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Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation

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Visual deprivation during a developmental sensitive period markedly alters visual cortical response properties, but the changes in intracortical circuitry that underlie these effects are poorly understood. Here we use a slice preparation of rat primary visual cortex to show that 2 d of prior visual deprivation early in life increases the excitability of layer 4 circuitry. Slice recordings showed that spontaneous activity of layer 4 star pyramidal neurons increased 25-fold after 2 d of visual deprivation between postnatal days (P) 15 and P17. This effect was mediated by increased net excitatory and decreased net inhibitory synaptic drive. Paired recordings showed that excitatory connections between star pyramidal neurons doubled in amplitude, whereas inhibitory connections decreased or increased depending on the interneuron class. These effects reversed when vision was restored. This dynamic adjustment of the excitation-inhibition balance may allow the networks within layer 4 to maintain stable levels of activity in the face of variable sensory input.

Visual deprivation has been a powerful tool for investigating the anatomical and physiological correlates of experience-dependent plasticity[1–3]. Visual deprivation during a developmental sensitive period induces profound changes in visual response properties, including reduced cortical responsiveness to visual stimulation[1,4–6], reduced visual acuity[7] and impaired orientation tuning of cortical neurons[5,8]. Many of these physiological changes can be induced quite rapidly[9,10] and can be reversed if deprivation is lifted within the sensitive period[11,12]. Despite the long history of these manipulations, the detailed changes that loss of vision produces in intracortical synaptic strengths, as well as the plasticity mechanisms that underlie these changes, are not fully understood. Here we examine how synaptic connectivity in rodent layer 4 is altered by brief periods of monocular lid suture.

Layer 4 is the main input layer to visual cortex, and excitatory star pyramidal neurons in layer 4 receive direct excitatory thalamic drive, as well as recurrent excitatory connections from other star pyramidal neurons[13,14]. This recurrent excitatory circuitry is kept in check by both feed-forward and feedback inhibition, which are mediated by distinct classes of inhibitory GABAergic interneuron[15,16]. Early in life, 2 d of monocular deprivation increases the quantal amplitude of excitatory synapses on star pyramidal neurons in layer 4 (ref. 17). In addition, cortical inhibition may be reduced by prolonged visual deprivation[18,19], although how rapidly this occurs and how these changes are expressed at the level of individual inhibitory connections remain unknown. Here we examine the possibility that visual deprivation rewires intracortical layer 4 circuitry through highly selective changes in the strengths of excitatory and inhibitory synapses.

To accomplish this, one eye of a rat was sutured shut between P14 and P17, and acute slices of primary visual cortex were cut from the deprived and control hemispheres. We then carried out a detailed analysis of connectivity in monocular layer 4 using quadruple whole-cell recordings. Because most of the rodent visual cortex is driven exclusively by the contralateral eye[20,21], monocular deprivation will lower visual drive to one hemisphere of the monocular portion of visual cortex, while leaving the other hemisphere unaffected[22,23]. We found that visual deprivation dramatically increased the spontaneous firing of star pyramidal neurons in layer 4, through a net increase in excitatory and a net decrease in inhibitory synaptic drive. This alteration in the balance between excitation and inhibition was achieved through a doubling in the strength of excitatory connections between star pyramidal neurons and a decrease in the strength of feedback, but not feed-forward, inhibition. This increase in spontaneous layer 4 activity was completely reversed by reopening the eye for 2 d before slice preparation. These data indicate that layer 4 excitability may be dynamically adjusted to compensate for alterations in sensory drive through highly selective changes in intracortical synaptic strength. This homeostatic regulation of the excitation-inhibition balance is likely to be important for maintaining stable cortical activity during normal development and may also contribute to the pathological changes in visual response properties that are induced by sensory deprivation.

RESULTS

Monocular deprivation increases spontaneous activity

To assess the effects of monocular deprivation on local circuit activity, we compared spontaneous firing rates in the monocular region of visual cortical slices from the deprived and nondeprived hemispheres. We initially targeted star pyramidal neurons, which are the major class of excitatory neuron in layer 4 of rat primary visual cor-

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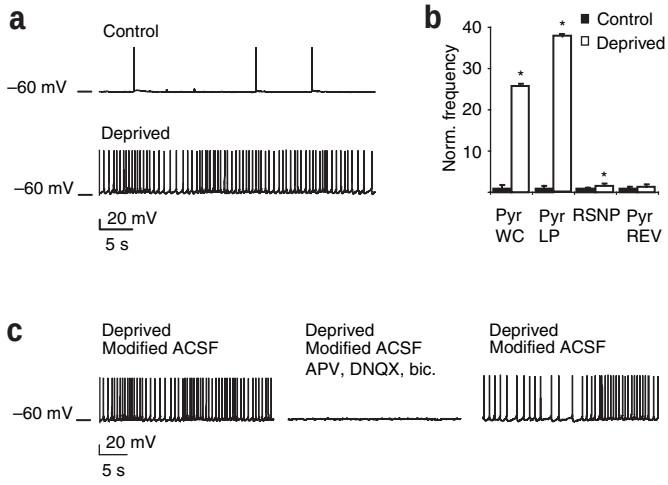

