridi1,5, Roberto de Pasquale1,5, Crystal L. Lantz2,5, Yu Gu3, Andrew Borrell3, Se-Young Choi6,1, Kaiwen He1, Trinh Tran1, Su Z. Hong1, Andrew Dykman1, Hey-Kyoung Lee1,4, Elizabeth M. Quinlan6,2,3* and Alfredo Kirkwood6,1,4*

Models of firing rate homeostasis such as synaptic scaling and the sliding synaptic plasticity modification threshold predict that decreasing neuronal activity (for example, by sensory deprivation) will enhance synaptic function. Manipulations of cortical activity during two forms of visual deprivation, dark exposure (DE) and binocular lid suture, revealed that, contrary to expectations, spontaneous firing in conjunction with loss of visual input is necessary to lower the threshold for Hebbian plasticity and increase miniature excitatory postsynaptic current (mEPSC) amplitude. Blocking activation of GluN2B receptors, which are upregulated by DE, also prevented the increase in mEPSC amplitude, suggesting that DE potentiates mEPSCs primarily through a Hebbian mechanism, not through synaptic scaling. Nevertheless, NMDA-receptor-independent changes in mEPSC amplitude consistent with synaptic scaling could be induced by extreme reductions of activity. Therefore, two distinct mechanisms operate within different ranges of neuronal activity to homeostatically regulate synaptic strength.

Homeostatic regulation of neuronal activity, the maintenance of neuronal spiking output within an optimal range, is widely considered essential to preserve neuronal processing in the face of changing inputs1–10. Multiple mechanisms that can accomplish this homeostasis have been identified, including synaptic scaling11 and the sliding modification threshold for Hebbian plasticity12. A common feature of these mechanisms is that deviations in neuronal activity away from a target set point elicit a compensatory response in postsynaptic function.

In the sliding threshold model, often referred to as metaplasticity, the threshold for the induction of long-term potentiation (LTP) and long-term depression (LTD) is determined by the history of neuronal spiking such that LTP is favored at the expense of LTD when activity is low, and LTD at the expense of LTP when activity is high12–14. This has been validated in the rodent primary visual cortex (V1), where complete visual deprivation via DE for as little as 2 d lowers the threshold for LTP induction15. Lowering the LTD threshold is thought to be mediated by the DE-induced increase in the number of synaptic NMDA receptors (NMDARs) containing the GluN2B subunit, which have slower decay kinetics and may favor LTD15–17.

In the synaptic scaling model, reduced neuronal spiking increases (and enhanced neuronal activity decreases) the postsynaptic strength of all synapses multiplicatively18. These changes result from the synaptic insertion (and removal) of AMPA receptors in a process that is independent of NMDAR activation19. Synaptic scaling was first demonstrated in cultured neurons, where bidirectional changes in neural activity elicited compensatory changes in mEPSC amplitude. Changes consistent with this model have also been demonstrated in vivo; in rodents, visual deprivation via DE15–17, intraocular tetrodotoxin injection18, lid suture19, enucleation13 and retinal lesions20 have all been demonstrated to increase the amplitude of mEPSCs in V1. Similarly, deafening by cochlear ablation and whisker trimming increase mEPSC amplitude in the primary auditory and somatosensory cortices, respectively18–20. However, recent studies suggest that changes in overall spike rate may not be sufficient to explain scaling of mEPSCs12,21.

Both the increase in mEPSC amplitude and the reduction in LTP threshold during sensory deprivation are thought to increase spontaneous activity to return the overall neuronal firing rate to a homeostatic set point, thus compensating for the loss of sensory drive. Indeed, long-term dark rearing from birth increases spontaneous neuronal firing in juvenile V121,22, and DE in adults increases spontaneous firing rates in V1 neurons23. However, recent studies indicate that changes in firing rates following changes in visual input can be fast24,25, likely via rapid changes in inhibitory GABAergic circuits26,27, potentially preceding changes at glutamatergic synapses. This raises the possibility that the increase in spontaneous activity may be a contributing factor to, rather than an effect of, homeostasis at glutamatergic synapses. In this study, we re-examined the causal relationship between the recruitment of synaptic homeostasis mechanisms and cortical spike rates by manipulating V1 activity in two models of visual deprivation: dark exposure (DE), which normally induces robust synaptic homeostasis, and binocular lid suture (BS), which does not. Reducing spontaneous firing in V1 prevented the increased mEPSC amplitude and decreased LTP threshold normally induced by DE, whereas increasing firing during BS promoted these changes. In addition, reducing spontaneous firing during DE prevented the upregulation of GluN2B. Blocking GluN2B activity prevented the enhancement of mEPSCs induced by DE, but not the one induced by extreme reductions in neuronal activity. Therefore we propose the following: (i) the deprivation-induced increase in spontaneous firing is not merely a consequence of, but most likely a necessary condition for, synaptic homeostasis;
(ii) increased mEPSC amplitude during DE reflects Hebbian potentiation of synaptic strength due to reduced induction threshold; and (iii) synaptic scaling can be engaged in vivo, but by reducing neuronal activity to very low levels.

**Results**

**Deprivation-induced metaplasticity does not depend on decreased overall firing activity.** Visual deprivation decreases cortical inhibition, which increases spontaneous activity in V1 and lowers the sliding modification threshold for plasticity to favor LTP\(^{26,27,29}\). We tested whether the increase in spontaneous activity is necessary for the DE induced reduction in the plasticity threshold. We first recorded single-unit activity in awake, head-fixed adult mice with chronically implanted multielectrode arrays (Supplementary Fig. 1). Compared to baseline, DE increased the overall spontaneous firing rate in V1 regular-spiking neurons (Wilcoxon rank sum test \(U(60) = 330.5, P = 0.035\) (Fig. 1a), spontaneous bursting \(U(60) = 211.0, P \leq 0.001\) and inter-burst spike rate \(U(60) = 235.0, P \leq 0.001\) (Supplementary Fig. 2). To counteract the disinhibition caused by DE, we employed diazepam, a positive allosteric modulator of GABA\(_{A}\) receptors used extensively to reduce cortical firing\(^{31,32}\). Diazepam treatment prevented these changes in spontaneous activity when delivered chronically during DE in both naïve (Supplementary Fig. 1c) and previously deprived mice (Fig. 1a) (naïve: \(U(54) = 366.0, P = 0.676\); non-naïve \(U(60) = 379.0, P = 0.155\), Wilcoxon rank sum test).

