Experience-dependent modification of synaptic plasticity in visual cortex

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In many regions of the cerebral cortex, Ca^{2+} influx through NMDA (*N*-methyl-D-aspartate) sensitive glutamate receptors (NMDA receptors) can trigger two forms of synaptic plasticity: long-term depression (LTD) and long-term potentiation (LTP)[1]. LTD is induced by low levels of postsynaptic NMDA-receptor activation, for instance in response to low-frequency stimulation, whereas LTP is induced by the stronger activation that occurs following high-frequency stimulation[2–4]. Theoretical studies have shown that the properties of synaptic LTD and LTP can account for many aspects of experience-dependent plasticity in the developing visual cortex, provided that the LTD–LTP crossover point (the modification threshold, θ_m) varies as a function of the history of cortical activity[5–7]. Here we provide direct experimental evidence that the value of θ_m depends on sensory experience. We find in visual cortex of light-deprived rats that LTP is enhanced and LTD diminished over a range of stimulation frequencies, and that these effects can be reversed by as little as two days of light exposure. Our findings support the idea that a variable synaptic-modification threshold allows synaptic weights in neural networks to achieve a stable equilibrium.

The Bienenstock–Cooper–Munro (BCM) theory was originally proposed to account for aspects of experience-dependent visual-cortical plasticity[6]. It assumes that active synapses undergo LTD or LTP depending on the level of postsynaptic response, an assumption for which there is now good evidence both in hippocampus and neocortex[2–4,8–11]. It also assumes that the value of the threshold θ_m is not fixed, but varies as a function of the previous activity of the postsynaptic cortical neuron. Thus, θ_m increases after a period of increased activity, promoting synaptic depression, and decreases after a period of decreased activity, promoting synaptic potentiation. We tested this hypothesis by studying LTD and LTP of layer III synaptic responses in slices of visual cortex prepared from light-deprived and control rats 4–6 weeks old.

The field potentials evoked in layer III by layer IV stimulation were of similar size and shape, regardless of the rearing history (light-deprived or not) of the visual cortex. There were no significant differences in the stimulation currents required to evoke half-maximal field potentials, the field-potential amplitudes at half-maximal stimulation intensity, the field-potential widths at half-amplitude, or the times to peak. In addition, a comparison of LTP induced by theta-burst stimulation (TBS) of layer IV in visual cortex of light-deprived ($n = 68$ slices from 29 rats) and control ($n = 70$ slices from 32 rats) animals revealed no significant difference (Fig. 1*a*).

Unlike the effects of TBS, however, light-deprived and control visual cortex responded differently to lower-frequency conditioning stimulation. Three 2-s trains of 20-Hz stimulation, which