Figure 1 Spontaneous activity of layer 4 star pyramidal neurons was increased after 2 d of visual deprivation. (**a**) Representative whole-cell recordings from star pyramidal neurons in slices from control and deprived hemispheres, illustrating the elevation in activity in the deprived hemisphere. (**b**) Average spontaneous firing rates from star pyramidal neurons (Pyr) in whole-cell (WC) and loose-patch (LP) configurations, RSNP cells in the whole-cell configuration and from star pyramidal neurons 48 h after reopening the eye (REV). Firing rates in the deprived hemisphere are represented relative to the control hemisphere to show the fold change. Asterisk indicates significant difference from control. Here and for all subsequent figures, error bars represent the s.e.m. (**c**) Example recording from a star pyramidal neuron from the deprived hemisphere during wash-in (middle panel) and washout (right panel) of synaptic blockers (APV, DNQX and bicuculline).

tex[13]. In standard artificial cerebral spinal fluid (ACSF), there was little or no spontaneous activity. Changing the solution to a modified ACSF, which is closer to the composition of rat CSF *in situ*[24,25], initiated low levels of spontaneous firing of star pyramidal neurons (**Fig. 1**), similar to what has been reported in ferret prefrontal cortical slices[26]. Whole-cell recordings showed that star pyramidal neurons in slices from the deprived hemisphere had firing rates that were 25-fold higher than those from the control hemisphere (**Fig. 1a**,**b**; control: 0.05 ± 0.03 Hz, $n = 10$; deprived: 1.28 ± 0.24 Hz, $n = 10$; $P < 0.006$). A similar increase was observed using loose-patch recordings to measure star pyramidal neuron firing rates extracellularly, to avoid modifying firing properties through whole-cell dialysis (**Fig. 1b**; control: 0.11 ± 0.06 Hz, $n = 8$; deprived: 2.9 ± 0.8 Hz, $n = 8$; $P < 0.003$). Firing activity in modified ACSF was completely abolished by blocking synaptic transmission with APV, DNQX and bicuculline (**Fig. 1c**), indicating that spontaneous firing was driven by synaptic activity.

This increased activity in the deprived hemisphere was highly selective for star pyramidal neurons. Monocular deprivation elevated spiking in layer 4 regular-spiking nonpyramidal (RSNP) neurons by only 1.6-fold (**Fig. 1b**; control: 1.57 ± 0.19 Hz, $n = 8$; deprived: 2.47 ± 0.38 Hz, $n = 7$; $P < 0.01$), a much smaller increase in firing than that produced in star pyramidal neurons. In addition, the effects of monocular deprivation on pyramidal neuron activity were selective for layer 4: the firing rates of layer 2/3 pyramidal neurons were unaffected by monocular deprivation at this age (control: 0.085 ± 0.045 Hz, $n = 11$; deprived: 0.077 ± 0.025 Hz, $n = 8$; $P = 0.88$). When 2 d of monocular deprivation was followed by removal of the suture for 2 d, star pyramidal neuron activity in layer 4 was restored to control values (**Fig. 1b**; control: 0.08 ± 0.03 Hz, $n = 8$; previously deprived: 0.11 ± 0.02 Hz, $n = 8$; $P = 0.55$). This reversibility suggests that these changes in excitability represent a dynamic, compensatory response to lowered sensory drive and is consistent with behavioral data showing that the effects of visual deprivation are reversible if deprivation is lifted early in life[11,12].

Increased firing is due to altered synaptic drive

At least three mechanisms could contribute to the increased spontaneous activity of star pyramidal neurons: increased intrinsic excitability[27], increased excitatory synaptic drive[17,28] and decreased inhibitory synaptic drive[29]. To test changes in intrinsic properties, we generated firing rate versus current (*F–I*) curves in the presence of the synaptic receptor blockers APV (50 μM), DNQX (20 μM) and bicuculline (20 μM; **Fig. 2a**,**b**). No significant differences were observed in the slopes of the linear portion of these curves (control, 1.53 ± 0.07; deprived, 1.49 ± 0.04; $P = 0.49$) or in the spike threshold (**Fig. 2b**). Monocular deprivation also did not significantly affect resting input resistances (control: 318 ± 40 MΩ, $n = 10$; deprived: 361 ± 56 MΩ, $n = 10$; $P = 0.56$) and resting membrane potentials (control: –66.4 ± 1.6 mV, $n = 10$; deprived: –67.3 ± 0.7 mV, $n = 10$; $P = 0.63$) measured in the presence of synaptic blockers.