When delivered acutely, diazepam reduced activity in V1 of controls housed in a normal lighted environment (Wilcoxon signed rank test \(Z(26) = -3.916, P \leq 0.001\) (Supplementary Fig. 3a). Acute diazepam also decreased spontaneous bursting (paired \(t\) test \(t(26) = 4.419, P \leq 0.001\) and inter-burst firing rates (Wilcoxon signed rank test \(Z(26) = -3.892, P \leq 0.001\) (Supplementary Fig. 3c,d). Additional single-unit recordings in anesthetized animals confirmed that the decrease in firing due to a single acute dose of diazepam was long-lasting, in accordance with reports of brain elimination half-life of 8–10 h\(^{33}\) (one-way repeated-measures ANOVA \(F(5, 15) = 12.694, P \leq 0.001\) (Supplementary Fig. 3b).

Next we asked how a decrease in spontaneous activity during activity would affect the sliding threshold for Hebbian plasticity. We administered diazepam (10 mg/kg i.p., twice daily) during brief DE (2 d). The sliding of the threshold for Hebbian plasticity was evaluated ex vivo, in V1 slices, by performing whole-cell voltage-clamp recordings of the responses of layer II/III pyramidal neurons to extracellular stimulation of layer IV. Hebbian plasticity was induced by pairing presynaptic stimulation with postsynaptic depolarization (see Methods). To ensure that we could detect a shift in the plasticity threshold in favor of LTP, we used a pairing (~10 mV) that was below the threshold for LTP induction in normally reared (NR) subjects. LTD was induced by pairing to ~40 mV, which induces LTD in NR subjects. As predicted by the sliding threshold model, and consistent with previous findings\(^6\), DE promoted the induction of LTP by DE (one-way ANOVA \(F(3, 33) = 8.759, P \leq 0.001\)) (Fig. 1b). Likewise, DE impaired layer IV/II/III LTD in slices from vehicle-treated mice. Diazepam treatment prevented the promotion of LTP by DE (one-way ANOVA \(F(3, 33) = 8.759, P \leq 0.001\)) (Fig. 1b). DE impaired layer IV/II/III LTD in slices from vehicle-treated, but not diazepam-treated, mice (one-way ANOVA \(F(3, 33) = 12.251, P \leq 0.001\) (Fig. 1c). Notably, diazepam did not affect the threshold for LTP or LTD in NR mice (Fig. 1b,c; Holm–Sidak post hoc test \(P = 0.93\) (LTP), \(P = 0.482\) (LTD)). The results indicate that reducing spontaneous activity (by enhancing GABAergic inhibition) during DE prevents the sliding of the modification threshold to favor of LTP and at the expense of LTD.

In a complementary set of experiments, we examined whether increasing neuronal spiking (by decreasing inhibition) could promote metaplasticity during BS, a milder form of visual deprivation that degrades but does not eliminates patterned vision\(^{41-43}\). Unlike DE, BS decreased spontaneous activity in V1 (Wilcoxon rank sum test \(U(64) = 366.5, P = 0.023\) (Fig. 1d). However, chronic treatment with flumazenil, which blocks endogenous activation of the benzodiazepine site of GABA\(_{A}\) receptors\(^7\), increased overall spontaneous firing rates (Wilcoxon rank sum test \(U(49) = 204.5, P = 0.015\) and spontaneous bursting (\(U(49) = 193.0, P = 0.013\)) in BS mice (Fig. 1d and Supplementary Fig. 2a,b). Acute flumazenil administration in NR controls increased overall spontaneous firing (Wilcoxon signed rank test \(Z(20) = 3.076, P = 0.002\), bursting ((\(Z(20) = -3.797, P \leq 0.001\) and inter-burst rates (\(Z(20) = 3.041, P = 0.002\), Wilcoxon signed rank test) (Fig. 1d and Supplementary Fig. 3a,c,d). Additional single-unit recordings in anesthetized animals confirmed that an acute dose of flumazenil produced a long-lasting increase in firing (one-way repeated-measures ANOVA \(F(5, 15) = 12.844, P \leq 0.001\) (Supplementary Fig. 3b).

Next we tested whether flumazenil treatment (10 mg/kg i.p., twice daily) would promote metaplasticity during BS. BS alone was not sufficient to slide the threshold for LTP, revealed by the absence of synaptic potentiation using the subthreshold pairing protocol (Fig. 1e). However, flumazenil treatment during BS lowered the threshold for LTP, allowing potentiation to occur (one-way ANOVA \(F(3, 29) = 9.848, P \leq 0.001\) (Fig. 1e). Conversely, BS (alone or with flumazenil) impaired layer IV/II/III LTD (one-way ANOVA LTD: \(F(3,28) = 15.724, P \leq 0.001\) (Fig. 1f). Flumazenil did not affect the threshold for LTD in NR controls (Fig. 1f; Holm–Sidak post hoc test \(P = 0.990\) (LTP), \(P = 0.710\) (LTD)). Together, these findings suggest that an increase in spontaneous activity combined with a decrease in patterned vision is necessary and sufficient to lower the synaptic modification threshold in favor of LTP.

**Decreased activity during visual deprivation does not increase mEPSC amplitude.** We next sought to determine whether spontaneous activity affects other types of plasticity induced by visual deprivation, such as the increase in mEPSC amplitude, thought to reflect homeostatic scaling up of synaptic strength. In these experiments, mEPSC amplitude was measured in layer II/III pyramidal neurons. As expected from previous studies\(^2\), 2 d of DE induced a robust increase in mEPSC amplitude in vehicle-treated mice (Fig. 2a,b). We then combined DE (to eliminate visually evoked activity) with diazepam (to decrease spontaneous activity). The synaptic scaling model predicts this combination would result in a larger increase in mEPSC amplitude than DE alone\(^44\). Surprisingly, we found that diazepam treatment (10 mg/kg, i.p. twice daily) during DE prevented the increase in mEPSC amplitude (Fig. 2a,b and Supplementary Table 1). Similarly, the increase in mEPSC amplitude by DE was blocked when diazepam was infused directly into the lateral ventricle (t test \(t(44) = 2.589, P = 0.013\) (Supplementary Fig. 4). Diazepam treatment had no effect on mEPSC amplitude in NR controls (Fig. 2a,b) (one-way ANOVA \(F(3,46) = 4.909, P = 0.005\); Holm–Sidak post hoc NR vehicle vs. diazepam, \(P = 0.831\).

To further test whether increased spontaneous neuronal activity during visual deprivation is required to increase mEPSC amplitude, we again used BS, a milder form of visual deprivation. BS alone is insufficient to increase mEPSC amplitude (Fig. 2c,d) or increase spontaneous neuronal spike rate (Fig. 1d). To ask whether the failure of BS to increase mEPSC amplitude was due to insufficient spontaneous activity, we increased spontaneous neuronal activity with flumazenil. Flumazenil treatment during BS did increase the mEPSC amplitude (one-way ANOVA \(F(3, 79) = 5.716, P \leq 0.001\) (Fig. 2c,d and Supplementary Table 2). In contrast, flumazenil alone did not affect the magnitude of mEPSCs in NR controls (Fig. 2d,e; Holm–Sidak post hoc test \(P = 0.995\) ). Together, these findings show that the homeostatic increase in mEPSC amplitude by visual deprivation requires an increase in spontaneous activity.