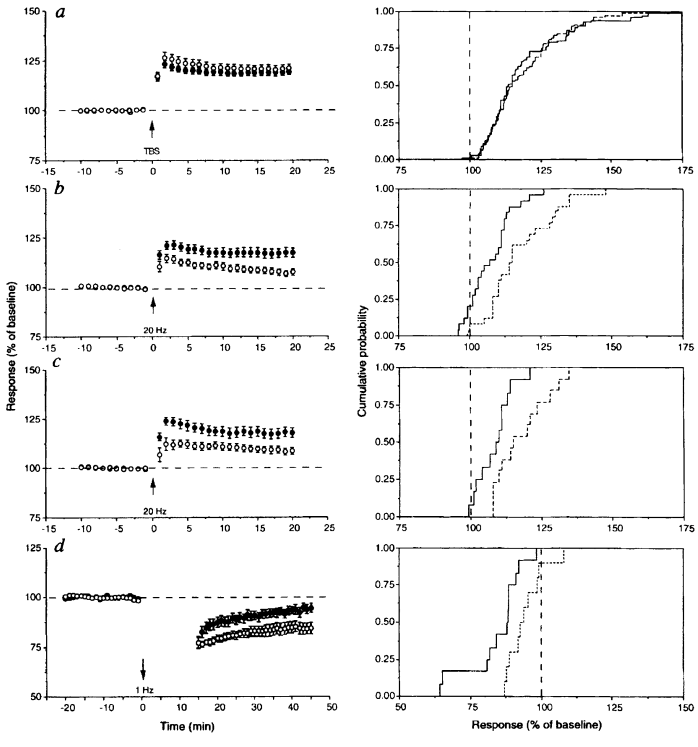

FIG. 1 Activity-dependent modification of synaptic responses in visual-cortical slices from light-deprived (filled symbols) and normal (open symbols) rats. Left, average (±s.e.m.) responses (amplitude of the maximum negative field potential in layer III normalized to average baseline value) and their modification by tetanic stimulation (arrows). Right, cumulative histograms showing the effects of conditioning stimulation in every slice from both groups (light-deprived, dotted lines; control, solid line). *a*, Effects of three trains of TBS (120 pulses total). *b*, Effects of three trains of 20-Hz stimulation (120 pulses total) in all cases studied. c, Effects of 20-Hz stimulation in experiments in which the investigator was 'blind' to the rearing history. *d*, Effects of 1-Hz stimulation for 15 min (900 pulses total). METHODS. Dark rearing and brain slice preparation were performed as described[15]. Slices of visual cortex were maintained in humidified 95% O_2, 5% CO_2, and superfused with 30 °C artificial cerebrospinal fluid (ACSF) at a rate of 1 ml min^{-1}. The ACSF was saturated with 95% O_2, 5% CO_2, and contained (in mM) NaCl, 124, KCl 5, NaH_2PO_4 1.25, $MgSO_4$ 1, $CaCl_2$ 2, $NaHCO_3$ 26, dextrose 10. A site in the middle of the cortical thickness, confirmed histologically to correspond with layer IV and upper layer V, was stimulated to evoke field potentials in layer III, as described[15,29]. The amplitude of the maximum negative field potential in layer III was used as a measure of the evoked population excitatory synaptic current. Changes in the amplitude of the maximum negative field potential reflect changes in the magnitude of a monosynaptic current sink, and correlate with changes in the initial slope of excitatory postsynaptic potentials recorded intracellularly in layer III neurons[3,29]. Baseline responses were obtained every 15 s with a stimulation intensity that yielded a half-maximal response. To study the stimulation requirements for inducing LTP, stimulus trains 2 s long were repeated every 10 s until 120 pulses had been delivered. Typically, either three 20-Hz trains or three trains of TBS were delivered; a train of TBS consists of brief bursts of stimuli delivered every 200 ms, with each burst containing 4 pulses at 100 Hz. In some experiments, six 10-Hz trains were also used. To study LTD, 900 pulses were delivered at 1 or 2 Hz. The stimulation pulse duration and intensity during conditioning stimulation were the same as for baseline stimulation. Induction of LTP and LTD with these stimulation protocols requires NMDA-receptor activation[3,29]. The group data were analysed as follows: the maximum negative field-potential amplitude data for each experiment were expressed as percentages of the preconditioning baseline average; the timescale in each experiment was converted to time from the onset of conditioning; and the time-matched, normalized data were averaged across experiments and expressed as the means (±s.e.m.). Light-deprived and control groups were compared 20 min after cessation of HFS, and 30 min after LFS, using a *t*-test. Cumulative histograms were also constructed to show the data from each slice in each experimental group, as described[15]. For the 'blind' experiments, one light-deprived and one control animal was used each day, studied simultaneously on two slice rigs. For each slice, LTP was first attempted with TBS, which previous experiments had shown to be unaffected (on average) by rearing history or age[15]. If plasticity was observed, LTP was attempted using 20-Hz trains at a distant, independent location on the same slice; if no plasticity was observed, the slice was not studied further. We used this criterion to ensure that only good-quality slices were used in the 'blind' study. The only criterion used for the non-blind study, however, was a stable baseline. The gross morphology of slices from light-deprived and control animals was indistinguishable[30].

