Long-Term Depression at Thalamocortical Synapses in Developing Rat Somatosensory Cortex

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Summary

Sensory experience during an early critical period guides the development of thalamocortical circuits in many cortical areas. This process has been hypothesized to involve long-term potentiation (LTP) and long-term depression (LTD) at thalamocortical synapses. Here, we show that thalamocortical synapses in rat barrel cortex can express LTD, and that LTD is most readily induced during a developmental period that is similar to the critical period for thalamocortical plasticity in vivo. Thalamocortical LTD is homosynaptic and dependent on activation of N-methyl-D-aspartate (NMDA) receptors. The age-related decline of LTD is not due to changes in inhibition nor to changes in NMDA receptor voltage dependence. Minimal stimulation experiments indicate that, unlike thalamocortical LTP, thalamocortical LTD is not associated with a significant change in failure rate. The existence of LTD and its developmental time course suggest that LTD, like LTP, may contribute to the refinement of thalamocortical inputs in vivo.

Introduction

Sensory experience during early postnatal development refines cortical circuitry and shapes the response properties of cortical neurons, transforming immature circuits into appropriately organized connections that mediate adult brain function (Wiesel and Hubel, 1963; Katz and Shatz, 1996). In many cortical areas, the initial stages of this process involve plasticity of thalamocortical synapses (Hubel et al., 1977; Fox and Zahs, 1994). A powerful system for studying thalamocortical synaptic plasticity is the whisker representation (barrel field) of the rat’s primary somatosensory cortex (S1), where abnormal whisker experience early in life causes changes in both the spatial patterning of thalamocortical afferents (Woolsey and Wann, 1976; Fox, 1992) and in thalamocortically mediated responses of layer IV neurons to whisker input (Fox, 1992). Thalamocortical synaptic plasticity in rat S1 exhibits a critical period in which the capacity for plasticity following experimental alterations in whisker use is maximal at postnatal day 0 (P0) and declines to a low level by P4–P6 (Fox, 1992; Schlaggar et al., 1993; Glazewski and Fox, 1996). Unlike the well-described critical period for plasticity at thalamocortical synapses in visual cortex (Hubel and Wiesel, 1970; Daw et al., 1992), however, the critical period for thalamocortical synapses in S1 is not absolute, since some residual plasticity appears to persist at these synapses into adulthood (Armstrong-James et al., 1994).

The synaptic basis for experience-dependent plasticity at thalamocortical synapses is not known. However, it is widely hypothesized that the underlying mechanism depends on correlated pre- and postsynaptic activity (Hebb, 1949; Stent, 1973; Shatz, 1990) and involves long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy (Bear et al., 1987; Miller et al., 1989; Fregnac et al., 1994; Fox, 1995; Singer, 1995). Indeed, homosynaptic LTD of thalamocortical synapses has been demonstrated in S1 cortical slices maintained in vitro (Crair and Malenka, 1995; Isaac et al., 1997) and has a developmental profile that is similar to the critical period for experience-dependent plasticity at thalamocortical synapses in vivo (Crair and Malenka, 1995; Fox, 1995). A similar correlation has been reported in the visual cortex, where LTP of synapses on layer III neurons is also restricted to a developmental period coinciding with the critical period for ocular dominance plasticity (Kirkwood et al., 1995). These observations suggest that LTD of thalamocortical synapses may contribute to experience-dependent plasticity in vivo.

A number of experimental and theoretical observations suggest that LTD may also play an important role in experience-dependent development and plasticity of thalamocortical responses. For example, the rapid and dramatic reduction of neuronal responses to visual stimuli that occurs following monocular deprivation or reverse occlusion (Blakemore and Van Sluyters, 1974; Mioche and Singer, 1989) is readily explained by a reduction in the efficacy of thalamocortical synapses. Consistent with this idea, computational models incorporating synaptic depression accurately reproduce many aspects of the development of visual cortical response properties (Bienenstock et al., 1982; Bear et al., 1987; Miller et al., 1989). It has also been suggested that LTD may be required to prevent the saturation of synaptic efficacy that might result if only LTP occurred during development (Stent, 1973; Fregnac et al., 1994). If LTD does play a role in cortical development, one prominent possibility is that it leads to the eventual withdrawal of inappropriately targeted synapses, whereas LTP would lead to the stabilization of appropriately targeted ones (Goodman and Shatz, 1993).