**Allosteric modulators of GABA\(_{A}\) receptors regulate activity by acting on synapses made by many types of interneurons.** However,
disinhibition during visual deprivation has been specifically attributed to a reduction in the output of parvalbumin-positive interneurons (PV-INs)\(^{29}\). Therefore, we asked whether targeting PV-IN activity is sufficient to induce changes in mEPSC amplitude during visual deprivation. In a first set of experiments, we increased the excitation of PV-INs by administering neuregulin-1 peptide (NRG1) to NR or DE mice. Systemic delivery of NRG1 increases excitatory drive onto PV-INs\(^{30}\) and decreases spontaneous firing rates in regular-spiking neurons in V1\(^{28}\). NRG1 treatment (10 ng/kg, i.p. twice daily) during DE blocked the increase in mEPSC amplitude (Fig. 3a,b) (one-way ANOVA \(F(3, 102) = 5.720, P \leq 0.001\)), in accordance with the effects of diazepam.

Second, we used viral expression of a G\(_i\)-coupled DREADD (designer receptor exclusively activated by designer drugs) to reduce the activity of PV-INs\(^{30}\). AAV9 encoding Cre-dependent G\(_i\)-DREADD and mCherry was injected into the left hemisphere of V1 (layer II/III) in PV-cre mice. As expected, activation of the DREADD via clozapine-N-oxide (CNO) administration (5 mg/kg, i.p.) increased spontaneous activity of pyramidal neurons in G\(_i\)-DREADD-infected hemispheres (two-tailed paired \(t\) test, \(t(30) = -4.094, P \leq 0.001\)) (Supplementary Fig. 5). We then subjected mice to either 2 d of normal rearing or 2 d of BS, with concurrent CNO delivery to both cohorts (5 mg/kg i.p. twice daily). mEPSC amplitude was significantly higher in G\(_i\)-DREADD-expressing hemispheres from BS mice compared to uninjected control hemispheres (one-way ANOVA \(F(3, 101) = 5.359, P = 0.002\)) (Fig. 3c,d). Thus, increasing spontaneous pyramidal neuron spiking by reducing PV-IN activity enables mEPSC amplitude to increase during BS.

**Decreased spontaneous activity during DE prevents GluN2B upregulation.** Changes in the composition and function of NMDARs mediate the sliding of the modification threshold for LTP and LTD induction\(^{11}\). DE increases the expression of the GluN2B subunit, and as little as 2 d of DE is sufficient to induce this change in layer II/III of V1\(^{14,29}\). Since reducing spontaneous activity prevents lowering the synaptic modification threshold during visual deprivation, we asked whether it also prevents the upregulation of GluN2B by DE. The GluN2B component of the evoked (layer IV→II/III) NMDAR current was measured in NR and DE mice treated with...
**Fig. 2** | Depivation-induced upregulation of mEPSC amplitude does not depend on reduced firing rates. **a.** Average mEPSC waveforms in NR and DE mice with and without diazepam treatment. **b.** The DE-induced increase in mEPSC amplitude observed in control, vehicle-treated mice was blocked by diazepam treatment during DE. *P* = 0.002, †*P* = 0.003, ‡*P* = 0.007, one-way ANOVA followed by Holm–Sidak post hoc test. **c.** Average mEPSC waveforms in NR and BS mice with and without flumazenil treatment. **d.** BS alone did not increase mEPSC amplitude in control, vehicle-treated mice. However, flumazenil treatment during BS increased mEPSC amplitude. *P* = 0.002, †*P* ≤ 0.001, ‡*P* = 0.004, one-way ANOVA followed by Holm–Sidak post hoc test. Dashed lines and error bars in **b,d** indicate mean ± s.e.m.; individual data points are displayed horizontally for clarity. Sample size is indicated in parentheses (neurons, mice).

**Fig. 3** | Manipulations of PV-IN activity regulate the deprivation-induced increase in mEPSC amplitude. **a.** Average mEPSC waveforms in NR and DE subjects with and without NRG1 treatment. **b.** The DE-induced increase in mEPSC amplitude was blocked by NRG1 treatment. *P* ≤ 0.001, †*P* ≤ 0.006, one-way ANOVA followed by Holm–Sidak post hoc test. **c.** Average mEPSC waveforms in NR and BS subjects with and without chemogenetic suppression of PV-IN activity (CNO i.p. twice daily; see Supplementary Fig. 5). Gi-DREADD was virally expressed in left hemisphere (LH) V1 PV-INS. The uninfected right hemisphere (RH) was used as control. **d.** BS did not increase mEPSC amplitude in control hemispheres (no Gi-DREADD injection). In the injected hemispheres, activation of Gi-DREADD in PV-INS during BS increased mEPSC amplitude. *P* ≤ 0.001, †*P* = 0.008, ‡*P* = 0.009; one-way ANOVA followed by Holm–Sidak post hoc test. Dashed lines and error bars in **b,d** indicate mean ± s.e.m.; some data points are displaced horizontally for clarity. Sample size is indicated in parentheses (neurons, subjects).

**GluN2B blockade during DE prevents the increase in mEPSC amplitude.** Our findings indicate that two of the consequences of visual deprivation, lowered threshold for LTP and increased mEPSC amplitude, are dependent on disinhibition and the resultant increase of spontaneous activity. The combination of lowered synaptic modification threshold and enhanced spontaneous activity in V1 of DE animals is predicted to strongly favor the induction of LTP. This prompted us to consider the possibility that the increase in mEPSC amplitude observed following DE may reflect an increase in synaptic AMPA receptor levels due to NMDAR-dependent LTP, rather than synaptic scaling. To ask whether GluN2B function is necessary for the DE-mediated increase in mEPSC amplitude, we administered the GluN2B-specific antagonist Ro 25-6981 (30 mg/kg per day s.c. via osmotic minipump) during normal vision and DE. Ro 25-6981 blocked the DE-mediated increase in mEPSC amplitude and had no effect on mEPSCs in NR mice (one-way ANOVA F(3, 10) = 8.155, *P* ≤ 0.001) (Fig. 4c,d and Supplementary Table 4). This suggests that lowering the synaptic modification threshold with DE enables an NMDAR-dependent increase in AMPA receptor mEPSCs. Notably, these results also rule out a role for synaptic scaling in increasing mEPSC amplitude during DE, as changes in synaptic strength due to synaptic scaling are independent of NMDAR activation**15**.