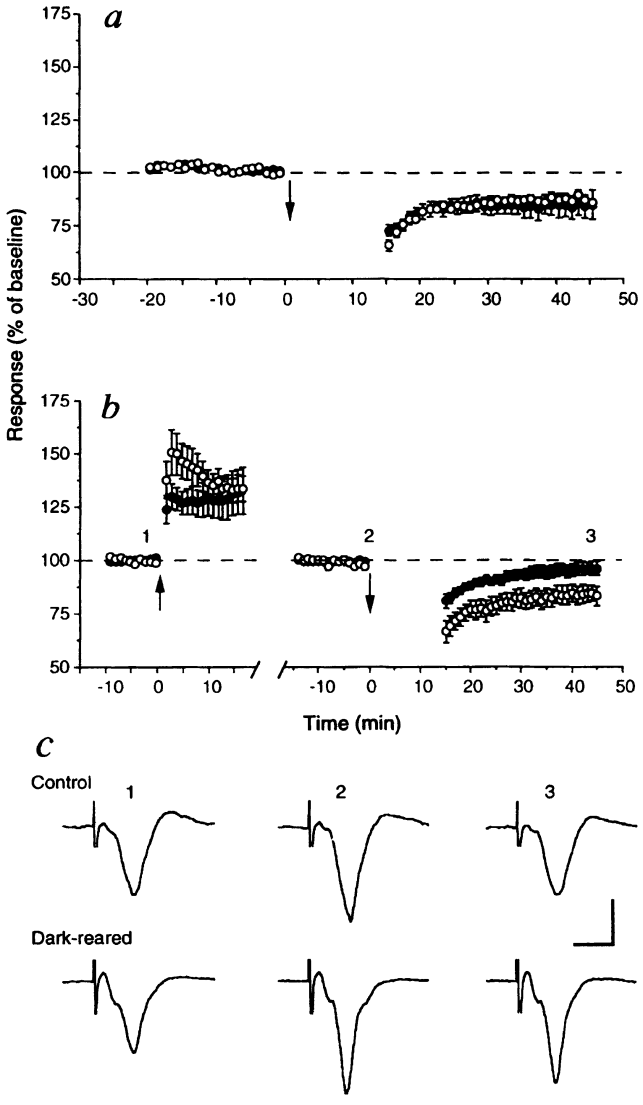

FIG. 2 *a*, Investigation of LTD induction in area CA1 of hippocampus in slices form light-deprived (filled symbols) and normal (open symbols) rats. *b*, Investigation of LTD induction following establishment of LTP in visual cortex of light-deprived and normal rats. LTP was induced with TBS, LTD with LFS (1 Hz for 15 min). Time elapsed between TBS and LFS varied from 25 to 45 min. Data shown to the right of the break in the *x*-axis were renormalized. *c*, Representative field potentials evoked in layer III by layer IV stimulation in visual cortex of light-deprived and control rats. Each trace is the average of 4 consecutive responses, collected at the times indicated by the numbers in *b*. Scale bars: 5 ms, 0.8 mV for control; 5 ms, 1.0 mV for light-deprived rats.

deliver the same number of pulses over the same time interval as TBS, produced stable LTP in slices from light-deprived animals ($117.4 \pm 2.3\%$ of baseline, $n = 26$ slices from 17 rats), but little potentiation in controls ($107.3 \pm 1.5\%$, $n = 25$ slices from 18 rats; Fig. 1*b*). The difference between light-deprived and control groups was significant at $P < 0.005$. To rule out any possible contribution of experimenter bias to the results, the effects of 20-Hz stimulation were re-examined in a series of experiments in which the investigator was 'blind' to the rearing history of the rats. These studies yielded essentially identical results ($117.8 \pm 2.5\%$, $n = 13$ slices from 9 light-deprived rats, compared to $108.6 \pm 1.8\%$, $n = 12$ slices from 9 control rats; $P < 0.01$; Fig. 1*c*). Potentiation following 10-Hz trains was also of greater magnitude in slices from light-deprived rats ($115.0 \pm 5.8\%$, $n = 5$ slices from 4 rats) compared with control ($105.0 \pm 2.7\%$, $n = 9$ slices from 8 rats; $P < 0.07$; data not shown).

Induction of homosynaptic LTD was studied by delivering 900 pulses at 1 Hz. In slices from control animals, low frequency stimulation (LFS) produced significant LTD ($84.3 \pm 2.4\%$, $n = 12$ slices from 10 rats). In light-deprived animals, however, the magnitude of depression after LFS was significantly less ($94.4 \pm 2.3\%$, $n = 9$ slices from 7 rats; $P < 0.02$; Fig. 1*d*). The difference in LTD was regionally specific; there was no significant difference in the magnitude of hippocampal LTD in slices from light-deprived ($n = 4$ from 4 rats) and control ($n = 5$ slices from 5 rats) animals (Fig. 2*a*).

