Critical periods for experience-dependent synaptic scaling in visual cortex

Niraj S. Desai[1,2], Robert H. Cudmore[1], Sacha B. Nelson[1] and Gina G. Turrigiano[1]

[1] *Department of Biology and Volen National Center for Complex Systems, MS 008, Brandeis University, 415 South Street, Waltham, Massachusetts 02454, USA*

[2] *Current address: The Neuroscience Institute, 10640 John Jay Hopkins Drive, San Diego, California 92121, USA*

Correspondence should be addressed to G.G.T. (turrigiano@brandeis.edu)

Published online: 24 June 2002, corrected 25 June 2002 (details online), doi:10.1038/nn878

The mechanisms underlying experience-dependent plasticity and refinement of central circuits are not yet fully understood. A non-Hebbian form of synaptic plasticity, which scales synaptic strengths up or down to stabilize firing rates, has recently been discovered in cultured neuronal networks. Here we demonstrate the existence of a similar mechanism in the intact rodent visual cortex. The frequency of miniature excitatory postsynaptic currents (mEPSCs) in principal neurons increased steeply between post-natal days 12 and 23. There was a concomitant decrease in mEPSC amplitude, which was prevented by rearing rats in complete darkness from 12 days of age. In addition, as little as two days of monocular deprivation scaled up mEPSC amplitude in a layer- and age-dependent manner. These data indicate that mEPSC amplitudes can be globally scaled up or down as a function of development and sensory experience, and suggest that synaptic scaling may be involved in the activity-dependent refinement of cortical connectivity.

Neuronal activity is important for the formation and maturation of neural circuits in the mammalian nervous system[1]. Very early in development, the basic connections that define these circuits are determined genetically, using a wide variety of molecular cues[2–4]. Refinement of these connections during later development, however, is strongly influenced by neuronal activity. At first, this activity is internally generated and spontaneous, as in the case of the retinal waves that influence early postnatal visual development[5,6]. Later, activity-dependent development is driven by sensory experience[1]. Experimental manipulations of this experience can have profound effects on the functioning of the resulting circuits[7–10]. In particular, these manipulations have been used to establish the existence of critical periods during which various aspects of nervous system development are acutely sensitive to experience.

Exactly how activity shapes synaptic connectivity during development, and the precise mechanisms underlying these critical periods, are still unknown. There is considerable evidence that correlation-based, or Hebbian, plasticity mechanisms such as long-term potentiation and depression (LTP and LTD) are centrally involved in synaptic refinement[11–13]. Hebbian plasticity may not be sufficient for understanding activity-dependent development, however, because the dramatic changes in synaptic strength produced by Hebbian mechanisms tend to threaten the stability of neural circuits[14–17]. In addition, it is not clear that Hebbian rules necessarily lead to competition between presynaptic inputs for postsynaptic influence[15], yet such synaptic competition is a ubiquitous feature of developmental plasticity.

How might stability and competition be promoted throughout development, if simple Hebbian rules are insufficient? A number of answers to this question have been proposed[14–18]. One idea is that the total synaptic strength of inputs to a given neuron can be regulated by ongoing activity. In the last several years, evidence for such homeostatic forms of synaptic plasticity has mounted, based primarily on data from the neuromuscular junction[19,20] and from cultured networks of central neurons[21–26]. These *in vitro* experiments have shown that the quantal amplitude of excitatory AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-mediated synaptic inputs to pyramidal neurons can be scaled up and down in order to compensate for changes in firing rates. In contrast to synapse-specific forms of plasticity like LTP and LTD, this synaptic scaling seems to globally adjust the strength of all of a neuron's synapses based on some average measure of postsynaptic activity[22,26]. Such global synaptic adjustment schemes have long been used in Hebbian network models on an *ad hoc* basis to maintain stability[15], and recent modeling studies have demonstrated that synaptic scaling can introduce competition between synapses[27].

Here we extend these studies of homeostatic synaptic plasticity to the intact central nervous system, showing that a process like synaptic scaling is at work during activity-dependent development *in vivo*. First, we examined how quantal excitatory currents in rodent visual cortex change during development, between postnatal days 12 and 23 (P12 and P23). This is a time of intense synaptogenesis, when normal synaptic input to cortical neurons rises continuously[28]. We found a marked reduction in the sizes of quantal excitatory synaptic currents during this period, which we were able to prevent by dark rearing. In addition, we used a

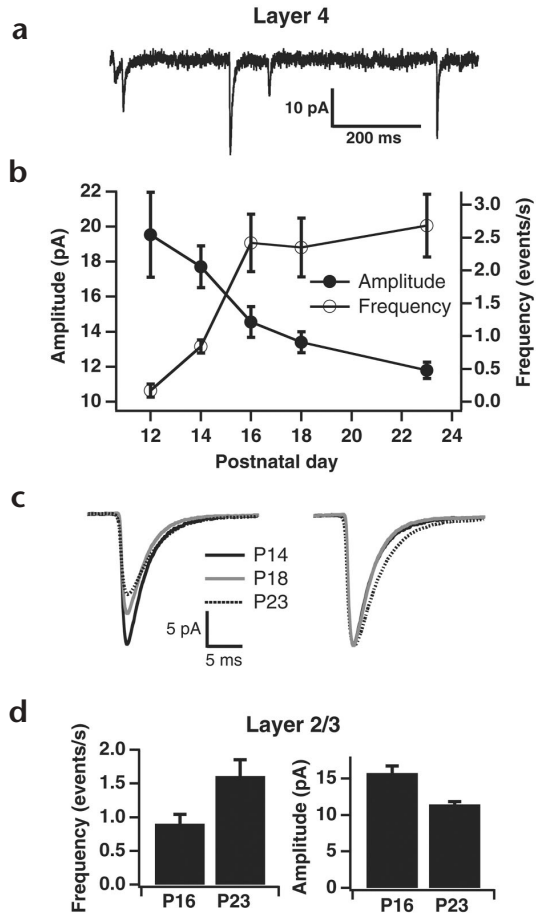

Fig. 1. Layer 4 and 2/3 mEPSC amplitudes decreased while mEPSC frequencies increased during the second and third postnatal weeks. (**a**) Representative whole-cell voltage clamp recording of mEPSCs from a principal neuron in layer 4 at P16. (**b**) Average mEPSC amplitude (filled circles) and frequency (open circles) in layer 4 neurons as a function of postnatal day. (**c**) Left, waveforms of the average AMPA-mediated mEPSCs measured in layer 4 neurons at postnatal days 14, 18 and 23, showing the progressive decrease in amplitude with age. Right, average mEPSCs shown at left were scaled to peak to allow a comparison of kinetics. (**d**) Average mEPSC amplitude and frequency for layer 2/3 pyramidal neurons at P16 and P23.