LTD has been widely observed at cortical synapses (Artola et al., 1990; Tsumoto, 1992; Kirkwood and Bear, 1994; Castro-Alamancos et al., 1995; Hess and Donoghue, 1996), including synapses onto layer IV neurons in visual cortex (Dudek and Friedlander, 1996). However, none of these studies examined thalamocortical responses in isolation; instead, preparations were used...
Figure 1. Representative Example of Thalamocortical LTD
(A) Plot of peak EPSC amplitude for each sweep (stimulation rate, 0.1 Hz) as a function of time. Dashed line, mean amplitude for the 50 stimuli immediately preceding pairing.
(B) Series resistance during this recording.
(C) Mean EPSC for the 50 stimuli immediately preceding pairing (Baseline) and the 50 stimuli starting 5 min after pairing (5±13 min after pairing). The magnitude of LTD for this cell was 24.5%.

in which heterogeneous populations of synapses were activated both orthodromically and antidromically. Here, we have investigated the ability to generate LTD at thalamocortical synapses in developing rat barrel cortex using a slice preparation that allows selective stimulation and study of thalamocortical synapses (Agmon and Connors, 1991; Crair and Malenka, 1995). After establishing that LTD can be elicited and is developmentally regulated, we go on to characterize some of its basic properties and mechanisms.

Results

Induction of LTD and Developmental Time Course
Thalamocortical synaptic currents were measured by holding layer IV cells at −70 to −80 mV while stimulating the ventrobasal (VB) nucleus of the thalamus at a slow rate (0.1–0.33 Hz). Because higher stimulation rates can cause fiber failure, particularly in immature tissue, we did not attempt to use prolonged low frequency (e.g., 1 Hz) stimulation to elicit LTD as is typically done when studying LTD in hippocampal (Dudek and Bear, 1992; Mulkey and Malenka, 1992) and cortical (Kirkwood and Bear, 1994; Dudek and Friedlander, 1996) slices. Instead, LTD was induced by raising the holding potential to −50 mV for 100 consecutive stimuli without changing the stimulation rate, a protocol termed “pairing” and one which has been used to elicit LTD in hippocampal CA1 neurons (Selig et al., 1995; Goda and Stevens, 1996). Figure 1 shows an example of a cell in which this protocol caused a decrease of 25% in the mean amplitude of the thalamocortical excitatory postsynaptic current (EPSC) (79 pA during the baseline; 59 pA following pairing). This depression was stable for the duration of the recording; that is, LTD was generated.

LTD at thalamocortical synapses was observed most reliably in slices prepared from younger animals (Figure 2). The relationship between the age of the animal from which slices were prepared and the magnitude of LTD is shown in Figure 2A. In all cells in which LTD was elicited, it was stable for the length of recording (up to 40 min after pairing). At P4–P5, LTD was observed with high probability (9 of 11 cells, 82%), while at P10–P12 LTD was observed in only 4 of 14 cells (29%). Correspondingly, the mean reduction in EPSC amplitude following pairing declined significantly with age (ANOVA, p < 0.02). The greatest mean LTD was observed at P4–P5 (−28.2% ± 4%, n = 11). At P10 and older, on average, little or no LTD was apparent (−3.8% ± 5%,
The dramatic difference in the mean effect of pairing on synaptic strength in P4±P5 versus P10±P12 animals is shown in Figure 2C. These data indicate that LTD induced using this pairing protocol exhibits a developmental profile that is similar to that observed for LTP at this synapse (Crair and Malenka, 1995).