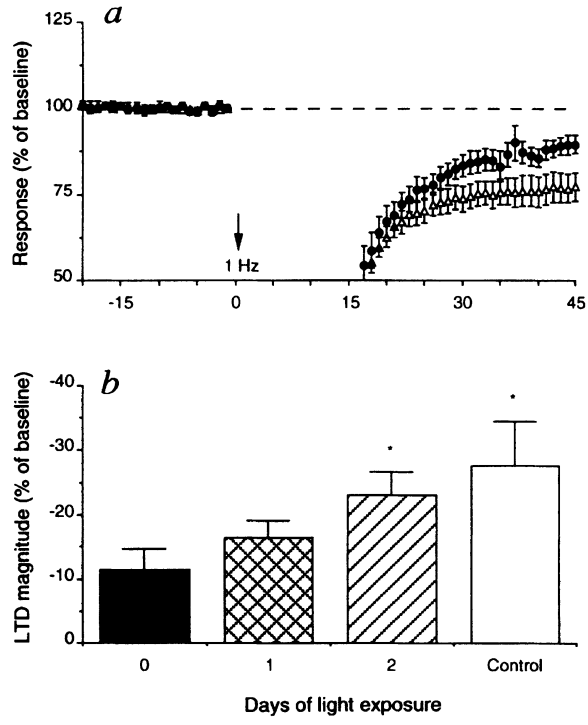

FIG. 3 Visual experience restores normal LTD to light-deprived visual cortex. *a*, The effects of 1-Hz stimulation (900 pulses) on synaptic responses in light-deprived cortex (filled symbols) and in visually deprived cortex receiving 2 days of light exposure before slice preparation (open symbols). *b*, Comparison of the magnitude of LTD 30 min after LFS in visual cortex of animals receiving various amounts of light exposure. Both the 2-day light-exposure and control groups significantly differ from light-deprived (asterisk, Tukey test, $P < 0.05$).
METHODS. Visually deprived rats 5–6 weeks old were killed 24 or 48 h after light exposure. Slices were prepared as described (Fig. 1) and, in these experiments, were maintained in a submersion chamber at 31 °C. The composition and flow rate of the ACSF was as Fig. 1. Data were collected, analysed and displayed as Fig. 1. All LFS cases were pooled, even those following application of a high-frequency tetanus, as the magnitude of LTD was independent of whether LTP had been previously induced or not (Figs 1*d* and 2*b*).

Visually evoked responses in light-deprived visual cortex are weak and unreliable[12]. The reduced LTD in slices from light-deprived animals could therefore be due to synaptic strengths already being near the 'floor' of their available dynamic range. To test this, we took advantage of the fact that the effectiveness of

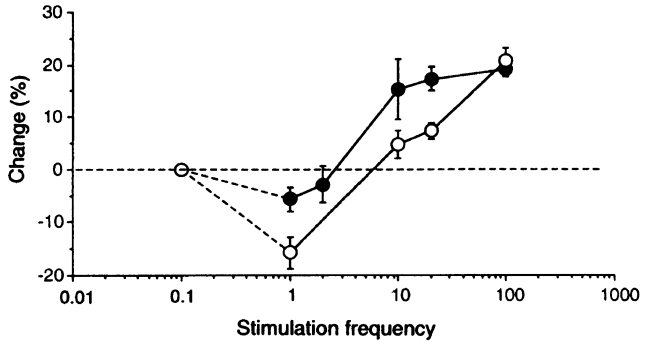

FIG. 4 Frequency-response functions derived from visual cortex of light-deprived (filled symbols) and normal (open symbols) rats. Data points for stimulation frequencies $\geqslant$ 10 Hz represent the average change (±s.e.m.) 20 min after the delivery of 120 pulses of conditioning stimulation. Data points for 1- and 2-Hz stimulation represent the average change (±s.e.m.) 30 min after delivery of 900 pulses of conditioning stimulation. The data point for 0.07 Hz is inferred as baseline stimulation once every 15 s does not appear to induce synaptic modification in light-deprived or normal cortex. All data are from slices maintained in an interface chamber (see Fig. 1).