To test whether changes in excitatory and inhibitory synaptic drive could explain the increased firing rates, we recorded spontaneous excitatory and inhibitory currents onto star pyramidal neurons by leaving ongoing circuit activity intact and voltage clamping individual postsynaptic neurons to different potentials. Excitatory and inhibitory currents were separated by holding the postsynaptic neuron at the measured reversal potential (E_{rev}) for inhibitory postsynaptic currents (IPSCs; –40 mV; **Fig. 3a**) or at the measured E_{rev} for excitatory postsynaptic currents (EPSCs; +10 mV; **Fig. 3b**). Integrating spontaneous excitatory and inhibitory currents over a 5-min period showed that monocular deprivation produced a 75% increase in excitatory synaptic charge (**Fig. 3a**,**c**; control, $n = 9$; deprived, $n = 9$; $P < 0.01$) and a 46% decrease in inhibitory synaptic charge (**Fig. 3b**,**c**; control, $n = 9$; deprived,

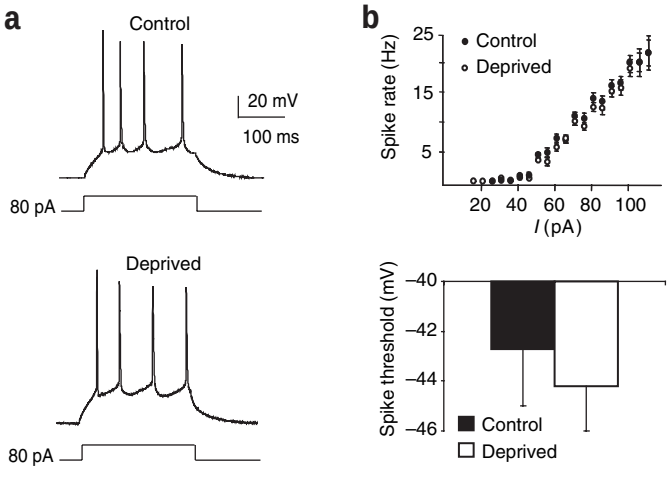

Figure 2 Visual deprivation did not affect the intrinsic excitability of star pyramidal neurons. (**a**) Representative recordings of layer 4 star pyramidal neurons in slices from control and deprived hemispheres in response to a DC depolarizing current injection (500 ms, 80 pA). Recordings were obtained in the presence of synaptic blockers. (**b**) Top panel: average current versus firing rate from star pyramidal neurons in control and deprived hemispheres. Bottom panel: average spike threshold (the interpolated membrane potential at which *dV/dt* equaled 20 V/s) for the control and deprived conditions.

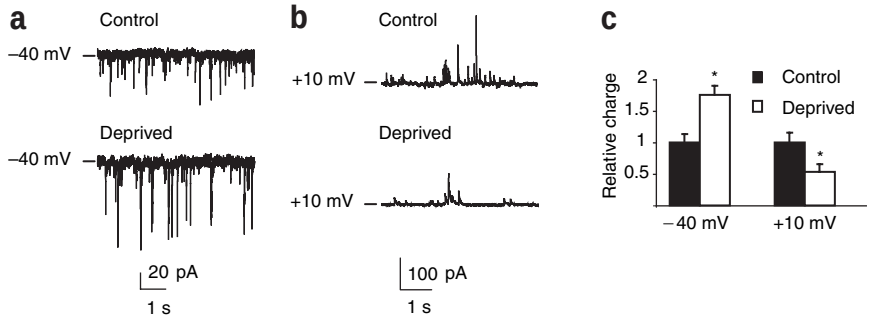

Figure 3 The balance between excitatory and inhibitory synaptic drive was altered by visual deprivation. (**a**) Representative recordings of spontaneous EPSCs (recorded at the Cl^- reversal potential) from star pyramidal neurons in slices from control and deprived hemispheres in modified ACSF. (**b**) Representative recordings of spontaneous IPSCs (recorded at the reversal potential for spontaneous EPSCs); examples are from the same control and deprived neurons as in **a**. (**c**) Average excitatory and inhibitory charge (obtained by integrating spontaneous EPSC or IPSC current over 5 min for each neuron) for star pyramidal neurons from control or deprived hemispheres. Deprived data are expressed relative to control to show the fold change. Asterisk indicates significant difference from control.

$n = 9$; $P < 0.01$). These results show that the balance between excitation and inhibition in the recurrent circuitry of layer 4 has shifted to favor excitation.