NMDA Receptor Dependence

At many excitatory synapses in the mammalian brain, LTD induction requires activation of N-methyl-D-aspartate receptors (NMDARs) (Dudek and Bear, 1992; Mulkey and Malenka, 1992; Kirkwood et al., 1993; Kirkwood and Bear, 1994; Castro-Alamancos et al., 1995; Dudek and Friedlander, 1996). However, NMDAR-independent forms of LTD have been observed in cortex and hippocampus (Kato, 1993; Bolshakov and Siegelbaum, 1994; Oliet et al., 1997), and these often require the activation of metabotropic glutamate receptors (mGluRs). To determine which receptor is required for induction of thalamocortical LTD, we attempted to induce LTD in P4±P5 slices in the presence of the NMDAR antagonist D-APV (50 μM). We found that induction of LTD by pairing was completely blocked by APV (n = 10; Figure 3A). In contrast, the broad spectrum mGluR antagonist MCPG (1 mM) had no effect on LTD induction (n = 5; Figure 3B). In four cells, we were able to wash out APV after LTD induction had been blocked; in each of these cells, subsequent pairing elicited significant LTD (Figure 3C). Together, these data demonstrate that induction of thalamocortical LTD requires activation of NMDARs but not mGluRs.

Basis for Developmental Time Course

Because induction of LTD depends on activation of NMDA receptors, we considered the possibility that cells in older slices may fail to exhibit LTD because disynaptic inhibitory currents, which become detectable in many layer IV neurons around P8 (Agmon and O'Dowd, 1992), might somehow interfere with the activation of NMDARs during the pairing protocol. Inhibition has been shown to prevent LTD induction in visual cortical layer IV cells that are not voltage clamped, probably by hyperpolarizing the cell and thus reducing NMDAR currents by virtue of their voltage sensitivity (Dudek and Friedlander, 1996). In our experiments, cells are electrotonically compact (Segev et al., 1995) and are voltage clamped during pairing, making this mechanism unlikely. However, it is possible that inhibitory postsynaptic currents (IPSCs) may lower membrane potential in inadequately voltage-clamped distal dendrites, thereby reducing NMDA receptor currents at some thalamocortical synapses. We tested this possibility explicitly by including 100 μM picROTOXIN in the external solution to block GABA_A currents in some experiments (cells marked with triangles in Figure 2A). PicROTOXIN induced sporadic paroxysmal discharges in the cortex at a low frequency but did not generally interfere with the measurement of thalamocortical EPSCs. PicROTOXIN applied to slices at P9–P10 failed to rescue LTD induction (n = 5 cells; mean LTD = −5.2% ± 7% with picROTOXIN, −15% ± 7% without picROTOXIN [n = 8]; t test, p > 0.05). Nor did picROTOXIN affect the magnitude of LTD observed at P4–P5 (n = 6 cells; −23.1% ± 7% with picROTOXIN, 28.2% ± 4% in normal solution [n = 11]; t test, p > 0.05). These data indicate that our failure to induce LTD reliably at older ages was not due to the presence of GABA_A conductances.

We also tested whether a developmental change in the properties of NMDAR-mediated synaptic currents might be responsible for the developmental profile for LTD. Previously, it was shown that the time course of NMDAR-mediated EPSCs decreases dramatically during early postnatal development at this synapse (Crair and Malenka, 1995) as at several others (e.g., Hestrin, 1992; Carmignoto and Vicini, 1992). This observation suggested that the reduced capacity for plasticity after P9 might reflect a general reduction in the magnitude of NMDAR currents. An alternative explanation for the relative ease of inducing LTD in neonatal animals is that,
as has been suggested for other brain regions (Ben-Ari et al., 1988; Kato and Yoshimura, 1993; Burgard and Hablitz, 1994), immature NMDARs may be relatively insensitive to Mg2+ and therefore less voltage dependent than NMDARs in older animals. If this were true in somatosensory cortex, pairing at −50 mV might generate an NMDA receptor current that was large enough to trigger LTD in a P4 neuron, whereas the same pairing in a P10 neuron might generate a smaller current that was insufficient to trigger LTD. Such a mechanism has been suggested to regulate the induction of LTP in slices of visual cortex (Kato and Yoshimura, 1993). To test this possibility, we measured the I-V relationship for the NMDAR-mediated component of thalamocortical EPSCs in slices from rats of different ages. NMDA EPSCs were isolated by bath application of 10 μM CNQX, 100 μM picROTOXIN, and 100 μM saclofen and exhibited a mean reversal potential of +3 mV and a region of negative slope conductance from about −80 to −20 mV. Both of these properties are characteristic of NMDAR currents. When the conductance was calculated as a function of membrane potential, it was evident that the voltage dependence of NMDA EPSCs was essentially identical in slices from P4–P5, P7–P8, and P11–P14 animals (Figure 4). Thus, changes in voltage dependence cannot account for the difficulty in eliciting LTD at older ages. Instead, the relative loss of plasticity observed after P9 may reflect the developmental reduction in the time course of NMDAR currents that has been observed at this synapse (Crair and Malenka, 1995) or, alternatively, some other change occurring downstream of calcium entry.

