independent of any change in the relative proportion of NMDA and AMPA receptors at S1 thalamocortical synapses, the properties of synaptic NMDA receptors have changed in a manner which would make it more difficult to elicit LTP.

Theoretical work[3,4,16,17] suggests that hebbian synaptic modifications are critical for the precise patterning of neuronal connectivity during development. However, experimental evidence in support of this hypothesis has been limited because it is difficult to find manipulations that block such modifications (e.g. LTP) without affecting normal neural activity (see ref. 18). We report here that, at the thalamocortical synapses which form the barrels of rodent S1, LTP is limited to the first postnatal week and essentially cannot be generated thereafter, a time period which closely matches the critical period for the topographic reorganization of VB afferents by sensory experience. Although this strong correlation does not prove causality, it provides compelling evidence that NMDA receptor-dependent LTP is critical for the rearrangement of cortical maps induced by sensory experience. Consistent with this hypothesis is earlier work which demonstrated developmental changes in LTP at unidentified synapses in visual cortex[19] and the occurrence of LTP at developing retinogeniculate synapses[20]. Unlike layer IV cells that are directly activated by thalamic afferents, the peripherally evoked responses of cells in infra- and supragranular layers can be modified by sensory perturbations after the critical period[21,22]. This may be attributable to the LTP which can be generated at intracortical connections in older animals[13].

During the course of development, the molecular composition of NMDA receptors can change[23,24] and thereby influence NMDA receptor inactivation kinetics[25,26]. Because LTP is thought to require an increase in intracellular calcium concentration beyond some critical threshold[27], changes in NMDA receptors that decrease the time course and relative contribution of NMDA receptor-mediated synaptic currents provide a mechanism for the difficulty in eliciting LTP at thalamocortical synapses in S1 after the critical period. Changes in local inhibitory circuits may also influence evoked NMDA receptor currents[28], although these cannot account for our results because inhibition was blocked when recording NMDA receptor-mediated synaptic currents. In visual cortex[15] and superior colliculus[14], the change in NMDA receptor properties appears to occur later in development than in S1, suggesting that the exact timing of critical periods in different brain areas may be dependent on the local molecular regulation of NMDA receptors. □

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Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience

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LONG-TERM potentiation (LTP) is a lasting enhancement of excitatory synaptic transmission that follows specific patterns of electrical stimulation[1]. Although the mechanism of LTP has been intensively studied, particularly in the hippocampus, its significance for normal brain function remains unproven. It has been proposed that LTP-like mechanisms may contribute to naturally occurring, experience-dependent synaptic modifications in the visual cortex[2–8]. The formation of normal binocular connections within the visual cortex requires simultaneous input from both eyes during a postnatal critical period[9–12] that can be delayed by rearing animals in complete darkness[13,14]. To explore the role of LTP in this experience-dependent maturation process, we induced LTP in visual cortical slices taken at different ages from light-reared and dark-reared rats. Susceptibility to LTP coincides with the critical period and, like the critical period, can be prolonged by rearing animals in darkness. These findings support the hypothesis that LTP reflects a normal mechanism of experience-dependent synaptic modification in the developing mammalian brain.

For many years the visual cortex has been used as a model to study experience-dependent synaptic plasticity. Modifications can be elicited *in vivo* with simple manipulations of visual experience, such as monocular deprivation, and these modifications have clear behavioural consequences, such as blindness of the deprived eye[15]. Although carnivores and primates have traditionally been used for studies of experience-dependent visual cortical development, the same principles apply to rodents[12]. In rat visual cortex, for example, visual response properties mature rapidly from postnatal day (P) 18, when the optics clear, to P45. As in other species, dark-rearing from birth postpones this maturation. Moreover, response properties in the small binocular region of rat visual cortex can be altered by monocular deprivation during a critical period. Susceptibility of binocular connections to monocular deprivation is greatest when deprivation is begun at approximately three weeks of age but declines rapidly, such that deprivation initiated at 5 weeks of age has little effect[12,16].