Monocular deprivation strengthens excitatory connections

Layer 4 star pyramidal neurons in the visual cortex receive direct thalamic input and recurrent excitatory connections from other star pyramidal neurons within layer 4. The average quantal amplitude of excitatory synapses on star pyramidal neurons is increased after 2 d of monocular deprivation[17]. To investigate in detail how monocular deprivation affects the properties of unitary excitatory synaptic connections within layer 4, we carried out quadruple recordings from star pyramidal neurons to find monosynaptically connected pairs (**Fig. 4a**). All paired recordings here and below were done in standard ACSF so there was minimal background synaptic activity. We found 25 connected pairs out of 234 tested (10.6%) from control hemispheres and 45 connected pairs out of 238 tested (18.9%) from deprived hemispheres (**Fig. 4b**; $P < 0.025$, χ^2). In addition to this increase in connection probability, the average amplitude of monosynaptic pyramidal-to-pyramidal connections approximately doubled in the deprived hemisphere (**Fig. 4c**,**d**; $P < 0.001$), with no significant changes in EPSC latency (control, 1.2 ± 0.2 ms; deprived, 1.1 ± 0.2 ms; $P = 0.31$) or kinetics (20–80% rise times: control, 0.69 ± 0.14 ms; deprived, 0.64 ± 0.19 ms; $P = 0.63$ and decay time constants: control, 7.2 ± 1.8 ms; deprived, 8.9 ± 1.6 ms; $P = 0.37$).

A small but significant change was observed in the short-term plasticity of pyramidal-to-pyramidal connections in response to trains of five precisely timed presynaptic spikes at 20 Hz. Connections from the deprived hemisphere showed slightly more depression (**Fig. 4c**,**e**; $n = 10$ control and 17 deprived pairs; $P < 0.001$, one-way ANOVA). Failure rates and coefficients of variation of EPSC amplitude were not, however, significantly different (**Fig. 4d**; $P = 0.56$), suggesting that probability of release was not much altered. Similarly, only modest changes in short-term plasticity have been observed in rodent somatosensory cortex after whisker deprivation[30,31], and activity deprivation in cortical and spinal cultures increases excitatory synaptic strength primarily through postsynaptic changes in receptor accumulation[28,32]. A higher connection probability between star pyramidal neurons, accompanied by a doubling of EPSC amplitude between connected pairs, should cooperate to increase the gain of excitatory feedback within layer 4.

Interneurons respond differently to monocular deprivation

Different cortical interneurons are functionally distinct within the cortical circuit[33]. A number of studies have suggested that activity deprivation in culture[29,34] or visual deprivation in species ranging from rodents to primates[18,35] can reduce cortical inhibition. Whether this reduction differentially affects inhibition that arises from different classes of interneuron has not been assessed. Here we examined changes in unitary connections from two classes of interneuron, fast-spiking and RSNP cells, onto star pyramidal neurons.

Fast-spiking neurons are nonadapting, high frequency–firing GABAergic inhibitory interneurons (**Fig. 5a**), which generate feedback inhibition onto star pyramidal neurons[36]. There were 16 connected