Input Specificity

To further characterize thalamocortical LTD, we tested whether its expression was specific to the synapses that had undergone the pairing protocol, or whether instead LTD was generalized to all synapses on the layer IV neuron. In P4–P5 slices, we performed a two-pathway experiment in which we measured, in the same neuron, both thalamocortical EPSCs and EPSCs evoked by stimulation of a cortical site at the layer III/IV boundary, 100–150 μm lateral of the recorded cell. These latter EPSCs were presumably mediated by horizontal intracortical connections. Horizontal and thalamic inputs were stimulated alternately during the baseline and postpairing periods. During pairing, horizontal stimulation was ceased, and cells were held at −50 mV while the thalamic input was stimulated at the normal (baseline) rate. The results of these experiments are shown in Figure 5. Pairing of thalamocortical inputs produced LTD of the thalamocortical EPSC (−32.3% ± 7%, n = 5), while no significant reduction was observed in the horizontal EPSC (−0.1% ± 7%). These results indicate that thalamocortical LTD is homosynaptic; that is, it is expressed at the synapses at which it is induced and not at other synapses on the same postsynaptic neuron. We did not determine whether horizontal inputs were capable of pairing-induced LTD. The sparseness of the thalamocortical connectivity in our slices prevented us from separately stimulating two thalamocortical inputs onto a single layer IV neuron, so we could not test whether LTD was specific to distinct groups of synapses within the thalamocortical projection.

Minimal Stimulation Experiments

In minimal stimulation experiments, long-term potentiation (LTP) at the thalamocortical synapse in barrel cortex has been shown to be associated with both a decrease in the failure rate and an increase in the mean amplitude of successes (Isaac et al., 1997), which is termed potency (Stevens and Wang, 1994). Because LTD is thought to represent a reversal of the expression mechanisms of LTP (Dudek and Bear, 1993; Mulkey et al., 1993; Bear and Malenka, 1994), we predicted that LTD should involve an increase in failure rate and a decrease in potency. However, when we used minimal stimulation
Figure 5. Thalamocortical LTD Is Pathway Specific

(A) Mean EPSCs from a representative experiment in which stimulation of the thalamocortical input was alternated with stimulation of a horizontal, intracortical pathway. After the control period, the thalamocortical input was paired with depolarization to −50 mV. Stimulation of the horizontal intracortical input was suspended during the pairing protocol. After selective pairing of the thalamic input in this manner, LTD was observed for the thalamocortical EPSC (top row). No significant reduction in the EPSC elicited by horizontal intracortical stimulation was observed (bottom row).

(B) Summary of two-pathway experiments in which LTD was induced successfully in the thalamocortical pathway (n = 5).

Figure 6. Representative Example of Thalamocortical LTD Using Minimal Stimulation

(A) EPSC amplitudes for individual sweeps (stimulation rate, 0.33 Hz) for a P4 neuron. Open circles represent visually identified failures. Note that after pairing, the proportion of failures is not increased, while the size of the successes (closed circles) is decreased.

(B) Mean EPSC during the baseline period and eight consecutive individual sweeps.

(C) Amplitude histograms (bin size, 1 pA) for baseline (thin, n = 97 sweeps) and postpairing (thick, n = 462 sweeps) periods.

(D) Mean of all successes during baseline and postpairing periods.

By a detectable change in failure rate (plotted as [1 − failure rate], the success rate). Instead, LTD in these cells was accompanied by a decrease in potency that completely accounted for the magnitude of LTD (Figure 7B). In the other two cells, each of which exhibited an unusually large amount of LTD, both the success rate and the potency decreased following pairing. This same result (the expression of LTD primarily by a change in potency, without a change in failure rate) was observed using several different induction protocols to induce thalamocortical LTD: either the standard protocol of 100 stimuli while pairing at −50 mV (Figures 7A and 7B, closed circles; n = 14), an extended protocol using 200 stimuli (circles with crosses; n = 3), or a depotentiation protocol using 100 stimuli while pairing at −50 mV in cells in which LTP had been induced previously by pairing at 0 to −10 mV (triangles, n = 4; see below).