Various forms of activity-dependent modifications have been described by studying synaptic plasticity in the visual cortex *in vitro*[2–6,17]. The common approach has been to record intracellular or population excitatory postsynaptic potentials in layer III that are evoked by electrical stimulation at the border of the white matter and layer VI. In the absence of treatments to reduce inhibition, conditioning stimulation of the white matter fails to produce robust LTP in layer III in slices of visual cortex prepared from normal adult (⩾P35) rats[3,7,17]. However, it has been reported that LTP may be elicited using this stimulation-

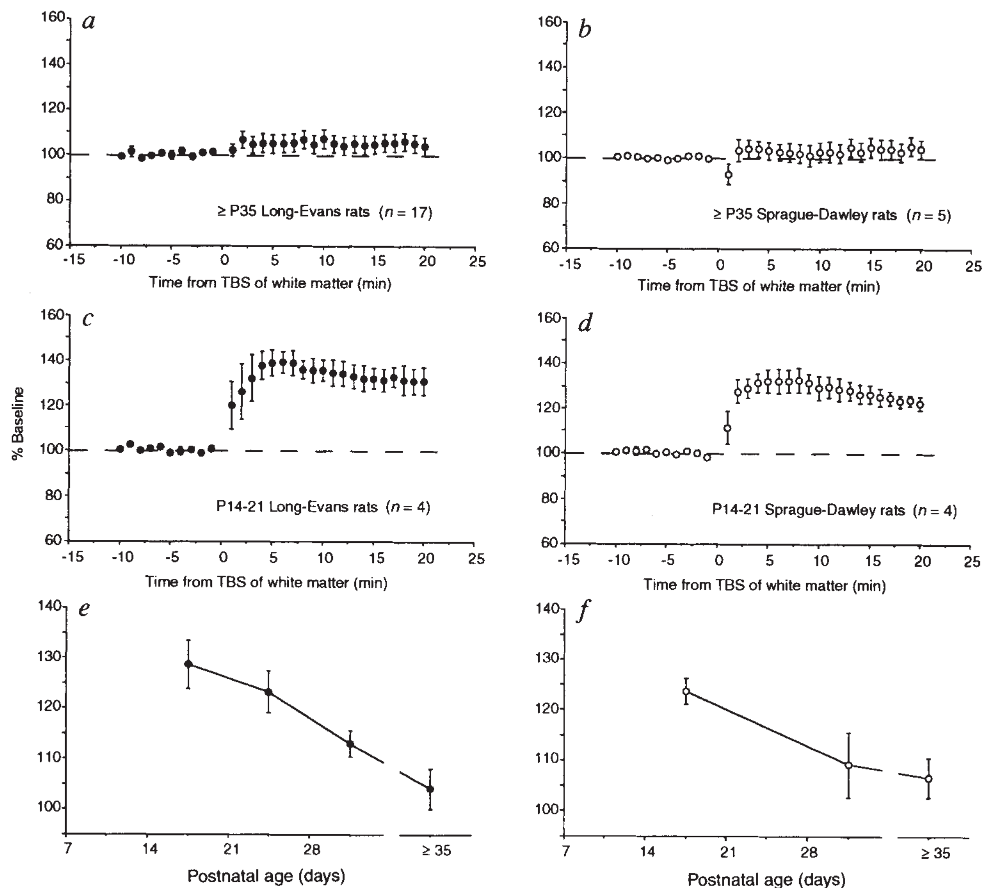

FIG. 1 Developmental decline in LTP in rat visual cortex. *a*, *b*, Average effect of TBS of the white matter on the field potential amplitude in layer III of visual cortical slices from *a*, pigmented, and *b*, albino rats aged ≥P35. *c*, *d*, Average effect of TBS of the white matter on the field potential amplitude in layer III of visual cortical slices from *c*, pigmented, and *d*, albino rats aged 2–3 weeks postnatal (P14–21). *e*, *f*, LTP evoked in layer III by TBS of white matter plotted as a function of age. Each point is the normalized field potential amplitude 20 min following TBS averaged across rats ($n \geq 4$) in each age group (by 20 min the measured LTP in visual cortex is stable for at least 1 h). For rats younger than P35, the data collected in the given postnatal week were collapsed and averaged to yield the single points shown in the graph. The range of ages in the P≥35 group was 35–49. Data in *a–d* are expressed as follows. For each slice exhibiting a stable baseline, the field potential amplitudes were calculated as percentages of the baseline average, and the timescale was converted to time from TBS. The time-matched, normalized data were averaged across slices to yield a single data set for each rat. These data were then averaged across rats to yield the values shown in the graph, expressed as the means±s.e.m.

METHODS. Slices of visual cortex (400 μm) were prepared as previously described[6]. The slices were maintained in an atmosphere of humidified 95% O_2 and 5% CO_2, and superfused with 35 °C artificial cerebrospinal fluid (ACSF) at a rate of 1 ml min^{-1}. The ACSF was saturated with 95% O_2 and 5% CO_2, and contained 124 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, 10 mM dextrose. Glass microelectrodes (1–2 MΩ) were filled with ACSF and positioned in layer III, approximately 400 μm below the pial surface. Field potentials were evoked with pulses of 10–100 μA amplitude lasting 0.2 ms delivered with a bipolar concentric stimulating electrode (outside diameter 200 