**Extreme reduction of firing rates increases mEPSC amplitude.** Our visual deprivation model differs significantly from conditions originally used to describe NMDAR-independent synaptic upscaling, in which action potential generation was completely blocked with tetrodotoxin11. We therefore hypothesized that an extreme reduction in neuronal firing rates in vivo would modify mEPSC amplitude via synaptic scaling. To test this hypothesis in vivo, we increased tonic inhibition in the cortex by administration of THIQ, a potent agonist of extrasynaptic δ-subunit-containing GABA receptors40.
THIP administration (10 mg/kg i.p.) strongly reduced spontaneous activity in V1 (Wilcoxon signed rank test \(Z(15) = 3.517, P \leq 0.001\)) (Supplementary Fig. 3a), as well as burst frequency (two-tailed paired \(t \) test \(t(15) = 9.508, P \leq 0.001\)) and non-burst firing rate (Wilcoxon signed rank test \(Z(15) = -3.516, P \leq 0.001\)) (Supplementary Fig. 3c,d). THIP reduced spontaneous activity to a significantly greater extent than did diazepam (Fig. 5a) (diazepam: 70.0 ± 7.3% of baseline; THIP: 26 ± 3.0%; \(U(44) = 22.0, P \leq 0.001\), Wilcoxon rank sum test). This extreme reduction in activity was accompanied by an increase in mEPSC amplitude in both NR and DE animals (Supplementary Fig. 3a), as well as burst frequency (two-tailed paired \(t \) test \(t(15) = 9.508, P \leq 0.001\)) and non-burst firing rate (Wilcoxon signed rank test \(Z(15) = -3.516, P \leq 0.001\)).

Together, this suggests that there are two distinct mechanisms of homeostatic plasticity in vivo that depend on the magnitude of the change in neuronal activity: (i) a simple linear scaling in parallel to the overall spike rate, and (ii) an increase in mEPSC amplitude induced by the reduced syn- aptic scaling. As a further test of this hypothesis, we tested whether THIP-induced scaling was NMDAR independent. We treated DE mice with Ro 25-6981 to block GluN2B-containing NMDARs and administered vehicle or THIP to induce an extreme reduction in spike rate. Blocking GluN2B receptors with Ro 25-6981 did not prevent the increase in mEPSC amplitude induced by THIP (Fig. 5d,e). Therefore, in contrast to the increase in mEPSC amplitude induced by DE, the increase in mEPSC amplitude observed following extreme reductions in neural activity by THIP is independent of GluN2B activity. Thus, two distinct mechanisms for experience-dependent homeostatic plasticity coexist in vivo and are engaged by different magnitudes of firing rate reduction (Fig. 6).

**Discussion**

Visual deprivation via DE reduces evoked activity in the visual cortex and triggers a compensatory increase in excitatory synapses. This homeostatic change is predicted by the sliding modification threshold and synaptic scaling models, which also predict that an additional reduction of activity would promote additional homeostatic compensation in synaptic function. In contrast, we found that reducing the spontaneous activity of pyramidal neurons (by increasing inhibition) during DE actually prevents rather than enhances further compensatory changes. Moreover, increasing spontaneous pyramidal neuron activity (by decreasing inhibition) during a milder form of visual deprivation, BS, lowers the synaptic modification threshold for LTP and increases mEPSC amplitude. In sum, our manipulations of visual input combined with manipulations of spontaneous neuronal activity reveal that (i) changes in overall neuronal firing rate alone are not sufficient to mediate the lowered modification threshold for LTP/LTD nor the increased mEPSC amplitude observed after visual deprivation, (ii) changes in mEPSC amplitude induced by visual deprivation reflect Hebbian potentiation of excitatory synapses enabled by the reduced synaptic modification threshold, and (iii) changes in mEPSC amplitude induced by extreme reductions in neuronal activity reflect NMDAR-independent synaptic scaling. Thus the two mechanisms of synaptic homeostasis, the sliding synaptic modification threshold and synaptic scaling, operate within different ranges of activity, and changes in the spiking output of pyramidal neurons, previously thought to be a consequence of metaplasticity, are a necessary component of the induction process.

The exact changes in neural activity that slide the LTP/LTD threshold remain to be determined. Our data show that it is not simply the overall spike rate. Rather, an increase in spontaneous activity (including bursting) in conjunction with reduced visual input is better correlated with a lowered LTP threshold. As mentioned, DE completely eliminates visual input and rapidly increases spontaneous activity in V1, likely due to a decrease in the output of GABAergic interneurons or increased intrinsic excitability. However, it is unclear how pyramidal neurons could monitor a balance of visual input and spontaneous activity. An attractive possibility is that correlated pre- and postsynaptic firing, which is
more frequent during evoked activity, is detected as NMDAR activation to affect the synaptic content of GluN2b and the threshold for the synaptic modification. Concomitantly, overall spike rate (or AMPA receptor activation) may be monitored to detect extreme decreases in activity and regulate NMDAR-independent synaptic scaling. Finally, we note that in order to manipulate activity we targeted synaptic inhibition. However, changes in GABA conductance per se are unlikely to directly mediate homeostasis of glutamatergic transmission, as there are no known mechanisms to translate Cl flow into intracellular signaling cascades. In contrast,

Fig. 5 | Synaptic scaling is engaged by extreme reductions in neuronal activity. a, THIP induced a larger decrease in spontaneous firing than diazepam (diazepam: 70.0 ± 7.3%; THIP: 26.2 ± 3.0% of baseline). *P ≤ 0.001, two-tailed Wilcoxon rank sum test. b, Average mEPSC waveforms from NR and DE mice treated with vehicle or THIP. c, Compared to vehicle, THIP increased mEPSC amplitude in NR controls. THIP during DE further increased mEPSC amplitude over DE or THIP alone. *P = 0.002; **P = 0.017; †P ≤ 0.001, one-way ANOVA followed by Holm–Sidak post hoc test. d, Average mEPSC waveforms from DE mice treated with Ro 25-6981 with and without THIP. e, Ro 25-6981 did not prevent THIP-induced upscaling of mEPSC amplitude. *P ≤ 0.001, two-tailed t test. Dashed lines indicate mean (± s.e.m.). Some data points are displaced horizontally for clarity. Sample size is indicated in parentheses (neurons, mice).

Fig. 6 | Model depicting two distinct mechanisms that increase mEPSC amplitude in response to decreased neuronal activity. When neuronal activity varies within the normal physiological range, such as during DE, the change in temporal activity patterns (evoked spikes: red; spontaneous: blue) increases the expression of GluN2B and slides the plasticity threshold to favor LTP. After the threshold is lowered, spontaneous activity is sufficient to induce LTP, causing synaptic AMPA receptor (AMPAR) insertion and increased mEPSC amplitude. In contrast, when neuronal activity is near zero, the neuron cannot engage Hebbian plasticity mechanisms (purple box). In this case synaptic scaling mechanisms increase mEPSC amplitude.
intracellular signaling cascades activated by Ca\(^{2+}\) influx in response to neuronal spiking and activation of glutamatergic synapses are well documented.