To verify that the expression mechanisms for LTD...
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and LTP at this synapse were significantly different, we compared quantitatively the changes in success rate that occurred in cells in which LTD had been induced versus cells in which LTP had been induced (Figure 7C). For this comparison, we selected cells from this study and from Isaac et al. (1997) in which the magnitude of LTD or LTP that had been induced was similar. The magnitude of LTP was defined as (mean EPSC amplitude after pairing)/mean amplitude before pairing), and the magnitude of LTD was defined as 1/(mean EPSC amplitude after pairing/mean amplitude before pairing). Eleven cells exhibited an LTD magnitude in the range 1.2–1.8, which corresponds to a decrease in EPSC amplitude of 17%–44%. Nine cells exhibited LTP magnitudes in the same range (four of these cells were from the present study and five from Isaac et al. [1997]). Figure 7C directly compares these cells and demonstrates that for an equivalent magnitude change in synaptic strength LTP was associated with a change in success rate, whereas LTD was not.

This difference in the expression of LTP and LTD was confirmed by experiments in which LTP and LTD (depotentiation) were induced sequentially in the same cells (Figures 7A and 7B, triangles; n = 4). In these cells, LTP was induced following a baseline period by pairing at −250 mV. Subsequently, LTD (depotentiation) was induced by pairing at −50 mV. As expected (Isaac et al., 1997), LTP (mean magnitude, 190% ± 40% of baseline) was consistently associated with both an increase in potency and a decrease in failure rate (39% ± 5% failure rate during baseline versus 19% ± 4% following LTP induction; paired t test, p < 0.02). In contrast, subsequent induction of LTD (depotentiation) (mean magnitude, −21% ± 2%) did not further change the failure rate (22% ± 5%; paired t test, p > 0.18). These results confirm that at the developing thalamocortical synapse, LTP is accompanied by changes in both potency and failure rate, whereas LTD is associated with a change in potency but no change in failure rate in the great majority of cells.

Discussion

Using the thalamocortical slice preparation (Agmon and Connors, 1991), it is possible to characterize the properties of thalamocortical synapses in developing and adult somatosensory cortex. Prior to the present study, it was known that thalamocortical synaptic transmission involves the activation of both NMDAR- and AMPAR-mediated currents (Agmon and Connors, 1991; Crair and Malenka, 1995; Gil and Amitai, 1996; Isaac et al., 1997), and that thalamocortical synapses are capable of LTP during a restricted developmental period (Crair and Malenka, 1995). In this paper, we have shown that identified changes in success rate. During LTD of the same magnitude, no significant success rate changes were observed (one-sample t test for difference from 1.0 mean, p > 0.50). The success rate changes observed for LTP and LTD were significantly different (t test, SRR for LTP versus 1/SRR for LTD, p < 0.0001).

(Left) Change in success rate for LTD (n = 11 cells) versus LTP (n = 9 different cells) for cells in which the magnitude of LTD or LTP was in the range 1.20–1.80. This range was chosen because it included the greatest number of cells exhibiting either LTD or LTP. (Left) Change in success rate for LTD is plotted as 1/SRR, so that for both LTD and LTP, changes in success rate that contribute to the EPSC amplitude change are plotted upwards. During LTP, cells showed significant changes in success rate. During LTD of the same magnitude, no significant success rate changes were observed (one-sample t test for difference from 1.0 mean, p > 0.50). The success rate changes observed for LTP and LTD were significantly different (t test, SRR for LTP versus 1/SRR for LTD, p < 0.0001).

(Right) Magnitude of LTD or LTP for these cells. The magnitude of EPSC amplitude change was the same for LTD and LTP cell populations (t test, p > 0.49).
thalamocortical synapses are also capable of exhibiting LTD. Thalamocortical LTD is dependent on activation of NMDARs, is homosynaptic, and exhibits a developmental time course that parallels that for LTP and experience-dependent plasticity at the thalamocortical synapse in vivo. The existence of LTD at thalamocortical synapses lends support to theoretical models of thalamocortical development that explicitly (Stent, 1973; Bienenstock et al., 1982) or implicitly (Miller et al., 1989) postulate the existence of thalamocortical LTD.