μm) placed either just below the border of the white-matter and layer VI, or in the middle of the cortical thickness. The amplitude of the maximum negative field potential in layer III was used as a measure of the evoked population excitatory synaptic current. As discussed in more detail elsewhere[7], changes in the amplitude of the maximum negative field potential reflect changes in the magnitude of a synaptic current sink, and correlate with changes in the initial slope of excitatory postsynaptic potentials recorded intracellularly in layer III neurons. In every experiment, a full input–output curve was generated, and baseline responses were obtained at 0.07–0.03 Hz with a stimulation intensity that yielded a half-maximal response. To induce LTP, 3 episodes of TBS were delivered at 0.1 Hz. TBS consisted of 10 to 13 stimulus trains delivered at 5–7 Hz; each train consisted of 4 pulses at 100 Hz.

recording configuration in immature animals[18-20], suggesting that visual cortical LTP, like experience-dependent plasticity, is more robust during a critical period. We investigated these observations, and then extended the analysis to animals that had been reared in complete darkness.

The results of the developmental study, performed using slices of visual cortex from both Long-Evans pigmented rats and Sprague-Dawley albino rats, are summarized in Fig. 1. In agreement with previous findings[7], theta burst stimulation (TBS) of the white matter did not produce substantial LTP in layer III of visual cortex from rats older than P35 (Fig. 1*a*, *b*). In contrast, TBS produced a robust synaptic enhancement in slices from animals in their third postnatal week (P14–21) (Fig. 1*c*, *d*). Figure 2*a* shows an example of LTP in one experiment. The developmental decline in LTP, expressed as the percentage change from baseline 20 min after TBS, is plotted for both strains of rat in Fig. 1*e*, *f*. This change in LTP from 3 to 5 weeks of age closely parallels the developmental decline in the effects of monocular deprivation on binocular connections *in vivo*[12].

To investigate whether the developmental decline in LTP is altered by the rearing history of the animals, litters of rats were placed in a dark room within a few days of birth until approximately 5 weeks of age. Slices from age-matched light-reared and dark-reared animals were studied simultaneously on two separate slice rigs. The experiments were performed in two series, depending on whether the experimenters knew the rearing history of the animals ($n = 19$ rats) or not ($n = 12$ rats). The results were virtually identical: the developmental decline in LTP evoked from the white matter was prevented by dark-rearing the animals.