variant of a classic manipulation of sensory experience, monocular deprivation[7], to reduce activity levels in monocular primary visual cortex. We found evidence for a global rescaling of synapses that is reminiscent of synaptic scaling previously seen in culture and that operates in a laminar- and age-dependent manner, as do other forms of activity-dependent plasticity observed *in vivo*[8–10]. We conclude that homeostatic synaptic scaling may play an important role in the activity-dependent refinement of central circuits.

Results

For all experiments, coronal brain slices containing primary visual cortex were prepared from postnatal Long-Evans rats. Visualized whole-cell patch recordings were confined to principal neurons of layer 4 or layer 2/3 of the monocular portion of visual cortex (Methods). Miniature EPSCs mediated by AMPA receptors were measured in the presence of tetrodotoxin (TTX, which blocks voltage-dependent sodium channels), the NMDA receptor antagonist APV, and the $GABA_A$ receptor blocker bicuculline (**Fig. 1a**). These mEPSCs arise randomly from among all of a neuron's synaptic contacts and so represent a sampling of all of the neuron's inputs.

Table 1. Passive neuronal properties.

Layer	Age	Condition	V_m (mV)	R_{in} (MΩ)	Decay time
4	12	Control	−65 ± 2	668 ± 96	2.6 ± 0.3
4	14	Control	−66 ± 1	536 ± 30	2.9 ± 0.2
4	16	Control	−67 ± 2	350 ± 25	3.6 ± 0.3
4	16	MD	−66 ± 2	400 ± 28	3.3 ± 0.2
4	16	DR	−63 ± 3	340 ± 34	3.6 ± 0.5
4	18	Control	−67 ± 1	396 ± 43	3.1 ± 0.2
4	18	DR	−66 ± 1	308 ± 40	3.5 ± 0.2
4	23	Control	−68 ± 1	297 ± 20	4.0 ± 0.2
4	23	MD	−68 ± 1	281 ± 15	4.0 ± 0.2
4	23	DR	−69 ± 1	245 ± 36	3.6 ± 0.3
2/3	16	Control	−68 ± 1	501 ± 46	3.4 ± 0.2
2/3	16	MD	−68 ± 1	576 ± 30	3.0 ± 0.2
2/3	23	Control	−74 ± 1	259 ± 10	4.2 ± 0.2
2/3	23	MD	−73 ± 1	251 ± 11	3.9 ± 0.3

Resting potential (V_m), resting input resistance (R_{in}) and mEPSC decay time (exponential fit) for neurons in layers 4 or 2/3, at postnatal ages between 12 and 23, for control, monocularly deprived (MD) or dark-reared (DR) animals. None of the differences between conditions for a given age group were significant ($P > 0.05$, *t*-test).

mEPSC amplitude decreases as frequency increases

In hippocampal cell cultures, the average quantal amplitude of AMPA currents is inversely correlated with the density of active synapses; that is, the more synapses a given neuron receives, the weaker its response to vesicle release at any one synapse[29]. This suggests that if the number of synapses between cortical neurons grows rapidly over developmental time, there may be a concomitant scaling down of the average quantal amplitude.

To examine this possibility, we measured mEPSCs between P12 and P23, a period of intense growth in synapse number[28] (**Fig. 1**). In layer 4 neurons, the average frequency of these spontaneous events increased dramatically, from 0.2 ± 0.1 events/s at P12 to 2.7 ± 0.5 events/s at P23 (n = 7–23 neurons per age, $P < 0.001$, one-way ANOVA; **Fig. 1b**). This increase was especially pronounced between P14 and P16, which is approximately when rats first open their eyes. Over the same developmental period, the average quantal amplitude decreased by 39%, from 19.5 ± 2.4 pA at P12 to 11.8 ± 0.5 pA at P23 ($P < 0.001$, one-way ANOVA; **Fig. 1b** and **c**). Again the period of eye opening saw the sharpest change, with amplitude decreasing by 25% between P14 and P18. The reduction in mEPSC amplitude between P14 and P18 (25%) occurred without any change in mEPSC kinetics (**Fig. 1c**, right), and with a proportional reduction in charge (23%). Thus, lower mEPSC amplitudes did not result from increased filtering of synaptic currents. The additional reduction in amplitude that occurred between P18 and P23 was accompa-