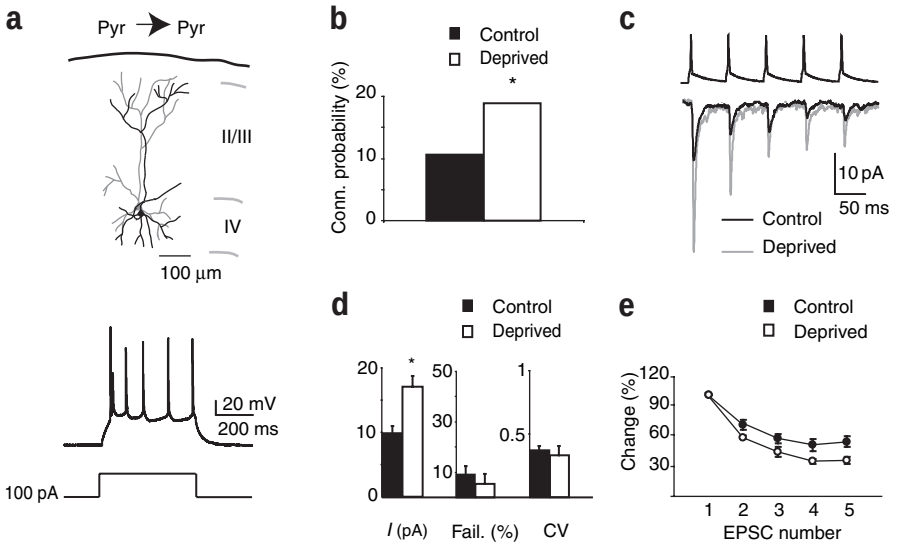

Figure 4 Visual deprivation increased the amplitude of monosynaptic connections between star pyramidal neurons. (**a**) Top: camera lucida reconstruction of a representative monosynaptically connected pair of star pyramidal neurons in layer 4, showing laminar position. Bottom: example of the typical firing pattern of star pyramidal neurons in response to a 500-ms depolarizing current step of 100 pA. (**b**) Connection probability in control and deprived hemispheres. (**c**) Representative recording of monosynaptic EPSCs (recorded at –80 mV) in response to precisely timed presynaptic spikes at 20 Hz. Each trace is the average of 30 repetitions. (**d**) EPSC amplitude (current; *I*), failure rate (Fail.) and coefficient of variation (CV), averaged across connections from control and deprived hemispheres. (**e**) Average EPSC amplitude for each response in the 20-Hz train (plotted as percentage of the first EPSC amplitude) for control and deprived pairs. Asterisk indicates significant difference from control.

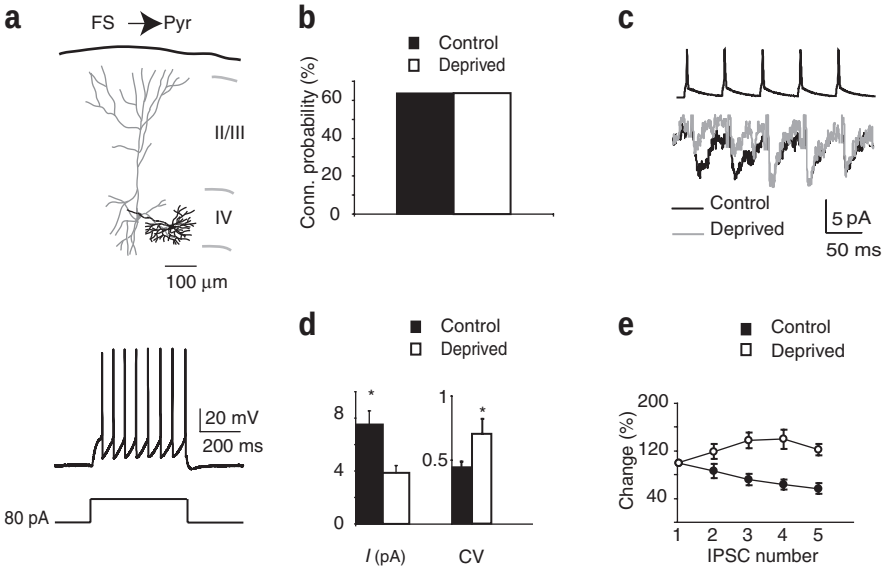

Figure 5 Visual deprivation decreased the amplitude of monosynaptic connections between fast-spiking and star pyramidal neurons. (**a**) Top: camera lucida reconstruction of a representative monosynaptically connected fast-spiking and star pyramidal neuron pair. Bottom: typical firing pattern of a fast-spiking interneuron in response to a 500-ms depolarizing current step of 80 pA. (**b**) Connection probability in control and deprived hemispheres. (**c**) Representative recording of monosynaptic IPSCs (recorded at –80 mV, so they appear as inward currents) in response to precisely timed presynaptic spikes at 20 Hz. (**d**) IPSC amplitude (current; *I*) and coefficient of variation (CV), averaged across connections from control and deprived hemispheres. (**e**) Average IPSC amplitude for each IPSC in the 20-Hz train (plotted as percentage of the first IPSC amplitude) for control and deprived pairs. Asterisk indicates significant difference from control.

pairs of fast-spiking and pyramidal neurons out of 25 tested in control hemispheres (64.0%) and 11 of 16 in deprived hemispheres (68.8%; $P = 0.9$, χ^2 test; **Fig. 5b**). Monocular deprivation induced a 50% decrease in the amplitude of monosynaptic connections between fast-spiking and star pyramidal neurons (**Fig. 5c**,**d**; control, $n = 16$; deprived, $n = 11$; $P < 0.003$). No significant changes were observed in synaptic delays (control, 1.8 ± 0.2 ms; deprived, 2.1 ± 0.3 ms; $P = 0.43$), rise times (control, 1.4 ± 0.2 ms; deprived, 1.4 ± 0.1 ms; $P = 0.85$) and decay time constants (control, 12.4 ± 1.4 ms; deprived, 11.7 ± 0.9 ms; $P = 0.69$). This decrease in amplitude was accompanied by a dramatic change in short-term plasticity. Although control connections showed short-term depression, connections from the deprived hemisphere showed pronounced short-term facilitation (**Fig. 5c**,**e**; $n = 16$ control and 11 deprived pairs; $P < 0.001$, ANOVA), and the coefficient of variation was significantly increased in the deprived hemisphere (**Fig. 5e**; $P < 0.01$), consistent with a reduction in release probability contributing to the reduction in IPSC amplitude. Because control connections depressed while deprived connections facilitated, the absolute amplitude of the last IPSC in a 20-Hz train was comparable between conditions (control, 3.4 ± 0.3 pA; deprived, 3.2 ± 0.5 pA).