Figure 3*a* shows the results from the 'blind' series of experiments. TBS of the white matter produced little change in the light-reared animals, but the age-matched dark-reared animals showed robust LTP (see Fig. 2*b*). The pooled data from both series of experiments are shown in Fig. 3*b*, and the data from each individual slice appear in a cumulative histogram[21] in Fig. 3*c*. The light-reared and dark-reared distributions are significantly different at $P < 0.02$ (Kolmogorov-Smirnov test). We observed no obvious differences, other than LTP, in the extracellular synaptic responses of slices in dark-reared animals compared with light-reared animals (no significant differences in field potential amplitude at half-maximal stimulation intensity, width at half-amplitude, or time to peak).

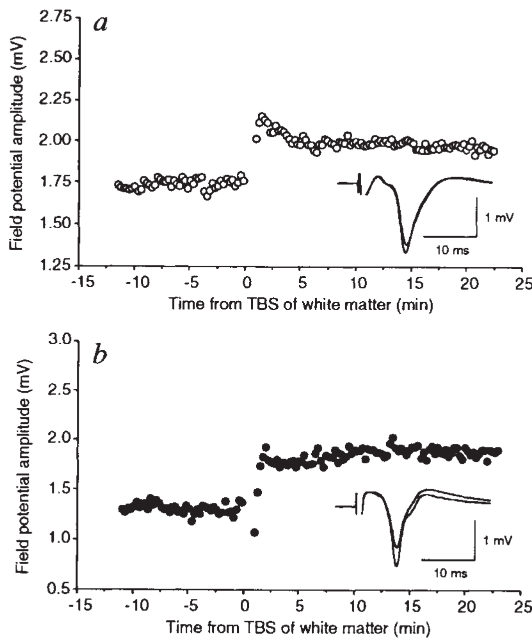

FIG. 2 Examples of LTP of the field potential evoked by white matter stimulation in visual cortical slices from *a*, a normal P16 rat, and *b*, a dark-reared P34 rat.

We conclude that white-matter-evoked LTP in layer III, and experience-dependent synaptic plasticity, are co-regulated in visual cortex by age and visual experience. Although it is not known precisely what is regulated, the results of additional experiments help to narrow the possibilities. We have previously shown that stimulation of the middle of cortex, corresponding roughly to layer IV, could yield LTP reliably in layer III of slices from adult visual cortex[7,17]. We therefore investigated whether there are quantitative effects of age and dark-rearing on layer-IV-evoked LTP in layer III. In striking contrast to the effect of white-matter stimulation, however, we found that TBS of the middle cortical layers yields LTP of comparable magnitude and probability, regardless of age (in the range examined) or rearing history (Fig. 4). These data suggest that the developmental regulation of plasticity does not occur at the level of the biochemical machinery of the layer III neurons expressing the LTP.

These forms of LTP in visual cortex require *N*-methyl-D-aspartate (NMDA) receptor activation[7,20], and there is evidence for a developmental decline in NMDA receptor function in layer IV of visual cortex, which is prevented by dark-rearing[22,23]. Because layer IV provides input to layer III, the LTP measured in layer III as a result of white-matter stimulation may be simply a reflection of a synaptic modification that actually occurs in layer IV neurons. However, dark-rearing has been shown to decrease, rather than increase, LTP of layer IV synaptic current sinks[24].