We propose that increased mEPSC amplitude following DE is due to NMDAR-dependent potentiation of synaptic strength induced by increased spontaneous activity and reduced LTP threshold, rather than synaptic scaling. Although the fact that mEPSC amplitude after DE increases in a multiplicative manner has been interpreted as scaling of all synapses by the same factor, this may not be the case; sizes of individual spines have been observed to remain unchanged or decrease, even when spine size increases multiplicatively on a population level. Furthermore, the increase in mEPSC amplitude after DE has been found to be non-multiplicative in some cases, such as in 3-month old mice. Although non-homogeneous synaptic changes could also result from local scaling (see ref. 41), NMDAR-dependent potentiation is better suited to explaining the circuit-level changes that occur during DE. DE increases the strength of synaptic inputs between highly spontaneously active layer II/III pyramidal neurons, but not the inputs from less spontaneously active layer IV neurons to layer II/III. This observation is incompatible with the synaptic scaling model, as layer IV neurons are less spontaneously active and therefore predicted to be more strongly affected by DE. Many other forms of deprivation, including intraocular tetrodotoxin, enucleation, lid suture, retinal lesion and monocular deprivation, have been reported to upregulate mEPSC amplitude via synaptic scaling. It will be important to determine whether Hebbian plasticity enabled by a reduced synaptic modification threshold and enhanced spontaneous activity also plays a role in these cases. Indeed, metaplasticity has been used to explain the strengthening of non-deprived eye responses observed after prolonged monocular deprivation, and Hebbian plasticity can explain input-specific upscaling in the deprived optic tectum.

Finally, we confirmed that NMDAR-independent synaptic scaling can be recruited, but by manipulations that reduce neuronal activity more severely than sensory deprivation. Together, these findings support a model in which two distinct mechanisms for homeostatic regulation of excitatory synaptic strength coexist in vivo. When cortical activity varies within the normal physiological range, homeostatic changes are mediated by the sliding the synaptic modification threshold for LTP/LTD. When cortical activity is reduced beyond the normal physiological range, homeostatic changes are mediated by synaptic scaling. The existence of two homeostatic mechanisms may be necessary because at very low levels of activity neurons are unable to undergo Hebbian plasticity and are therefore unable to utilize the sliding threshold mechanism (Fig. 6). The coexistence of distinct homeostatic mechanisms operating at distinct dynamic ranges of activity resonates with the hypothesized necessity of regulation at different timescales and argues that neural homeostasis is achieved through multiple complementary mechanisms.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41593-018-0150-0.

Received: 16 January 2018; Accepted: 3 April 2018; Published online: 14 May 2018

References

1. Marder, E., O’Leary, T. & Shruti, S. Neuromodulation of circuits with variable parameters: single neurons and small circuits reveal principles of state-dependent and robust neuromodulation. Annu. Rev. Neurosci. 37, 329–346 (2014).
2. Davis, G. W. Homeostatic control of neural activity: from phenomenology to molecular design. Annu. Rev. Neurosci. 29, 307–323 (2006).
3. Turrigiano, G. G. The self-tuning neuron: synaptic scaling of excitatory synapses. Cell 135, 422–435 (2008).
4. Cooper, L. N. & Bear, M. F. The BCM theory of synapse modification at 30: interaction of theory with experiment. Nat. Rev. Neurosci. 13, 798–810 (2012).
5. Abraham, W. C. & Bear, M. F. Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci. 19, 126–130 (1996).
6. Abraham, W. C. Metaplasticity: tuning synapses and networks for plasticity. Nat. Rev. Neurosci. 9, 387–399 (2008).
7. Kirkwood, A., Rioul, M. C. & Bear, M. F. Experience-dependent modification of synaptic plasticity in visual cortex. Nature 381, 526–528 (1996).
8. Philpot, B. D., Espinosa, J. S. & Bear, M. F. Evidence for altered NMDA receptor function as a basis for metaplasticity in visual cortex. J. Neurosci. 23, 5583–5588 (2003).
9. Guo, Y. et al. Dark exposure extends the integration window for spike-timing-dependent plasticity. J. Neurosci. 32, 15027–15035 (2012).
10. Philpot, B. D., Sekhar, A. K., Slouf, H. Z. & Bear, M. F. Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. Neuron 29, 157–169 (2001).
11. Chen, W. S. & Bear, M. F. Activity-dependent regulation of NR2B translation contributes to metaplasticity in mouse visual cortex. Neuropharmacology 52, 200–214 (2007).
12. He, H.-Y., Hodos, W. & Quinlan, E. M. Visual deprivation reactivates rapid ocular dominance plasticity in adult visual cortex. J. Neurosci. 26, 2951–2955 (2006).
13. Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C. & Nelson, S. B. Activity-dependent scaling of quantaal amplitude in neocortical neurons. Nature 391, 892–896 (1998).
14. Goel, A. & Lee, H. K. Persistence of experience-induced homeostatic synaptic plasticity through adulthood in superficial layers of mouse visual cortex. J. Neurosci. 27, 6692–6700 (2007).
15. He, K., Petrus, E., Gammon, N. & Lee, H.-K. Distinct sensory requirements for monomodal and cross-modal homeostatic synaptic plasticity. J. Neurosci. 32, 8469–8474 (2012).
16. Gao, M. et al. A specific requirement of Arc/Arg3.1 for visual experience-induced homeostatic synaptic plasticity in mouse primary visual cortex. J. Neurosci. 30, 7168–7178 (2010).
17. Goel, A. et al. Phosphorylation of AMPA receptors is required for sensory deprivation-induced homeostatic synaptic plasticity. PLoS One 6, e18264 (2011).
18. Desai, N. S., Cudmore, R. H., Nelson, S. B. & Turrigiano, G. G. Critical periods for experience-dependent synaptic scaling in visual cortex. Nat. Neurosci. 5, 783–789 (2002).
19. Hengen, K. B., Lambo, M. E., Van Hooser, S. D., Katz, D. B. & Turrigiano, G. Firing rate homeostasis in visual cortex of freely behaving rodents. Neuron 80, 335–342 (2013).
20. Keck, T. et al. Synaptic scaling and homeostatic plasticity in the mouse visual cortex in vivo. Neuron 80, 327–334 (2013).
21. Kostal, V. C. et al. Hearing loss raises excitability in the auditory cortex. J. Neurosci. 25, 3908–3918 (2005).
22. Glazewski, S., Greenhill, S. & Fox, K. Time-course and mechanisms of homeostatic plasticity in layers 2/3 and 5 of the barrel cortex. Philos. Trans. R. Soc. Lond. B Biol. Sci. 372, 20160150 (2017).
23. Greenhill, S. D., Ramson, A. & Fox, K. Hebbian and homeostatic plasticity mechanisms in regular spiking and intrinsically bursting cells of cortical layer 5. Neuron 88, 539–552 (2015).
24. Fong, M. F., Newman, J. P., Potter, S. M. & Wenner, P. Upward synaptic scaling is dependent on neurotransmission rather than spiking. Nat. Commun. 6, 6339 (2015).
25. Barnes, S. J. et al. Deprivation-induced homeostatic spine scaling in vivo is localized to dendritic branches that have undergone recent spine loss. Neuron 96, 871–882.e5 (2017).
26. Gianfranceschi, L. et al. Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF. Proc. Natl Acad. Sci. USA 100, 12486–12491 (2003).
27. Benevento, L. A., Bakkum, B. W., Port, J. D. & Cohen, R. S. The effects of dark-rearing on the electrophysiology of the rat visual cortex. Brain Res. 572, 198–207 (1992).
28. Yu, G. et al. Neuregulin-dependent regulation of fast-spiking interneuron excitability controls the timing of the critical period. J. Neurosci. 36, 10285–10295 (2016).
29. Kuhlman, S. J. et al. A disinhibitory microcircuit initiates critical-period plasticity in the visual cortex. Nature 501, 543–546 (2013).
30. Huang, S., Hokenson, K., Bandyopadhyay, S., Russek, S. J. & Kirkwood, A. Brief dark exposure reduces tonic inhibition in visual cortex. J. Neurosci. 35, 15916–15920 (2015).
31. Hensch, T. K. et al. Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science 282, 1504–1508 (1998).
32. Yu, G. et al. Obligatory role for the immediate early gene NARPs in critical period plasticity. Neuron 79, 335–346 (2013).
33. Greenblatt, D. J. & Sethy, V. H. Benzodiazepine concentrations in brain directly reflect receptor occupancy: studies of diazepam, lorazepam, and oxazepam. *Psychopharmacology (Berl.)* **102**, 373–378 (1990).