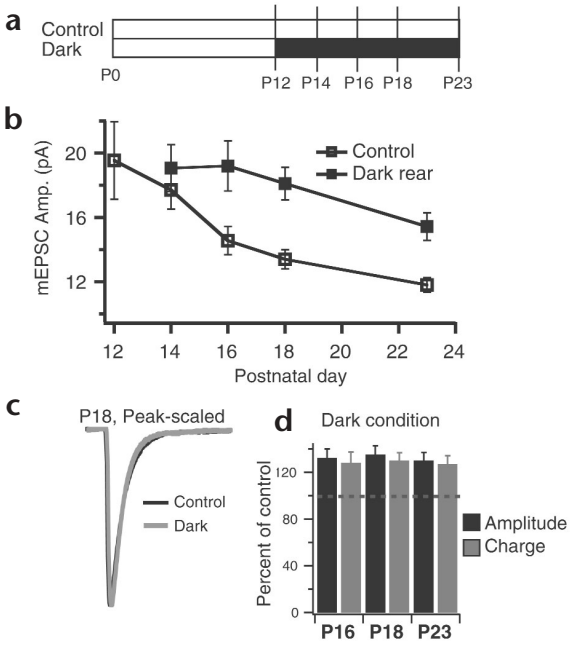

Fig. 2. Dark rearing prevented the developmental reduction in mEPSC amplitude in layer 4. (**a**) Dark-rearing protocol. Control animals were maintained in a normal light-dark cycle; dark-reared animals (dark) were maintained in the dark from P12. (**b**) Average AMPA quantal amplitudes for control or dark-reared animals at various developmental ages. Dark rearing significantly elevated mEPSC amplitudes at P16, 18 and 23 relative to values from age-matched controls (*t*-test, $P < 0.02$, 0.005 and 0.001, respectively). (**c**) Average mEPSCs from control or dark-reared animals at P18, scaled to peak to allow a comparison of kinetics. (**d**) Average mEPSC amplitudes and charge for dark-reared animals, expressed as a percentage of age-matched control values for the ages indicated. There was a proportional increase in amplitude and charge in the dark condition at all three ages.

nied by a slowing of mEPSC kinetics and no reduction in charge, so was probably due to changes in the passive properties of the neurons as they matured (**Table 1**).

A more restricted analysis of developmental changes in mEPSCs in layer 2/3 showed that, between P16 and P23, there was a similar inverse relationship between mEPSC frequency and amplitude, with the former increasing by 79% between P16 and P23 and the latter decreasing in amplitude by 27% and in charge by 15% (**Fig. 1d**, $n = 18$). Thus, in layer 2/3, about half the decrease in amplitude between P16 and P23 was attributable to increased filtering. The developmental decrease in mEPSC amplitude in layers 4 and 2/3 was not as large as the increase in frequency, possibly because a number of mechanisms cooperate to compensate for changes in excitatory drive, including an increase in intracortical inhibition[30,31].

Dark rearing prevents decline in mEPSC amplitude

The inverse relationship between mEPSC frequency and amplitude in layer 4 suggests that the increased activity associated with eye opening and increased synaptogenesis may drive a compensatory reduction in AMPA quantal amplitude. To directly test this hypothesis, we reduced visual system activity by rearing animals in the dark beginning at P12, and measured AMPA quantal amplitude in layer 4 at various developmental ages (**Fig. 2a**). Miniature EPSC frequency increased in dark-reared animals 3.5-fold between P14 and P18, similar to the increase seen in control animals (**Fig. 1b**).

In contrast, the developmental reduction in quantal amplitude that normally occurs between P14 and P18 was prevented by dark rearing (**Fig. 2b**). Whereas in control animals there was a significant reduction in amplitude with age, there was no significant reduction between P14 and P23 in dark-reared animals (ANOVA, $P > 0.12$, $n = 6$–12 neurons/age). At P16, P18 and P23, mEPSC amplitudes from dark-reared animals were significantly higer than control values from the same age (*t*-test, $P < 0.02$, 0.005 and 0.001, respectively). There were no differences in mEPSC kinetics between the control and dark-reared condition (**Fig. 2c**) and passive neuronal properties were not significantly affected by dark rearing (**Table 1**). There was a small and parallel reduction in mEPSC amplitude from both dark-reared and control animals between P18 and P23, but the difference in amplitude between dark-reared and control was relatively constant at 30% between P16 and P23, as was the difference in charge (also approximately 30%; **Fig. 2d**). These data indicate that visual experience between P12 and P18 is necessary for the normal developmental decrease in AMPA quantal amplitude.

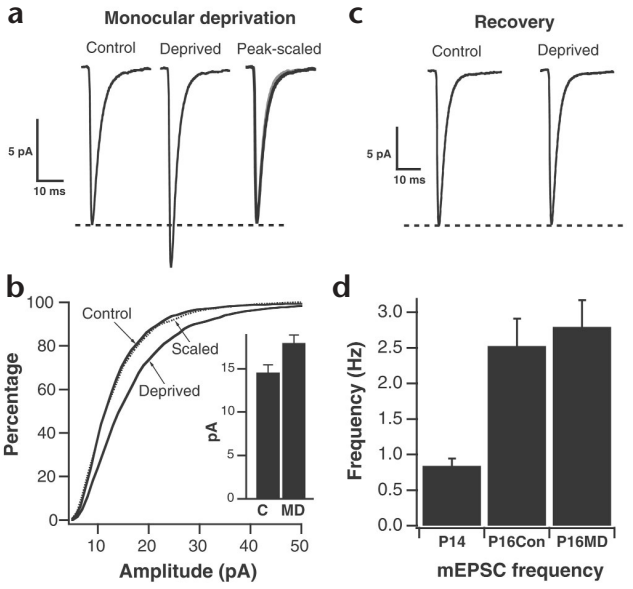

Fig. 3. Monocular deprivation (MD) via intraocular TTX injections between P14 and P16 increased average quantal amplitude in layer 4. (**a**) Average AMPA-mediated mEPSCs from layer 4 in monocular cortex ipsilateral (control) and contralateral (deprived) to the intraocular TTX injection. Amplitudes were increased by MD, but kinetics were not affected (peak-scaled). (**b**) Cumulative histogram of mEPSCs in control and deprived neurons (50 events/neuron). Deprivation shifted the entire distribution of mEPSCs toward larger values. The deprived distribution could be collapsed onto the control distribution by dividing every point in the deprived distribution by a factor of 1.19 (scaled). Inset, average quantal amplitude in deprived neurons was significantly larger than in control neurons ($P < 0.01$, *t*-test). (**c**) When retinal activity was allowed to resume by ceasing to refresh the TTX, mEPSCs returned to control values within 72 h. (**d**) MD did not prevent the normal increase in mEPSC frequency that occurs between P14 and P16 (MD not different from control at P16; $P > 0.74$).