Several observations suggest that the synaptic currents we measured in layer IV neurons following stimulation of the VB nucleus of the thalamus are due to the activation of thalamocortical synapses, as opposed to synapses on recurrent collaterals of corticthalamic layer VI neurons activated by antidromic stimulation of their axons in the thalamus. First, the cell bodies of cortically projecting VB neurons should have a lower threshold for electrical stimulation than corticthalamic axons and axon terminals in the VB. Second, before many experiments, we measured field potentials in both layer IV and layer V/VI while stimulating the VB at a slightly higher stimulus intensity than that used during whole-cell recordings. In every case, VB stimulation caused a large, short-latency current sink in layer IV but only a very weak field potential, or none at all, in layer V/VI (data not shown). Similarly, optical recording using a voltage-sensitive dye has shown that VB stimulation causes strong, monosynaptic responses in layer IV but only weak responses in layer V/VI (Crair et al., 1993, Soc. Neurosci., abstract). Therefore, it seems likely that VB stimulation does not strongly activate corticthalamic axons and instead activates thalamocortical synapses rather selectively.

**Induction Mechanism**

Like LTD at several other glutamatergic synapses (Linden and Connor, 1995; Bear and Abraham, 1996), thalamocortical LTD is dependent on activation of NMDA receptors (Figure 3) and therefore is likely to involve an increase in postsynaptic calcium. LTD induction has been shown to require postsynaptic calcium at a wide variety of synapses (Bear and Abraham, 1996). Because LTP is also triggered by an increase in calcium at many synapses (Madison et al., 1991), it has been proposed that a modest rise in postsynaptic calcium may trigger LTD, whereas a more substantial or prolonged rise would trigger LTP (Lisman, 1989; Artola and Singer, 1993; Malenka and Nicoll, 1993). Our data are consistent with this type of model for thalamocortical synaptic plasticity, since depolarization to −50 mV induces LTD in slices from P4–P5 animals (Figure 2), whereas depolarization to between −10 and 0 mV reliably induces LTP (Crair and Malenka, 1995; Isaac et al., 1997). Based on the voltage dependence of the NMDA receptor conductance (Figure 4), NMDA receptor activation at −50 mV would be predicted to elicit −15% of the calcium influx elicited at 0 mV (assuming a Nernst potential for calcium of −100 mV). Thus, the conditions that induce LTD are associated with less calcium influx through the NMDA receptor ionophore than the conditions that induce LTP.

Regardless of the underlying mechanism, our findings demonstrate that a critical membrane potential exists between −50 mV and −10 mV, above which NMDA receptor activation induces LTP, and below which activation induces LTD. This finding suggests that during the critical period for thalamocortical plasticity in vivo, synapses will undergo LTP when they are activated in a correlated fashion with excitatory inputs that depolarize a layer IV cell above this critical membrane potential. In contrast, synapses would be predicted to undergo LTD when they are activated in a manner less correlated with other excitatory inputs, resulting in only a small depolarization of the postsynaptic cell. A learning rule of this type has been used to explain experience-dependent plasticity during development of the visual cortex (Stent, 1973; Bienenstock et al., 1982; Bear, 1996).

**Developmental Time Course**

LTD at thalamocortical synapses exhibited a pronounced developmental time course, being most reliably induced in slices from animals at or before P4 and being absent, on average, after P9 (Figure 2). This time course is delayed slightly relative to that for LTP at this synapse, which could not be induced (with the exception of a single cell) after P7 (Crair and Malenka, 1995). The time course for both LTP and LTD matches reasonably well the developmental window for experience-dependent changes in the spatial patterning of thalamocorticalafferents, which ends at P6 (Schlaggar et al., 1993), and for experience-dependent changes in short-latency vibrissae responses of layer IV neurons in vivo, which end at P4–P5 (Fox, 1992, 1995; Glazewski and Fox, 1996).