34. Rittenhouse, C. D. et al. Stimulus for rapid ocular dominance plasticity in visual cortex. *J. Neurophysiol.* **95**, 2947–2950 (2006).

35. Rittenhouse, C. D., Shouval, H. Z., Paradiso, M. A. & Bear, M. F. Monocular deprivation induces homosynaptic long-term depression in visual cortex. *Nature* **397**, 347–350 (1999).

36. Frenkel, M. Y. & Bear, M. F. How monocular deprivation shifts ocular dominance in visual cortex of young mice. *Neuron* **44**, 917–923 (2004).

37. Christian, C. A. et al. Endogenous positive allosteric modulation of GABAA receptors by diazepam binding inhibitor. *Neuron* **78**, 1063–1074 (2013).

38. Wen, L. et al. Neuregulin 1 regulates pyramidal neuron activity via ErbB4 in parvalbumin-positive interneurons. *Proc. Natl Acad. Sci. USA* **107**, 1211–1216 (2010).

39. Cho, K. K. A., Khubnik, L., Philpot, B. D. & Bear, M. F. The ratio of NR2A/B NMDA receptor subunits determines the qualities of ocular dominance plasticity in visual cortex. *Proc. Natl Acad. Sci. USA* **106**, 5377–5382 (2009).

40. Drasbek, K. R. & Jensen, K. THIP, a hypnotic and antinociceptive drug, enhances an extrasynaptic GABAA receptor-mediated conductance in mouse neocortex. *Cereb. Cortex* **16**, 1134–1141 (2006).

41. Sun, Y. et al. Neuregulin-1/ErbB4 signaling regulates visual cortical plasticity. *Neuron* **92**, 160–173 (2016).

42. Pratt, K. G. & Aizenman, C. D. Homeostatic regulation of intrinsic excitability and synaptic transmission in a developing visual circuit. *J. Neurosci.* **27**, 8268–8277 (2007).

43. Gambrill, A. C., Storey, G. P. & Barria, A. Dynamic regulation of NMDA receptor transmission. *J. Neurophysiol.* **105**, 162–171 (2011).

44. Lee, K. F. H., Soares, C. & Béïque, J.-C. Tuning into diversity of homeostatic synaptic plasticity. *Neuropharmacology* **78**, 31–37 (2014).

45. Petrus, E. et al. Vision loss shifts the balance of feedforward and intracortical circuits in opposite directions in mouse primary auditory and visual cortices. *J. Neurosci.* **35**, 8790–8801 (2015).

46. Kaneko, M., Stellwagen, D., Malenka, R. C. & Stryker, M. P. Tumor necrosis factor-alpha mediates one component of competitive, experience-dependent plasticity in developing visual cortex. *Neuron* **58**, 673–680 (2008).

47. Ranson, A., Cheetham, C. E. J., Fox, K. & Sengpiel, F. Homeostatic plasticity mechanisms are required for juvenile, but not adult, ocular dominance plasticity. *Proc. Natl Acad. Sci. USA* **109**, 1311–1316 (2012).

48. Deeg, K. E. & Aizenman, C. D. Sensory modality-specific homeostatic plasticity in the developing optic tectum. *Nat. Neurosci.* **14**, 548–550 (2011).

49. Frémaux, N. & Gerstner, W. Neuromodulated spike-timing-dependent plasticity, and theory of three-factor learning rules. *Front. Neural Circuits* **9**, 85 (2016).

50. Zenke, F., Agnes, E. J. & Gerstner, W. Diverse synaptic plasticity mechanisms orchestrated to form and retrieve memories in spiking neural networks. *Nat. Commun.* **6**, 6922 (2015).

Acknowledgements

Research reported in this article was supported by the National Eye Institute of the National Institutes of Health under award number R01EY012124 (to A.K.), R01EY016431 (to E.Q.), R01EY025922 (to E.Q., A.K. and H.-K. L.) and R01-EY014882 (to H.-K. L.). A.K. was also supported by NIH grant P01 AG009973. M.C.D.B. was supported by grants T32EY007143 and T32HL110952.

Author contributions

M.C.D.B., R.d.P., T.T., K.H. and S.Z.H. collected slice electrophysiology data. M.C.D.B. and R.d.P. analyzed slice electrophysiology data. S.-Y.C. provided initial data for the inception of the project. C.L.L., Y.G. and A.B. collected and analyzed the in vivo unit recording data. A.D. and H.-K.L. provided code for analysis of single-unit firing properties. M.C.D.B., E.M.Q. and A.K. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41593-018-0150-0.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to E.M.Q. or A.K.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Methods

Animals. C57BL/6 and PV-cre mice, equal numbers of males and females, were raised (5 or fewer per cage) on a 12:12 light:dark cycle, with food and water ad libitum. All procedures conformed to the guidelines of the US Department of Health and Human Services Office of Laboratory Animal Welfare (OLAW) and were approved by the Institutional Animal Care and Use Committees of the University of Maryland and/or Johns Hopkins University. Sample sizes were chosen to correspond with previous studies in which the effects of visual manipulation were measured. For each experiment, animals within a litter were randomly distributed across groups. See the Nature Research Reporting Summary for details.