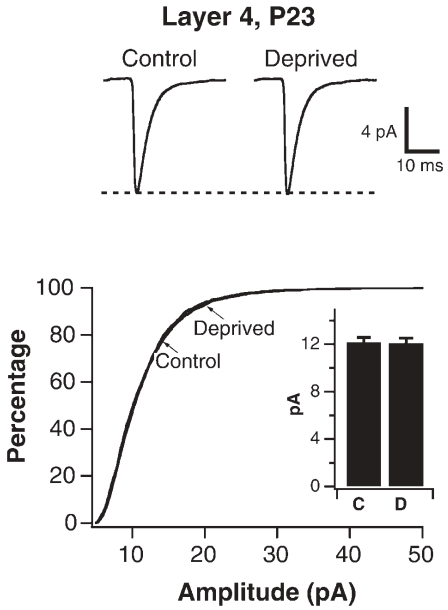

Fig. 4. Monocular deprivation between P21 and P23 did not affect mEPSCs from layer 4. Top, average mEPSCs from control and deprived neurons. Bottom, cumulative histogram of mEPSCs from control and deprived neurons (50 events/neuron). There were no significant differences between the two conditions.

Monocular deprivation scales mEPSC amplitudes

As a second means of lowering visually driven activity, we subjected rat pups to monocular deprivation for 48 hours by intraocular injections of TTX, a technique widely used to study ocular dominance plasticity in binocular cortex[7,32–35]. Here we used it to investigate the effects of activity deprivation, as blocking retinal activity reduces visual cortical activity[32,33]. The rodent visual system is particularly well-suited to this protocol because a substantial portion of visual cortex is monocular, receiving sensory input only from the contralateral eye[36,37]. By confining these recordings to monocular cortex, we were able to examine the effects of lowered activity without the potential complication of also altering competition between inputs from the two eyes, as would be the case in binocular cortex. In addition, each animal served as its own control, as blocking activity in one eye left activity unchanged in the ipsilateral monocular cortex.

We monocularly deprived P14 rat pups for 48 hours, and at P16, prepared brain slices and measured mEPSCs from layer 4 neurons in monocular cortex (both contralateral and ipsilateral to the eye injection). Eye opening occurs in the rat around P14, so this procedure effectually deprived one hemisphere of a major sensory event that would normally increase thalamic drive to cortex. We found that 48 hours of activity deprivation increased average mEPSC amplitude by 23% relative to the control hemisphere, from 14.6 ± 0.9 pA to 18.0 ± 0.9 pA (n = 14 and 19, $P < 0.01$, t-test; **Fig. 3a** and **b**), without changing mEPSC kinetics (**Fig. 3a**, peak-scaled) or passive cell properties (**Table 1**). Because mEPSC kinetics were unaltered, the amplitude increase was not a consequence of differential cable filtering but instead represented a change in quantal synaptic transmission. The increase in the average current resulted from a shift in the entire distribution of quantal currents toward larger values (**Fig. 3b**). As found previously for synaptic scaling in culture[22], these data are consistent with a model in which all synaptic inputs to a given neuron are scaled up by a single multiplicative factor, as dividing the amplitude of each event from deprived neurons by the same factor (1.19) produced a good fit to the control distribution (**Fig. 3b**).

Monocular deprivation using intraocular TTX requires repeated reinjection to maintain retinal blockade because TTX breaks down over time, allowing visual responses to resume approximately 24 hours after injection[33]. We used this fact to investigate whether the effect of deprivation on mEPSC amplitude is reversible. After injecting TTX into one eye of rats at P14 and again at P15, we ceased the TTX injections, allowing retinal activity to resume. At P18, we prepared coronal slices and measured mEPSCs in layer 4 neurons in both hemispheres. The recovery period was sufficient to reverse the effect of the 48 h deprivation: mEPSCs were no different in deprived neurons than in control ones, 15.0 ± 0.6 pA versus 14.9 ± 0.7 pA (**Fig. 3c**).

Monocular deprivation did not prevent the developmental changes in neuronal and synaptic properties that normally occur between P14 and P16. For example, the reduction in input resistance still occurred (**Table 1**), as did the increase in mEPSC frequency (**Fig. 3d**).

A critical period for layer 4 scaling

A common feature of plasticity in sensory cortex is the existence of critical periods during which rearrangements in circuitry can occur. For ocular dominance plasticity in visual cortex and for whisker deprivation effects in barrel field cortex, layer 4 has critical periods during the first several weeks of life[8–10,38]. To investigate whether the effect of activity deprivation on mEPSCs in layer 4 was also developmentally regulated, we began monocular deprivation at P21, and then at P23 we measured mEPSCs in layer 4 monocular cortex. We found that monocular deprivation had no effect (**Fig. 4**). Average mEPSC amplitudes, kinetics and frequencies were unchanged by monocular deprivation (12.1 ± 0.4 pA and 11.8 ± 0.5 pA for monocularly deprived and control, respectively; n = 14 for both conditions), and quantal amplitudes were distributed similarly in both conditions (**Fig. 4b**). These findings indicate that synaptic scaling is developmentally regulated in layer 4, with the window for plasticity closing fairly early.

Deprivation's effects are delayed in layer 2/3

Whereas the critical periods for several forms of developmental plasticity in layer 4 close early, layer 2/3 can exhibit plasticity well into adulthood[8–10,38]. To determine whether mEPSC scaling shares the layer-dependence of ocular dominance and whisker deprivation plasticity, we measured mEPSCs in layer 2/3 pyramidal neurons after subjecting rats to monocular deprivation from P14 to P16 or from P21 to P23. We found that plasticity was delayed relative to layer 4 neurons. Deprivation between P14 and P16 left mEPSCs unaltered by all of our measurements, including average amplitude (control, 15.7 ± 1.0 pA, n = 10; deprived, 15.7 ± 1.1 pA, n = 11; **Fig. 5a**). In contrast, deprivation between P21 and P23 increased average mEPSC amplitude by 14%, from 11.4 ± 0.4 pA to 13.0 ± 0.6 pA (n = 18 each, $P < 0.05$, t-test; **Fig. 5b**). There were no changes in mEPSC kinetics, frequency, or passive cell properties (**Table 1**). Again, the increase in the average amplitude was produced by a shift in the entire distribution of events (**Fig. 5b**, lower panel). These results show that the critical period for mEPSC scaling, like other forms of developmental plasticity, occurs later in layer 2/3 than in layer 4.