Importantly, we continued to observe robust LTD in a few cells after P9, indicating that the developmental time course for LTD does not constitute an absolute critical period but instead reflects a capacity for plasticity that declines to a reduced level after the first postnatal week. This observation is consistent with in vivo data showing that thalamocortical synaptic plasticity may occur in older animals, well after P9, following especially long periods of altered vibrissae experience (Armstrong-James et al., 1994).

The fact that LTD induction and experience-dependent plasticity at thalamocortical synapses occur during similar developmental windows suggests that LTD, like LTP, may contribute to experience-dependent refinement of receptive fields in vivo. In this view, the slight (1–3 day) discrepancy between the closing of the developmental windows for in vitro and in vivo plasticity may reflect any of several factors. First, the relatively modest LTP and LTD observed between P7 and P9 in vitro may be insufficient to cause plasticity that is detectable by the anatomical and physiological methods that have been used in vivo. Second, because the definition of the critical period in vivo is highly dependent on the type of sensory alteration that is used, the capacity for plasticity in vivo may in fact extend somewhat beyond P6 (Armstrong-James et al., 1994). Third, induction of LTD and LTP may be influenced by factors such as temperature, level of neuromodulators, and amount of network activity, each of which may be different in vivo than in vitro. Alternatively, it is possible that the similarity in developmental time course is coincidental, and the...
end of the critical period in vivo is determined by cellular processes other than LTP and LTD, or perhaps by a synergism between such processes and LTP and LTD.

Many neural systems exhibit a period of enhanced plasticity early in postnatal life. However, the factors responsible for the age-related loss of plasticity that brings such periods to a close remain largely unknown. In systems where plasticity is dependent on NMDARs, one possible cause of the developmental decline in plasticity is a decrease in the calcium signal generated by NMDAR channels. This could occur via several mechanisms, including a decrease in the amplitude or time course of NMDAR currents (Kato et al., 1991; Carmignoto and Vicini, 1992; Heston, 1992; Crair and Malenka, 1995), an increase in GABAergic inhibition that would reduce NMDAR currents by virtue of their voltage sensitivity (Luhmann and Prince, 1990; Agmon and O’Dowd, 1992), or an increase in the voltage-dependent blockade of the NMDAR ionophore by Mg$^{2+}$ (Ben-Ari et al., 1988; Kato and Yoshimura, 1993; Burgard and Hablitz, 1994). Alternatively, NMDAR currents could remain unchanged during development, but the effect of the calcium signal could be reduced by increased postsynaptic calcium buffering or by a reduction in the efficacy of factor(s) downstream of calcium entry that are required for induction of plasticity.

At the thalamocortical synapse, the development of GABA$\_A$-mediated inhibitory conductances cannot be responsible for the loss of LTD after P9, since blockade of these receptors by picrotoxin does not restore the ability to induce LTD. The same conclusion was reached by Dudek and Friedlander (1996) for LTD at unidentified synapses onto layer IV neurons in visual cortex. In addition, a change in the voltage dependence of the NMDAR current is unlikely to contribute to the decline in plasticity, since we detected no change in this property of the NMDAR current over the first 2 postnatal weeks. Instead, a developmental reduction in the duration of NMDAR currents may be involved, since the duration of NMDAR EPSCs decreases dramatically between P4 and P8 (Crair and Malenka, 1995), as it does during maturation of other glutamatergic synapses (Heston, 1992; Carmignoto and Vicini, 1992; Cline et al., 1996). Moreover, if plasticity were reduced due to a gradual reduction in the time course of NMDAR-mediated currents, the capacity for LTD would be predicted to be lost before the capacity for LTD, since LTP induction is thought to require a higher threshold level of calcium. Consistent with this prediction, we found that the ability to induce LTD was lost at P9, ~2 days after the ability to induce LTP (Crair and Malenka, 1995). Together, these findings suggest that a developmental decrease in calcium influx through the NMDAR ionophore may regulate induction of thalamocortical LTP and LTD. Whether additional developmental changes occur downstream of calcium entry to regulate plasticity at this synapse remains unknown.