Visual manipulations. Naive mice were deprived of vision for 2 d beginning at postnatal day 24–30. Mice were age-matched across groups. Dark-exposed animals were placed in a light-tight dark room. Animal care and drug administration was performed under infrared illumination. Binocular lid suture was performed under isoflurane anesthesia. The margins of the upper and lower lids of both eyes were trimmed and sutured together. Animals were disqualified in the event of suture opening or infection.

Intraperitoneal drug administration. Diazepam (10 mg/kg) (Sigma-Aldrich, St. Louis, MO) and flumazenil (10 mg/kg) (Tocris Bioscience, Bristol, UK) were dissolved in 10% Tween 80, 20% DMSO and 70% saline. THIP (10 mg/kg) (Tocris) and NRGI (10 mg/kg) were dissolved in saline. CNO (5 mg/kg) (Enzo Life Sciences, Farmingdale, NY) was dissolved in 5% DMSO and 95% saline. Each drug or its vehicle was administered twice daily i.p.

Minipump drug administration. Subjects receiving intracerebroventricular diazepam infusions were implanted with osmotic minipumps (Alzet 1007D, DurACT Corp., Cupertino, CA) filled with diazepam (2 mg/mL) or vehicle (50% propylene glycol, 50% saline) and a 30-gauge cannula was attached. The pump/cannula assembly was primed with 0.9% NaCl at 37 °C. Animals were anesthetized with isoflurane, and the minipump was implanted subcutaneously. The tip of the attached cannula was placed in the left lateral ventricle and the assembly was affixed using dental cement. For mice receiving Ro 25-6981 (Cayman Chemical, Ann Arbor, MI) infusions (due to its relatively short half-life in vivo11), minipumps were filled with the concentration required to deliver 30 mg/kg per day, dissolved in 20% DMSO/80% saline, and implanted subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.

Viral injection. Viral injections were conducted under isoflurane anesthesia. V1 was located in the left hemisphere using stereotaxic coordinates (3.6 mm rostral to the lambda) to a depth of 1 mm, to center the electrode shank in the middle of the cortical thickness and used to evoke responses of at least 100 pA. Slices were stimulated every 15 s until a stable baseline of 30 consecutive responses was obtained. Ifenprodil (3 μM; Tocris) was then delivered to the recording chamber. After a new stable amplitude was reached, 30 consecutive responses were recorded. The average peak amplitude was compared before and after ifenprodil.

Intraperitoneal drug administration. Subjects receiving intracerebroventricular diazepam infusions were implanted with osmotic minipumps (Alzet 1007D, DurACT Corp., Cupertino, CA) filled with diazepam (2 mg/mL) or vehicle (50% propylene glycol, 50% saline) and a 30-gauge cannula was attached. The pump/cannula assembly was primed with 0.9% NaCl at 37 °C. Animals were anesthetized with isoflurane, and the minipump was implanted subcutaneously. The tip of the attached cannula was placed in the left lateral ventricle and the assembly was affixed using dental cement. For mice receiving Ro 25-6981 (Cayman Chemical, Ann Arbor, MI) infusions (due to its relatively short half-life in vivo11), minipumps were filled with the concentration required to deliver 30 mg/kg per day, dissolved in 20% DMSO/80% saline, and implanted subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.

Stereotaxic coordinates. Slices 300 μm thick were cut in ice-cold dissection buffer containing (in mM) 212.7 sucrose, 1.2 NaCl, 1.25 NaH2PO4, 0.5 CaCl2, 26 NaHCO3, 10 dextrose, buffered with 95% O2/5% CO2 (pH 7.4). Slices were transferred to artificial cerebrospinal fluid (ACSF) and incubated at 30 °C for 30 min, then dissolved in 10% Tween 20 and 20% DMSO saline, and incubated subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.

Stereotaxic coordinates. Slices 300 μm thick were cut in ice-cold dissection buffer containing (in mM) 212.7 sucrose, 1.25 NaCl, 1.25 NaH2PO4, 0.5 CaCl2, 26 NaHCO3, 10 dextrose, buffered with 95% O2/5% CO2 (pH 7.4). Slices were transferred to artificial cerebrospinal fluid (ACSF) and incubated at 30 °C for 30 min, then dissolved in 10% Tween 20 and 20% DMSO saline, and incubated subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.

Stereotaxic coordinates. Slices 300 μm thick were cut in ice-cold dissection buffer containing (in mM) 212.7 sucrose, 1.25 NaCl, 1.25 NaH2PO4, 0.5 CaCl2, 26 NaHCO3, 10 dextrose, buffered with 95% O2/5% CO2 (pH 7.4). Slices were transferred to artificial cerebrospinal fluid (ACSF) and incubated at 30 °C for 30 min, then dissolved in 10% Tween 20 and 20% DMSO saline, and incubated subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.

Stereotaxic coordinates. Slices 300 μm thick were cut in ice-cold dissection buffer containing (in mM) 212.7 sucrose, 1.25 NaCl, 1.25 NaH2PO4, 0.5 CaCl2, 26 NaHCO3, 10 dextrose, buffered with 95% O2/5% CO2 (pH 7.4). Slices were transferred to artificial cerebrospinal fluid (ACSF) and incubated at 30 °C for 30 min, then dissolved in 10% Tween 20 and 20% DMSO saline, and incubated subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.

Stereotaxic coordinates. Slices 300 μm thick were cut in ice-cold dissection buffer containing (in mM) 212.7 sucrose, 1.25 NaCl, 1.25 NaH2PO4, 0.5 CaCl2, 26 NaHCO3, 10 dextrose, buffered with 95% O2/5% CO2 (pH 7.4). Slices were transferred to artificial cerebrospinal fluid (ACSF) and incubated at 30 °C for 30 min, then dissolved in 10% Tween 20 and 20% DMSO saline, and incubated subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.

Stereotaxic coordinates. Slices 300 μm thick were cut in ice-cold dissection buffer containing (in mM) 212.7 sucrose, 1.25 NaCl, 1.25 NaH2PO4, 0.5 CaCl2, 26 NaHCO3, 10 dextrose, buffered with 95% O2/5% CO2 (pH 7.4). Slices were transferred to artificial cerebrospinal fluid (ACSF) and incubated at 30 °C for 30 min, then dissolved in 10% Tween 20 and 20% DMSO saline, and incubated subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.

Stereotaxic coordinates. Slices 300 μm thick were cut in ice-cold dissection buffer containing (in mM) 212.7 sucrose, 1.25 NaCl, 1.25 NaH2PO4, 0.5 CaCl2, 26 NaHCO3, 10 dextrose, buffered with 95% O2/5% CO2 (pH 7.4). Slices were transferred to artificial cerebrospinal fluid (ACSF) and incubated at 30 °C for 30 min, then dissolved in 10% Tween 20 and 20% DMSO saline, and incubated subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.