Discussion

We have used two classic manipulations of visual experience, dark rearing and monocular deprivation, to demonstrate that the quantal amplitude of excitatory currents in primary visual cortex can be globally scaled up or down as a function of development and sensory experience. In binocular cortex, monocular deprivation both lowers activity and creates an imbalance in activity between the two eyes, making it difficult to untangle the relative contributions of Hebbian and homeostatic plasticity to any changes in synaptic strength. To circumvent this problem, we used monocular cortex to examine how visual cortical neurons adapted to prolonged periods of reduced retinal and sensory drive. Experiments monitoring immediate early gene expression have established that intraocular TTX injections have pronounced effects on activity in the monocular zone[32,33]. Using this method, we discovered that brief (48 h) activity deprivation produced significant increases in the amplitudes of excitatory quantal currents in visual cortical neurons. The increase affected both large and small synaptic currents, and was consistent with a model in which the entire distribution of currents in a given neuron was shifted toward larger amplitude values.

Such adaptive changes in overall synaptic strength have heretofore been studied at the neuromuscular junction[19,20] or in cultured central neurons[16,21–26]. Several groups working in different culture preparations—neocortical[22,25,26], hippocampal[21,23] and spinal cord[24]—have found that excitatory synaptic currents or postsynaptic receptors are sensitive to average activity levels. In particular, blocking spiking or synaptic activity pharmacologically results in a compensatory increase in current amplitude or receptor number; conversely, increasing activity above control levels results in a decrease in amplitude or receptor number. It has been proposed that this plasticity acts as a homeostatic regulatory mechanism, damping network activity when synaptogenesis or correlation-based plasticity drives it too high, boosting activity when it falls too low. Also, modeling studies have suggested that synaptic scaling promotes competition between presynaptic inputs, as high postsynaptic activity by one set of inputs weakens the effects of a second set after global rescaling[27]; if synaptic weakening is a prelude to synapse elimination[39], this would provide a means for strong inputs to force the pruning of weaker ones. Our data indicate that a process like synaptic scaling also operates *in vivo*. During development, as sensory and synaptic drive increases, mEPSC amplitudes are reduced in an activity-dependent manner, and after short periods of activity deprivation, quantal currents are globally upregulated.

Prolonged dark rearing starting at birth extends the critical period for monocular deprivation[40] and delays the maturation of visual response properties[41]. This raises the question of whether the effects of visual deprivation are simply due to arrested development in the absence of visually-driven activity. Although we cannot entirely rule this possibility out, several considerations argue against this interpretation. First, we found that a two-day period of deprivation is sufficient to modify quantal amplitude. This is a much shorter period of deprivation than that which produces a generalized developmental delay of visual response properties[40,41]. Second, the effects of monocular deprivation occur at very different developmental stages in different layers, suggesting that monocular deprivation is not simply causing a cortex-wide developmental delay. Finally, all other developmental parameters that we have monitored have continued to change apace during the period of deprivation, including cellular properties such as input resistance, and synaptic properties such as mEPSC frequency and kinetics. Taken together, these results suggests that brief periods of sensory deprivation do not produce a generalized developmental delay, but rather have a selective effect on the amplitude of AMPA-mediated synaptic transmission.

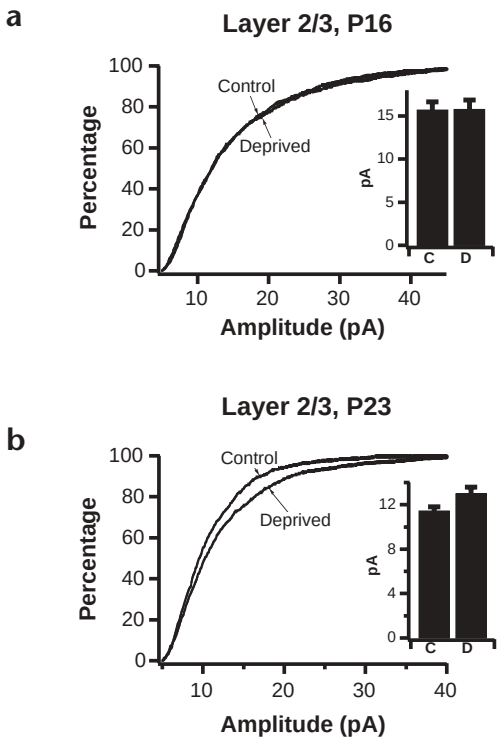

Fig. 5. Sensitivity to monocular deprivation was shifted developmentally in layer 2/3 relative to layer 4. (**a**) 48 h of deprivation had no effect on mEPSCs when begun at P14 and terminated at P16. There were no significant differences between the two conditions. (**b**) 48 h deprivation did significantly ($P < 0.05$, *t*-test) increase mEPSC amplitude when begun at P21 and terminated at P23. Cumulative histograms were constructed from 50 events/neuron.

A hallmark of activity-dependent cortical development is the existence of layer-specific critical periods for plasticity. In keeping with this, we found that deprivation scaled quantal currents in a layer- and age-specific manner. In layer 4, the principal input layer, quantal amplitudes increased when deprivation started at P14, immediately before eye opening, but not when it started at P21. On the other hand, deprivation's effects in layer 2/3 were delayed, with no change at the younger age but with a significant increase at the older one. This is strongly reminiscent of the critical periods for ocular dominance plasticity in binocular visual cortex[7,8] and for whisker deprivation plasticity in somatosensory cortex[9,10,38]. In each of those cases, the critical period for layer 4 plasticity closes after the first week or several weeks of life, whereas layer 2/3 plasticity extends much later in development. Notably, the critical period for synaptic scaling in layer 4 closes before the beginning of the critical period for ocular dominance plasticity in binocular cortex, suggesting that scaling at these synapses is unlikely to contribute

to ocular dominance plasticity. The opening of scaling in layer 2/3, by contrast, is coincident with the opening of the critical period for ocular dominance plasticity.