More work will be required to determine how LTP is developmentally regulated in visual cortex, but robust white-matter-

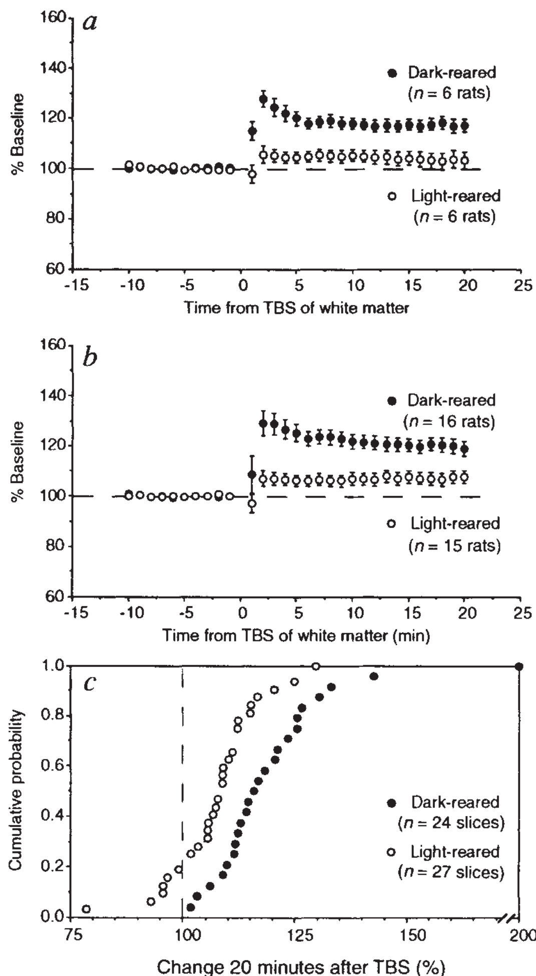

FIG. 3 Effect of dark-rearing on LTP evoked from the white matter in 4–6-week-old rat visual cortex. *a*, Comparison of LTP in visual cortical slices from 4–5-week-old rats reared in complete darkness with LTP in rats reared in a normal lighted environment. In this series, the experimenters were 'blind' to the rearing history of the animals until all analyses had been performed. Data are expressed as in Fig. 1*a–d*; the difference between groups at 20 min post-TBS is significant at $P<0.02$ (*t*-test). *b*, The same as *a*, except that additional animals, not studied 'blind', are included in the averages. *c*, Cumulative histogram using all slices from dark-reared and light-reared animals. These distributions are significantly different at $P<0.02$ (Kolmogorov–Smirnov test).

METHODS. Pregnant albino rats were purchased from Charles River laboratories, always timed so that two would give birth on approximately the same day. Within a week of giving birth, a mother and her pups were placed in a light-tight room while another was kept in a normal lighted laboratory environment (12 h light/dark cycle). To provide care for animals in the dark, or to remove them for experiments, they were illuminated with dim infrared light and viewed using infrared vision equipment. In the 'blind' series of experiments, one dark-reared and one light-reared animal was used each day, studied simultaneously on two slice rigs. For each slice, LTP was first attempted with middle layer stimulation, which other experiments had shown to be unaffected (on average) by rearing history or age (Fig. 4). If plasticity was observed then LTP was attempted from the white matter at a different location on the same slice; if no plasticity was observed, the slice was not studied further. We used this criterion to ensure that only high-quality slices were used in the 'blind' study. The only criterion used for the non-blind study, however, was a stable baseline. To construct cumulative histograms, the percentage change in the response 20 min after TBS was calculated for each slice, and these values were rank-ordered to show the fraction of cases showing changes of various magnitudes.