Stereotaxic coordinates. Slices 300 μm thick were cut in ice-cold dissection buffer containing (in mM) 212.7 sucrose, 1.25 NaCl, 1.25 NaH2PO4, 0.5 CaCl2, 26 NaHCO3, 10 dextrose, buffered with 95% O2/5% CO2 (pH 7.4). Slices were transferred to artificial cerebrospinal fluid (ACSF) and incubated at 30 °C for 30 min, then dissolved in 10% Tween 20 and 20% DMSO saline, and incubated subcutaneously without an attached cannula. Meloxicam (5 mg/kg, s.c.) was administered postoperatively to all animals to prevent inflammation and pain.
Statistical analysis. Normality was determined using the D’Agostino test and variance was compared using Levene’s median test. Groups with normally distributed data were compared using two-tailed paired or unpaired t tests, one-way ANOVAs or one-way repeated-measures ANOVAs, as indicated. Holm–Sidak post hoc tests were used for multiple comparisons following one-way ANOVAs. Groups that were not normally distributed were compared using nonparametric Wilcoxon rank sum or signed rank tests or ANOVAs on ranks (followed by Dunn’s post hoc test for multiple comparisons) (SigmaPlot, Systat Software, Inc, San Jose, CA). Statistical outliers were detected using preestablished criteria (ROUT test) and excluded from analysis.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Code availability. Custom code is available upon reasonable request to the corresponding author.

Data availability. Raw data used to produce Figs. 1–5 and Supplementary Figs. 1–6 are available upon reasonable request to the corresponding author.

References
51. Tallaksen-Greene, S. J., Janiszewska, A., Benton, K., Ruprecht, L. & Albin, R. L. Lack of efficacy of NMDA receptor-NR2B selective antagonists in the R6/2 model of Huntington disease. Exp. Neurol. 225, 402–407 (2010).
52. Rumbaugh, G. & Vicini, S. Distinct synaptic and extrasynaptic NMDA receptors in developing cerebellar granule neurons. J. Neurosci. 19, 10603–10610 (1999).
53. Murase, S. et al. Matrix metalloproteinase-9 regulates neuronal circuit development and excitability. Mol. Neurobiol. 53, 3477–3493 (2016).
54. Niell, C. M. & Stryker, M. P. Highly selective receptive fields in mouse visual cortex. J. Neurosci. 28, 7520–7536 (2008).
55. Chen, L., Deng, Y., Luo, W., Wang, Z. & Zeng, S. Detection of bursts in neuronal spike trains by the mean inter-spike interval method. Prog. Nat. Sci. 19, 229–235 (2009).
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

☐ n/a  Confirmed

☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement

☐ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ The statistical test(s) used AND whether they are one- or two-sided

☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☐ A description of all covariates tested

☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☐ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

☐ Give P values as exact values whenever suitable.

☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☐ Clearly defined error bars

☐ State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Data were collected using:

- IgorPro 6.37 (Wavemetrics)
- MATLAB 2013b (+ Psychtoolbox) (Mathworks)
- OpenWorkBench 2.20.1 (Tucker Davis Technologies)

Data analysis

Data were analyzed using:

- IgorPro 6.37 (WaveMetrics)
- Mini Analysis 6.0.7 (Synaptosoft)
- MATLAB 2013b and R2014b (Mathworks)
- Excel 2013 & 2016 (Microsoft)
- Sigmaplot 11.0 (Systat Software)
- OpenSorter 2.20.0 (Tucker Davis Technologies)
- OpenEx 2.28 (Tucker Davis Technologies)
- OpenExplorer 2.28 (Tucker Davis Technologies)
- SPSS version 24 (IBM)
- Custom codes used for data analysis in Matlab are available upon request.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Raw data used to produce Figures 1-5 and S1-S6 are available upon reasonable request to the corresponding authors.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample sizes were chosen to correspond with previous studies in which the effects of visual manipulation were measured (see Methods).

Data exclusions
Exclusion criteria are described for each experiment (see Methods). Animals were excluded from the lid suture experiments in the case of eye opening or infection. Data were excluded from whole cell recordings if the recording conditions (i.e. input and series resistance) did not meet the cutoff thresholds or if the values changed by more than 20% during the recording. Additionally, mini recordings were excluded if the RMS noise exceeded 2; LTP/D recordings were excluded if baselines were not stable. Statistical outliers were detected using pre-determined criteria (ROUT test) and excluded.

Replication
To ensure reproducibility, we reproduced previously published findings in our control groups to confirm that conditions in our lab are appropriate to produce the basic phenomenon of homeostasis in vivo. For example, we confirmed that DE shifts the threshold for LTP, increases mini amplitude, and increases NR2B content, whereas BS alone does not increase mini amplitude or shift the LTP threshold; all of these findings are previously published results (see text for references). We applied our novel experimental manipulations (manipulating neuronal firing rate during visual deprivation) in experiments interleaved with the control experiments. Furthermore, we have used multiple approaches to manipulating neuronal activity (pharmacological and molecular; see Fig. 2 & 3) and different diazepam delivery methods (see Fig. 2 & S4) and confirmed that these approaches have the same result on our main finding, the change in mini amplitude. Furthermore, different experimenters carried out these experiments (R. d. P. and S.H. collected data in Fig. 2; M.C.D.B collected data in Fig. 3; and T.T. and K.H collected data in Fig. S4) with consistent findings. Finally, we have used methods that are readily available to other labs and that are easily implemented (e.g. use of pharmacological agents that do not require sophisticated delivery methods; use of standard patch-clamp recording techniques, etc) so that our experiments can easily be reproduced.

Randomization
Animals were randomly placed into experimental groups. Animals from each litter were equally distributed across experimental groups.

Blinding
Due to the nature of the experiments, it was not possible to blind the experimenter to treatment condition (e.g. it is immediately obvious to the experimenter whether an animal is lid sutured, dark reared, or has normal vision).

Materials & experimental systems

Policy information about availability of materials
n/a

n/a Involved in the study

Unique materials

Antibodies

Eukaryotic cell lines

Research animals

Human research participants

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials
CS7BL/6 and PV-Cre mice, equal numbers of males and females, were raised (5 or fewer per cage) on a 12:12 light:dark
Animals/animal-derived materials

cycle, with food and water ad libitum. All procedures conform to the guidelines of the U.S. Department of Health and Human Services Office of Laboratory Animal Welfare (OLAW) and were approved by the Institutional Animal Care and Use Committees of the University of Maryland and/or Johns Hopkins University. For each experiment, animals within a litter were randomly distributed across groups. Animals were visually deprived beginning at postnatal day 24-30 (slice experiments) or as adults (in vivo recordings) (See Methods).

Method-specific reporting

| ChIP-seq | Flow cytometry | Magnetic resonance imaging |
|----------|----------------|---------------------------|
| ☒        | ☐              | ☒                         |