Why the critical period for layer 4 should close early is not clear for any form of activity-dependent plasticity. The potential for instability in layer 4 is probably greatest between P14 and P18, because of increased synaptogenesis and the sudden increase in sensory drive around the time of eye opening. Thalamocortical LTP also closes early in layer 4 of somatosensory cortex[42]. It is possible that synaptic scaling is no longer needed once synaptogenesis has slowed and Hebbian mechanisms have decreased. Whatever the reason, it is a consistent finding that many forms of layer 4 plasticity are only transiently expressed during development, and that in older animals the major locus of cortical plasticity shifts to other layers[7–10,43].

Layer 2/3 is only poorly driven by visual activity before the third postnatal week[41]. The lack of effect of retinal blockade on layer 2/3 quantal amplitudes before this time may simply reflect this lack of peripheral drive. The onset of sensitivity of AMPA quantal amplitudes to monocular deprivation in layer 2/3 coincides with the beginning of the classical critical period for ocular dominance plasticity in rodent binocular cortex[35,41,44], suggesting that the expression of these two forms of plasticity is closely linked during development. The precise end of the critical period for ocular dominance plasticity in layer 2/3 is still under debate, but it is thought to extend well into early adulthood[35,41,44], as does the capacity for axonal remodeling of horizontal connections in layer 2/3 (ref. 45). We do not yet know whether the capacity for synaptic scaling in layer 2/3 also extends into adulthood.

The magnitudes of the experience-dependent changes in quantal amplitude seen here (15–30%) are smaller than those seen in culture[22–26], probably because the reduction in cortical activity caused by sensory deprivation is smaller than the complete activity blockade of the culture experiments. However, because this scaling operates in a global manner on all excitatory synapses, the functional consequences of a 15–30% change in total excitation are likely to be pronounced. Alterations in the balance between excitation and inhibition can strongly influence activity and information flow within cortical networks[45–47], as well as the ability to induce LTP and LTD[48]. Experience-dependent scaling of intracortical synapses may therefore have a profound effect on the expression of other forms of activity-dependent plasticity, including higher-order processes such as ocular dominance plasticity, that are thought to rely on Hebbian and competitive cellular plasticity mechanisms.

Our results indicate that sensory experience can globally regulate the strengths of excitatory intracortical synapses in the direction needed to stabilize firing rates. This, in turn, may allow Hebbian mechanisms to selectively modify individual synaptic strengths without the loss of selectivity that can arise from the positive-feedback nature of correlation-based plasticity. Our data raise the possibility that synaptic scaling and Hebbian plasticity cooperate during development to selectively refine cortical connectivity.

Methods

Intraocular TTX injections and dark rearing. All experiments were approved by the Brandeis Animal Use Committee and were in accordance with the National Institutes of Health guidelines. Long-Evans rat pups, at postnatal days 14 or 21, were subjected to monocular deprivation by intraocular injection of TTX using a standard protocol[32–34]. Animals were first anesthetized with isoflurane. The eyes of the younger animals had not yet opened, and so the eyelids over both eyes were gently pulled apart with tweezers. The injection solution consisted of 1 mM (for P14 rats) or 4 mM (for P23 rats) TTX in a citrate buffer. A volume of 1–2 μl was injected into the vitreous of the right eye by inserting a pulled micropipette at the ora serrata. The micropipette was filled with solution and connected to a microinjector, allowing the solution to be injected slowly (1–2 min). This procedure blocks retinal activity completely for approximately 24 h, but activity recovers over longer times[34]. Therefore, to maintain blockade for 48 h, the procedure was repeated 24 h after the first injection. For dark rearing, entire litters were raised on a normal light/dark cycle until P12, when they were moved into the dark and maintained in total darkness. Pups were transferred to a light-tight box and anaesthetized in the dark before decapitation. Control animals were raised on a 12 hr light/12 hr dark cycle.

Slice preparation. Coronal brain slices containing primary visual cortex were prepared as described[49]. Animals were deeply anesthetized with isoflurane and decapitated and their brains were quickly removed and placed in chilled (1°C) artificial cerebrospinal fluid (ACSF) containing TTX. Slices of 400 μm thickness were cut on a vibratome. Slices were maintained at room temperature on semipermeable membranes covered by a thin layer of ACSF containing TTX and continuously oxygenated (with 95% O_2, 5% CO_2). Slices equilibrated for 1 h prior to the start of recording and were used up to 7 h after preparation.

Electrophysiology. Slices were transferred one at a time to a submerged chamber mounted on a fixed-stage upright microscope and slowly warmed to 30–32°C. They were continuously perfused with warmed, oxygenated ACSF at a rate of 2–3 ml/min. Low-power transillumination was used to visualize the location of monocular visual cortex[36,37] and the positions of layer 2/3 and 4; in most cases this was confirmed *post hoc* using biocytin reconstructions.

Visualized whole-cell patch recordings were obtained as previously described[25,49]. Slices were illuminated obliquely through an infrared filter and viewed with standard optics using a 40× long-working distance water immersion objective lens. Layer 2/3 neurons were identified as pyramidal based on their prominent apical dendrites and pyramidal somata; layer 4 neurons were predominantly star pyramids, identified by the presence of a thin apical dendrite extending into the upper layers. *Post hoc* reconstruction of biocytin fills confirmed that 96% of successfully filled layer 4 neurons had a star pyramidal morphology. Pipette resistances were 3–5 MΩ in the bath. Voltage-clamp recordings were done with an Axopatch 1D (Axon Instruments, Union City, California). Seal resistances were 2–8 GΩ. Recordings were discarded if the resting potential was more positive than –60 mV, the series resistance larger than 20 MΩ, the resting input resistance smaller than 200 MΩ, or if any of these quantities changed during the course of the recording by more than 10%.