RSNP neurons in rat visual cortical layer 4 are regular-spiking, moderately adapting GABAergic interneurons with a bipolar morphology (**Fig. 6a**). They receive direct thalamic drive and generate feed-forward inhibition onto star pyramidal neurons[37]. The probability of finding connected pairs in the deprived hemisphere was less than half that of the control (**Fig. 6b**; control, 16 of 50 or 32.0%; deprived, 8 of 58 or 13.8%; $P < 0.05$, χ^2 test). In contrast to the effects of monocular deprivation on fast-spiking-to-pyramidal connections, the amplitude of RSNP-to-pyramidal connections more than doubled after monocular deprivation (**Fig. 6c**,**d**; $P < 0.01$). Like pyramidal-to-pyramidal connections, deprivation produced a modest increase in short-term depression with no significant change in failure rate or coefficient of variation (**Fig. 6c**,**e**; $P = 0.62$). No changes were observed in synaptic delays (control, 1.8 ± 0.3 ms; deprived, 1.7 ± 0.2; $P = 0.91$), rise times (control, 1.2 ± 0.2 ms; deprived, 1.3 ± 0.2 ms; $P = 0.73$) and decay time constants (control, 11.4 ± 0.7; deprived, 10.9 ± 0.5 ms; $P = 0.3$). These data indicate that star pyramidal neurons receive inhibition from a smaller fraction of RSNP neurons, but that each unitary connection is more potent.

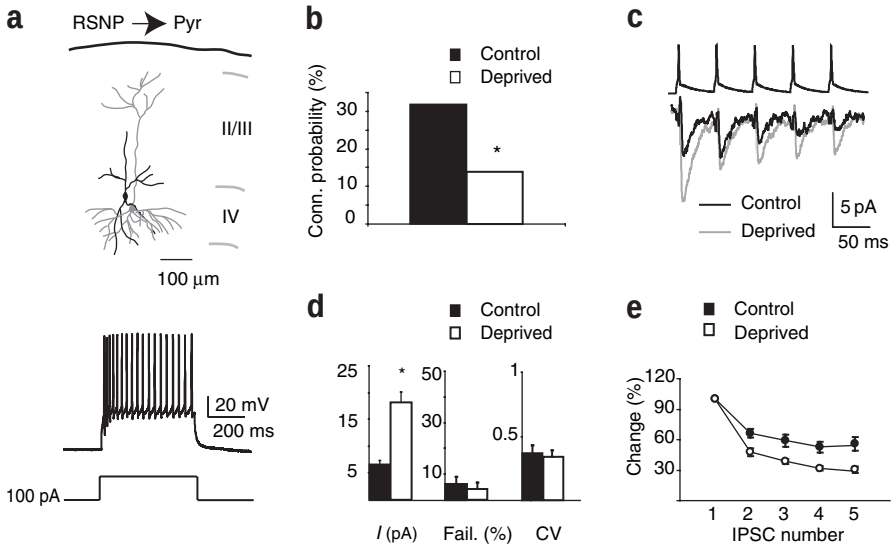

Figure 6 Visual deprivation increased the amplitude of monosynaptic connections between RSNP and star pyramidal neurons but decreased the connection probability. (**a**) Top, camera lucida reconstruction of a representative monosynaptically connected RSNP and star pyramidal neuron pair. Bottom, typical firing pattern of a RSNP interneuron in response to a 500-ms depolarizing current injection. (**b**) Connection probability for control and deprived hemispheres. (**c**) Representative recording of monosynaptic IPSCs (recorded at –80 mV, so they appear as inward currents) in response to precisely timed presynaptic spikes at 20 Hz. (**d**) IPSC amplitude (current; *I*), failure rate (Fail.) and coefficient of variation (CV), averaged across connections from control and deprived hemispheres. (**e**) Average IPSC amplitude for each IPSC in the 20-Hz train (plotted as percentage of the first EPSC amplitude) for control and deprived pairs. Asterisk indicates significant difference from control.

DISCUSSION

Here we show that a pronounced effect of early visual deprivation in layer 4 is a highly selective adjustment of intracortical synaptic strengths that results in increased layer 4 excitability. Two days of lid suture just at eye opening doubled the amplitude of local excitatory connections and doubled the probability of finding connected excitatory pairs. In contrast, inhibitory synaptic drive was reduced through selective changes in inhibitory circuitry, and these two effects cooperated to dramatically increase spontaneous activity within layer 4. This indicates that reducing sensory drive early in life increases the excitability of local layer 4 circuitry.