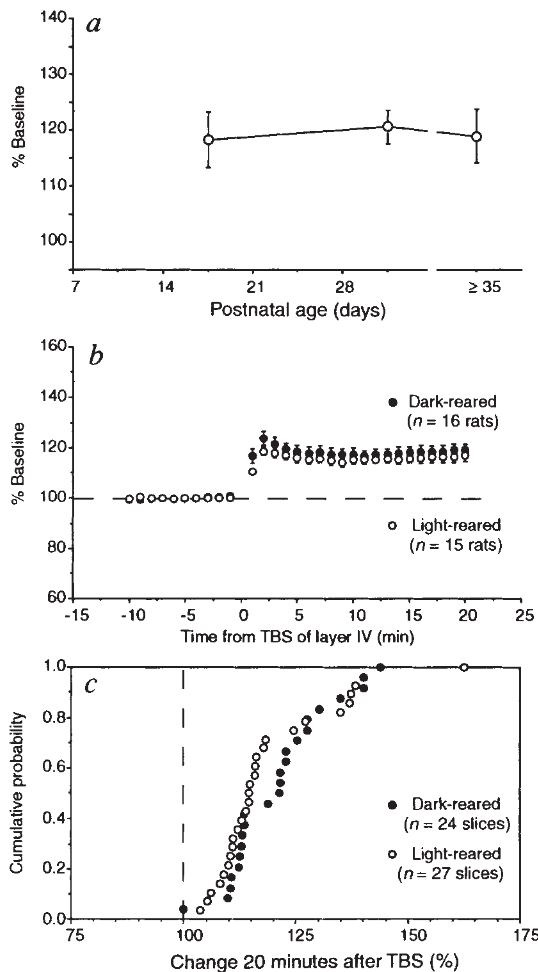

FIG. 4 Effect of age and visual experience on LTP evoked from the middle cortical layers. *a*, LTP evoked in layer III by TBS of the middle cortical layers plotted as a function of age. Data are calculated and expressed as in Fig. 1e, *f*. *b*, Comparison of LTP in visual cortical slices from 4–5-week-old rats reared in complete darkness with LTP in rats reared in a normal lighted environment. *c*, Cumulative histogram using all slices from age-matched dark-reared and light-reared animals. These distributions are not significantly different from one another.

evoked LTP can be restored in slices from adult animals if $GABA_A$-receptor-mediated inhibition is reduced[3,7]. Inhibitory circuits may contribute to a 'plasticity gate' which controls the activity of middle-layer cells that must be recruited to induce LTP in layer III[7]. Our observations suggest that the gate is open early in postnatal life and closes during the critical period under the influence of experience. Closure of the hypothetical plasticity gate could result from maturation of excitatory or inhibitory synapses in the middle cortical layers; indeed, there is evidence for changes due to development and experience in both types of transmission[25–30].

Our experiments have shown that activity-dependent synaptic plasticity *in vitro*, like experience-dependent synaptic plasticity *in vivo*, is regulated by age and previous sensory experience. These findings are significant for three reasons. First, they support the relevance of LTP for understanding naturally occurring synaptic modifications in the mammalian brain. Second, they indicate that the stimulation history of the synapses *in vivo* leaves a trace that can be detected *in vitro*. Third, they suggest that the study of LTP in visual cortex may be useful for understanding the factors that regulate experience-dependent plasticity during critical periods of development. □

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Soluble antigen can cause enhanced apoptosis of germinal-centre B cells

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GERMINAL centres are dynamic microenvironments of B-lymphocyte differentiation, which develop in secondary lymphoid tissues during immune responses[1–3]. Within germinal centres, activated B lymphocytes proliferate and point mutations are rapidly introduced into the genes encoding their immunoglobulin receptors[4–10]. As a result, new specificities of B cells are created, including those with a heightened capacity to bind the immunizing antigen[4–11]. Immunoglobulin gene mutation can also lead to reactivity to self antigens[12–14]. It has been suggested that any newly formed self-reactive B cells are eliminated within the germinal centre in order to avoid autoimmunity[15,16]. Here we present evidence that antigen-specific, high-affinity, germinal-centre B cells are rapidly killed by apoptosis *in situ* when they encounter soluble antigen. The effect seems to act directly on the B cells, rather than through helper T cells. Furthermore, the apoptosis is unique to germinal-centre cells, and is only incompletely impeded by constitutive expression of the proto-oncogene *bcl-2*. This phenomenon may reflect clonal deletion of self-reactive B cells within germinal centres.

A mouse model was developed to simulate a situation in which hypermutating, germinal centre B cells would encounter soluble self-antigen, not complexed to antibody on the surface of follicu-