To record AMPA-mediated mEPSCs, neurons were held in voltage clamp at –70 mV in the presence of TTX (1 μM), APV (50 μM) and bicuculline (20 μM). In-house software was used to detect and measure mEPSCs; detection criteria included amplitudes greater than 5 pA and 20–80% rise times less than 1 ms. All data are reported as mean ± s.e.m. for the number of neurons indicated. Data were obtained from a total of 43 animals, and from at least 3 animals for each developmental time point.

Solutions. The ACSF contained 126 mM NaCl, 3 mM KCl, 2 mM $MgSO_4$, 1 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$ and 14 mM dextrose; the pH was buffered to 7.4 by bubbling continuously with 95% O_2/5% CO_2. The internal pipette solution contained 100 mM potassium gluconate, 20 mM KCl, 10 mM HEPES/K-HEPES, 0.5 mM EGTA, 0.3 mM Na(GTP), 4 mM Mg(ATP) and 0.1% biocytin; the pH was adjusted to 7.3 with KOH and/or HCl.

Acknowledgments

Supported by National Institutes of Health grants RO1 NS 36853 and EY 11116. We are grateful for the technical assistance of T. Casimiro and M. Miller.

Competing interests statement

The authors declare that they have no competing financial interests.

RECEIVED 29 APRIL; ACCEPTED 4 JUNE 2002

1. Katz, L. C. & Shatz, C. J. Synaptic activity and the construction of cortical circuits. *Science* **274**, 1133–1138 (1996).
2. Goodman, C. S. & Shatz, C. J. Developmental mechanisms that generate precise patterns of neuronal connectivity. *Cell* **72**, 77–98 (1993).
3. Tessier-Lavigne, M. & Goodman, C. S. The molecular biology of axon guidance. *Science* **274**, 1123–1133 (1996).
4. Crowley, J. C. & Katz, L. C. Development of ocular dominance columns in the absence of retinal input. *Nat. Neurosci.* **2**, 1125–1130 (1999).
5. Feller, M. B. Spontaneous correlated activity in developing neural circuits. *Neuron* **22**, 653–656 (1999).
6. McCormick, D. A. Spontaneous activity: signal or noise? *Science* **285**, 541–543 (1999).
7. Hubel, D. H. & Wiesel, T. N. The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J. Physiol.* **206**, 419–436 (1970).
8. Daw, N. W., Fox, K., Sato, H. & Czepita, D. Critical period for monocular deprivation in the cat visual cortex. *J. Neurophysiol.* **67**, 197–202 (1992).
9. Fox, K. A critical period for experience-dependent synaptic plasticity in rat barrel cortex. *J. Neurosci.* **12**, 1826–1838 (1992).
10. Stern, E. A., Maravall, M. & Svoboda, K. Rapid development and plasticity of layer 2/3 maps in rat barrel cortex *in vivo*. *Neuron* **31**, 305–315 (2001).
11. Shatz, C. J. Impulse activity and the patterning of connections during CNS development. *Neuron* **5**, 745–756 (1990).
12. Malenka, R. C. & Nicoll, R. A. NMDA receptor–dependent synaptic plasticity: multiple forms and mechanisms. *Trends Neurosci.* **16**, 521–527 (1993).
13. Bear, M. F. & Malenka, R. C. Synaptic plasticity: LTP and LTD. *Curr. Opin. Neurobiol.* **4**, 389–399 (1994).
14. Abraham, W. C. & Bear, M. F. Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci.* **19**, 126–130 (1996).
15. Miller, K. D. Synaptic economics: competition and cooperation in synaptic plasticity. *Neuron* **17**, 371–374 (1996).
16. Turrigiano, G. G. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci.* **22**, 221–227 (1999).
17. Abbott, L. F. & Nelson, S. B. Synaptic plasticity: taming the beast. *Nat. Neurosci.* **3**, 1178–1183 (2001).
18. Desai, N. S., Rutherford, L. C. & Turrigiano, G. G. Plasticity in the intrinsic excitability of cortical pyramidal neurons. *Nat. Neurosci.* **2**, 515–520 (1999).
19. Davis, G. W. & Goodman, C. S. Genetic analysis of synaptic development and plasticity: homeostatic regulation of synaptic efficacy. *Curr. Opin. Neurobiol.* **8**, 149–156 (1998).
20. Davis, G. & Goodman, C. S. Synapse-specific control of synaptic efficacy at the terminals of a single neuron. *Nature* **392**, 82–86 (1998).
21. Rao, A. & Craig, A. M. Activity regulates the synaptic localization of the NMDA receptor in hippocampal neurons. *Neuron* **19**, 801–812 (1997).
22. Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C. & Nelson, S. B. Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* **391**, 892–896 (1998).
23. Lissen, D. V. *et al.* Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. *Proc. Natl. Acad. Sci. USA* **95**, 7097–7102 (1998).
24. O'Brien, R. J. *et al.* Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron* **21**, 1067–1078 (1998).
25. Watt, A. J., van Rossum, M. C. W., MacLeod, K. M., Nelson, S. B. & Turrigiano, G. G. Activity coregulates quantal AMPA and NMDA currents at neocortical synapses. *Neuron* **26**, 659–670 (2000).
26. Leslie, K. R., Nelson, S. B. & Turrigiano, G. G. Postsynaptic depolarization scales quantal amplitude in cortical pyramidal neurons. *J. Neurosci.* **21**, RC170 (2001).
27. Van Rossum, M. C. W., Bi, G. Q. & Turrigiano, G. G. Stable Hebbian learning from spike-timing dependent plasticity. *J. Neurosci.* **20**, 8812–8821.
28. Blue, M. E. & Parnavelas, J. G. The formation and maturation of synapses in the visual cortex of the rat. 1. Quantitative analysis. *J. Neurocytol.* **12**, 599–616 (1983).
29. Liu, G. & Tsien, R. W. Properties of synaptic transmission at single hippocampal synaptic boutons. *Nature* **375**, 404–408 (1995).
30. Hensch, T. K., Fagiolini, M., Mataga, N., Stryker, M. P., Baekkeskov, S. & Kash, S. F. Local GABA circuit control of experience-dependent plasticity in the developing visual cortex. *Science* **282**, 1504–1508 (1998).
31. Micheva, K. D. & Beaulieu, C. Quantitative aspects of synaptogenesis in the rat barrel field cortex with special reference to GABA circuitry. *J. Comp. Neurol.* **373**, 340–354 (1996).
32. Worley, P. F. *et al.* Constitutive expression of zif268 in neocortex is regulated by synaptic activity. *Proc. Natl. Acad. Sci. USA* **88**, 5106–5110 (1991).
33. Caleo, M., Lodovichi, C., Pizzorusso, T. & Maffei, L. Expression of the transcription factor Zif268 in the visual cortex of monocularly-deprived rats: effects of nerve growth factor. *Neuroscience* **91**, 1017–1026 (1999).
34. Caleo, M., Lodovichi, C. & Maffei, L. Effects of nerve growth factor on visual cortical plasticity require afferent electrical activity. *Eur. J. Neurosci.* **11**, 2979–2984 (1999).
35. Gordon, J. A. & Stryker, M. P. Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. *J. Neurosci.* **16**, 3274–3286 (1996).
36. Zilles, K., Wree, A., Schleicher, A. & Divac, I. The monocular and binocular subfields of the rat's primary visual cortex: a quantitative morphological approach. *J. Comp. Neurol.* **226**, 391–402 (1984).
37. Reid, S. N. M. & Juraska, J. M. The cytoarchitectonic boundaries of the monocular and binocular areas of the rat primary visual cortex. *Brain Res.* **563**, 293–296 (1991).
38. Diamond, M. E., Armstrong-James, M. & Ebner, F. F. Experience-dependent plasticity in adult rat barrel cortex. *Proc. Natl. Acad. Sci. USA* **90**, 2082–2086 (1993).
39. Colman, H., Nabekura, J. & Lichtman, J. W. Alterations in synaptic strength preceding axon withdrawal. *Science* **275**, 356–361 (1997).
40. Cynader, M. & Mitchell, D. E. Prolonged sensitivity to monocular deprivation in dark-reared cats. *J. Neurophysiol.* **43**, 1026–1054 (1980).
41. Fagiolini, M., Pizzorusso, T., Berardi, N., Domenici, L. & Maffei, L. Functional postnatal development of the rat primary visual cortex and the role of visual experience: dark-rearing and monocular deprivation. *Vis. Res.* **34**, 709–720 (1994).
42. Crair, M. C. & Malenka, R. C. A critical period for long-term potentiation at thalamocortical synapses. *Nature* **375**, 325–328 (1995).
43. Gilbert, C. D. Adult cortical dynamics. *Physiol. Rev.* **78**, 467–485 (1998).
44. Guire, E. S., Lickey, M. E. & Gordon, B. Critical period for the monocular deprivation effect in rats: assessment with sweep visually evoked potentials. *J. Neurophysiol.* **81**, 121–128 (1999).
45. Sillito, A. M. The contribution of inhibitory mechanisms to the receptive field properties of neurones in the striate cortex of the cat. *J. Physiol. (Lond.)* **250**, 305–329 (1975).
46. Changnac-Amitai, Y. & Connors, B. W. Synchronized excitation and inhibition driven by intrinsically bursting neurons in neocortex. *J. Neurophysiol.* **62**, 1149–1162 (1989).
47. Shadlen, M. N. & Newsome, W. T. Noise, neural codes and cortical organization. *Curr. Opin. Neurobiol.* **4**, 568–579 (1994).
48. Kirkwood, A. & Bear, M. F. Hebbian synapses in visual cortex. *J. Neurosci.* **14**, 1634–1645 (1994).
49. Varela, J. A., Song, S., Turrigiano, G. G. & Nelson, S. B. Differential depression at excitatory and inhibitory synapses in visual cortex. *J. Neurosci.* **19**, 4293–4304 (1999).