Theoretical work has suggested that compensatory, or homeostatic, plasticity mechanisms that stabilize neuronal or network activity are essential for the process of activity-dependent refinement[38,39], and several forms of homeostatic plasticity have been identified experimentally[40]. The data presented here indicate that, in highly recurrent layer 4 networks, this stabilization may be achieved through a coordinated set of changes in excitatory and inhibitory circuitry. When sensory drive is reduced, recurrent excitatory connections are increased in strength, whereas feedback inhibition is reduced in strength (**Supplementary Fig. 1** online). These synaptic changes are likely to act synergistically to produce the dramatic increase (25-fold) in spontaneous star pyramidal firing rates that was observed, although we cannot rule out that other synaptic changes also contribute to this increased excitability. This compensatory increase in layer 4 excitability in response to lowered sensory drive may enhance the ability of layer 4 to amplify the sensory signals it does receive.

Prolonged periods of visual deprivation (using lid suture, dark rearing or tetrodotoxin injections) have long been known to reduce inhibition in primary visual cortex of rodents and primates[18,19,35,41], but it was not known whether different classes of interneuron are differentially affected by sensory deprivation. Here we show that brief visual deprivation early in life has fundamentally different effects on different classes of inhibitory synapse. Fast-spiking interneurons generate classic feedback inhibition on star pyramidal neurons[36,42], and this class of connection was reduced in the deprived hemisphere. Interestingly, the short-term plasticity of this synapse underwent a dramatic change from depressing to facilitating. This suggests that fast-spiking connections are preferentially reduced in amplitude at low firing rates, but that at high firing rates this synapse will facilitate and more inhibition will be recruited. This may help to set an upper limit on spontaneous activity.

In contrast to fast-spiking neurons, RSNP neurons receive direct thalamic input and generate feed-forward inhibition onto star pyramidal neurons[37]. The average amplitude of RSNP-to-pyramidal connections more than doubled in the deprived hemisphere, whereas the connection probability was reduced to less than half of control values. This indicates that total inhibition from RSNP neurons remains roughly constant but that star pyramidal neurons receive more potent inhibition from fewer RSNP neurons. These results show that functionally distinct classes of inhibitory connection are adjusted in a highly selective manner by altered sensory drive, and that the effects of early sensory deprivation in layer 4 depend critically on the identity of the synapse and on its role in cortical function.

The effects of sensory deprivation are strongly developmentally regulated, and different cortical layers can have different sensitive periods during development. For example, in rodents the sensitive periods for ocular dominance plasticity in binocular visual cortex and for whisker deprivation plasticity in somatosensory cortex close early in layer 4 (after the first few weeks of life) but persist significantly later in layer 2/3. The effects of visual deprivation on the quantal amplitude of excitatory currents on pyramidal neurons also have layer-specific critical periods[17]. Lowering retinal activity between P14 and P17 scaled up excitatory quantal currents in layer 4 but not in layer 2/3, whereas the same deprivation carried out between P21 and P23 had no effect in layer 4 but instead scaled up excitatory quantal amplitudes in layer 2/3 (ref. 17). These data are consistent with the findings reported here that spontaneous activity in layer 4, but not in layer 2/3, is increased by lid suture between P14 and P17. Taken together, these studies indicate that homeostatic plasticity begins in layer 4 but (like other forms of plasticity) shifts during development to layer 2/3. The first few weeks of development are likely to represent the period of greatest instability in layer 4, as thalamocortical and intracortical synapses are rapidly forming and there is robust thalamocortical long-term potentiation, so homeostatic plasticity in layer 4 may be especially important during this developmental window. Whether the synaptic mechanisms underlying increased layer 4 excitability that are described here will generalize to other cortical layers at later developmental times remains to be determined.

In addition to serving a homeostatic function, shifts in the balance between excitation and inhibition could have a large impact on activity-dependent circuit refinement and function. Changes in this balance alter the ease with which synapse-specific forms of cortical plasticity (such as long-term potentiation and depression) can be elicited[43] and can modify or prolong the developmental periods during which visual response properties are sensitive to experience[44,45]. The initiation and propagation of activity in cortical networks is also influenced by the excitation-inhibition balance[46], and so the selectivity of cortical responses may be strongly affected when this balance is altered. Our data indicate that homeostatic regulation of the excitation-inhibition balance may be important in maintaining stable cortical activity during normal development and may also contribute to the pathological changes in visual response properties that are induced by sensory deprivation.