individual synapses can be bidirectionally modified by high- and low-frequency stimulation[8,13]. Thus, in an additional series of experiments, synaptic strengths were first raised by inducing LTP with TBS, and then, after waiting for a stable baseline to be reestablished (25–45 min), 1-Hz stimulation was delivered. LFS was still significantly less effective in producing synaptic depression in visual cortex from light-deprived animals (Fig. 2*b*, *c*). The response 30 min after 1-Hz stimulation, measured as a percentage of the stable pre-LFS baseline, was 83.3 ± 4.5% in control slices (n = 6 slices from 4 rats), but only 96.0 ± 3.2% in slices from light-deprived animals (n = 6 slices from 4 rats; $P < 0.05$). Together, the data strongly suggest that the LTD-induction mechanism, or its recruitment by 1-Hz stimulation, is altered in light-deprived cortex.

Total light deprivation can slow many aspects of visual cortical development[12,14,15], so it is possible that the altered frequency-response function in light-deprived cortex simply reflects an immature state. We consider this explanation unlikely, however. Both in hippocampus[13,16,17] and in visual cortex (unpublished observations), LFS produces significantly greater LTD in neonatal animals than in young adults. In contrast, dark rearing for several weeks appears to result in diminished LTD. A more likely explanation is that the frequency-response function has shifted during postnatal development as a specific consequence of cortical inactivity.

As an additional test of the sliding-θ_m hypothesis, visually deprived rats were exposed to light for various times, and the effects of LFS were investigated in visual cortex. In these experiments, the response 30 min after 1-Hz stimulation was 88.6 ± 3.2% of baseline in slices from light-deprived animals (n = 15 slices from 7 rats). The slightly greater average LTD magnitude in this group might be due to the slices being maintained in a submersion chamber instead of the interface chamber used in the previous experiments. In any case, LTD in slices from light-deprived animals was still significantly less than in controls studied under identical conditions (72.4 ± 6.8%; n = 5 slices from 5 rats; $P < 0.02$). Remarkably, however, the magnitude of LTD in light-deprived visual cortex returned nearly to control levels after only 2 days of light exposure (Fig. 3). The response 30 min after LFS was 77.0 ± 3.6% of baseline in cortex exposed to light for 2 days (n = 11 slices from 5 rats), which is significantly greater than in light-deprived cortex ($P < 0.02$). These data are consistent with the hypothesis that θ_m 'slides' as average cortical activity increases. The time course of the observed change in LTD closely corresponds to that predicted for θ_m in modelling studies[18].

Activity-dependent regulation of NMDA-receptor-dependent synaptic plasticity can occur at many levels, ranging from changes in network inhibition to alterations in postsynaptic NMDA receptors and Ca^{2+}-binding proteins[4,19–23]. Our data do not directly address which mechanism(s) accounts for the shift of the frequency-response function in light-deprived visual cortex. It is also unclear to what extent the present findings relate to the increased susceptibility to LTD that occurs transiently following synaptic stimulation in hippocampus *in vitro*[4,24]. Indeed, in visual cortex the magnitude of LTD caused by a single episode of LFS is the same regardless of whether LTP had previously been induced (Figs 1*d* and 2*b*).

Nonetheless, at a macroscopic level, synaptic plasticity is altered by light deprivation such that LTP is promoted over LTD over a range of stimulation frequencies (Fig. 4). The consequences of such an alteration on visual-cortical development have been investigated in theoretical studies[18,25]. For example, enhanced LTP enables initially weak visual responses to undergo rapid potentiation on subsequent light exposure, a trait characteristic of visual cortex in light-deprived animals[26,27].

Our findings lend experimental support to the concept that θ_m is not fixed, but varies according to the activation history of the cortex. Thus, key assumptions of the BCM theory have now been shown to have a plausible physiological basis. Analysis and computer simulations have shown that the BCM theory can account for diverse modifications of visual-cortical receptive fields that follow various manipulations of visual experience[18,25]. Our findings therefore provide additional support for the unifying hypothesis that the mechanisms of LTD and LTP account for key aspects of experience-dependent synaptic modifications in the visual cortex[7]. More generally, our results demonstrate that the properties of activity-dependent synaptic plasticity are themselves dependent on experience. Such plasticity of synaptic plasticity, or metaplasticity[28], may be critical for information storage by synapses in the brain. □

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