Critical periods for experience-dependent synaptic scaling in visual cortex

Niraj S. Desai, Robert H. Cudmore, Sacha B. Nelson and Gina G. Turrigiano

Nat. Neurosci. **5** (2002). DOI:10.1038/nn878

A corrupted electronic file led to an error in the AOP version of this paper. Parts of the axis lines and the figure labeling were missing in Fig. 5. This mistake has been corrected in the HTML version and will appear correctly in print. The PDF version available online has been appended.

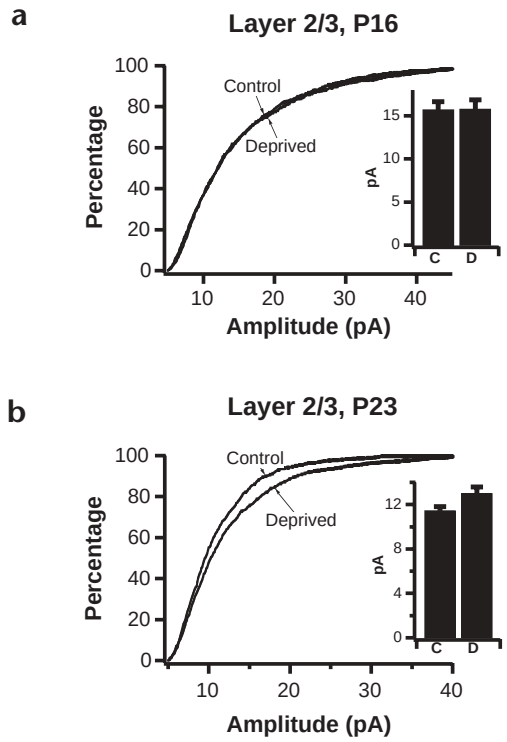

Fig. 5. Sensitivity to monocular deprivation was shifted developmentally in layer 2/3 relative to layer 4. (**a**) 48 h of deprivation had no effect on mEPSCs when begun at P14 and terminated at P16. There were no significant differences between the two conditions. (**b**) 48 h deprivation did significantly ($P < 0.05$, *t*-test) increase mEPSC amplitude when begun at P21 and terminated at P23. Cumulative histograms were constructed from 50 events/neuron.