METHODS

Monocular deprivation. Eyelid suture was done late on P14 (just before eye opening) during brief anesthesia with 70 mg/kg ketamine, 3.5 mg/kg xylazine hydrochloride and 0.7 mg/kg acepromazine maleate, intraperitoneally. The eyelid was covered with a thin layer of xylocaine gel, and the lid was secured with three mattress sutures. Sutures were checked every day until animals were killed for recording early on P17. All methods were approved by the Brandeis Animal Use Committee and were carried out in accordance with the National Institutes of Health guidelines.

Electrophysiology. Coronal slices containing primary visual cortex were prepared from the deprived and control hemispheres, and visualized patch-clamp recordings were obtained from layer 4 of the monocular region of V1, as described[17]. Neurons were targeted visually using infrared–differential interference contrast optics and were filled with biocytin during the recording session for *post hoc* morphological reconstruction. Neurons were identified based on laminar position, morphology, synaptic properties and firing properties. Neurons that were included in the analyses had a membrane potential (V_m) below –60 mV, an input resistance (R_{in}) above 200 MΩ and a series resistance below 15 MΩ; for the included neurons, these parameters did not change more than 10% during the recording. To measure spontaneous firing rates, a small amount of DC current (generally depolarizing) was injected to adjust the interspike potential to –60 mV. For loose-patch recordings, we used 2–3 MΩ pipettes filled with ACSF. A seal of 60–200 MΩ was obtained by patching onto visually identified star pyramidal neurons, and extracellular spikes were recorded. Recordings were analyzed if spikes exceeded 5 mV and if the seal remained stable for >5 min. For generating *F-I* curves, a series of depolarizing current steps was delivered in the presence of synaptic blockers. For measuring spontaneous synaptic currents, the reversal potential (E_{rev}) was determined by voltage clamping the postsynaptic neuron to different potentials (in 5 mV increments) between –55 and –30 mV for inhibitory currents and between –5 and +15

mV for excitatory currents. The average reversal potentials for EPSCs (control, 8.4 ± 1.5 mV; deprived, 9.2 ± 1.0 mV; $P = 0.46$) and IPSCs (control, –41.0 ± 1.0 mV; deprived, –40.1 ± 1.1 mV; $P = 0.32$) were not different between conditions, and when corrected for junction potentials, they were within 1 mV of those predicted with our internal and external solutions.

Monosynaptic paired recordings were obtained in layer 4 by quadruple whole-cell recordings of nearby neurons as described[47]. Firing properties were assessed in current clamp by delivering 500 ms depolarizing current steps. Reversal potentials of synaptic currents were determined by stimulating presynaptic neurons while voltage clamping the postsynaptic neuron to different potentials. To measure short-term plasticity, 30 trains of 5 presynaptic action potentials were elicited at 20 Hz (every 20 s), and postsynaptic currents were monitored at –80 mV.

Classification of neurons. Star pyramidal neurons were identified as previously described[17]. Based on morphology, firing properties and short-term plasticity of synaptic connections, we identified two populations of connections between interneurons and star pyramidal neurons. Fast-spiking neurons were multipolar, fast spiking and nonadapting, and their IPSCs on pyramidal neurons showed moderate depression[13,15,42,48]. RSNP neurons had a bipolar morphology and were regular spiking with frequency adaptation; their IPSCs on pyramidal neurons showed marked depression[13,15,42,49]. Note that interneuronal properties in layer 4 of visual cortex differ in some respects from those reported in layer 4 of somatosensory cortex[50].

Statistical analysis. All data are expressed as the mean ± s.e.m. for the number of neurons (or connected pairs) specified, and all statistical tests were unpaired two-tailed *t*-tests, except as noted in text.

Solutions. Standard ACSF consists of 126 mM NaCl, 3 mM KCl, 2 mM $MgSO_4$, 2 mM $CaCl_2$, 1 mM $NaHPO_4$, 25 mM $NaHCO_3$ and 25 mM dextrose. For paired recordings, the Mg^{2+} concentration was 1 mM. Modified ACSF (3.5 mM KCl, 0.5 mM $MgCl_2$ and 1 mM $CaCl_2$) was used to record spontaneous firing and spontaneous synaptic currents. The internal solution was 20 mM KCl, 100 mM potassium gluconate, 10 mM HEPES, 0.1% biocytin, 4 mM magnesium ATP, 0.3 mM sodium GTP and 10 mM sodium phosphocreatine. For measuring spontaneous synaptic currents, the internal solution contained 20 mM KCl, 100 mM cesium methylsulfonate, 10 mM HEPES, 0.1% biocytin, 4 mM Mg-ATP, 0.3 mM Na-GTP, 10 mM sodium phosphocreatine and 3 mM QX-314.

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

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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