**Mechanism of LTD Expression**

Because LTD and LTP in the CA1 region of the hippocampus reversibly regulate synaptic efficacy and mutually unsaturate each other (Dudek and Bear, 1993; Mulkey et al., 1993), it has been suggested that these two processes represent reversible modifications of common effector mechanisms. In this study, we present evidence that this may not be the case for LTP and LTD at thalamocortical synapses. Specifically, we found that whereas LTP is expressed by changes in both potency and failure rate (Isaac et al., 1997), LTD is expressed in most cells by a change in potency without a detectable change in failure rate (Figures 6 and 7). The implication of this finding is that LTD may reverse only a subset of the expression mechanisms of LTP, at least within 30 min after induction.

Several hypothetical molecular mechanisms for LTD and LTP expression could underlie these observations. It has been suggested that the decrease in failure rate observed during LTD at thalamocortical synapses has been postulated. By silencing functional synapses.

**Role of LTP and LTD in Development of Thalamocortical Circuits**

The specific functions performed by LTP and LTD during experience-dependent refinement of thalamocortical circuits are unknown. One reasonable possibility is that LTP and LTD act to modulate the efficacy of thalamocortical synapses that were previously formed via activity-independent mechanisms. Functionally, this would allow the receptive fields of layer IV neurons to be adjusted within limits determined by the architecture of
the existing circuitry (Singer, 1995; Gilbert et al., 1996). A second, not mutually exclusive possibility is that LTP and LTD guide anatomical restructuring of thalamocortical axonal arbors to allow receptive field adjustments outside the limits imposed by the original circuitry. In this model, which has been proposed to explain activity-dependent axonal remodeling in the developing visual system (Cline, 1991; Katz and Shatz, 1996), LTP at a subset of synapses would lead to the stabilization of axonal and dendritic branches bearing those synapses, whereas LTD would lead to branch retraction. Finally, LTP and LTD are also likely to regulate the maturation of the nonfunctional, silent synapses that are thought to exist during this early developmental period (Isaac et al., 1997). By converting silent synapses into functional synapses, LTP may effectively construct the first functional thalamocortical circuits. LTD may act to regulate the efficacy of synapses once they have been made functional or, perhaps, to resilience functional synapses under appropriate conditions.

Experimental Procedures

Slices (500 μm thick) were prepared from Sprague-Dawley rat pups 4–12 days after birth (P4-P12; day of birth was defined as P0). Animals were anesthetized with halothane and decapitated, and the brain was rapidly removed and placed in ice-cold extracellular solution (composition in mM: NaCl119, KCl 2.5, CaCl 2.5, MgSO 4 1.3, NaH2PO 4 1.0, NaHCO3 26.2, and glucose 11, saturated with 95% O2/5% CO2). Slices were cut using a vibrotome at an angle of 45° from the mid sagittal plane and 0°–10° from the coronal plane, a plane of section that leaves thalamocortical axons intact (Agmon and Connors, 1991; Crair and Malenka, 1995). Slices were allowed to recover 1–4 hr at room temperature before being placed in the recording chamber under a continuous stream of superfusing extracellular solution. In some experiments, picrotoxin (100 μM) was added to the extracellular solution. Recordings were made at room temperature (20°C–23°C).

Whole-cell voltage clamp recordings were made from neurons in layer IV of somatosensory cortex. Somatosensory cortex was identified by visualizing the barrels using transillumination, and by evoking short-latency field EPSPs by stimulation of the ventrobasal nucleus of the thalamus (Agmon and Connors, 1991; Crair and Malenka, 1995) with a concentric bipolar stimulating electrode (FHC, Brunswick, ME). Whole-cell recordings were made using 3-6 MΩ electrodes containing (in mM): cesium gluconate 117, HEPES 20, EGTA 0.2–0.4, TEA-Cl 5, MgSO4 1.3, NaH2PO4 1.0, NaHCO3 26.2, and glucose 11, saturated with 95% O2/5% CO2. Slices were cut using a vibrotome at an angle of 45° from the mid-sagittal plane and 0°–10° from the coronal plane, a plane of section that leaves thalamocortical axons intact (Agmon and Connors, 1991; Crair and Malenka, 1995). Slices were allowed to recover 1–4 hr at room temperature before being placed in the recording chamber under a continuous stream of superfusing extracellular solution. In some experiments, picrotoxin (100 μM) was added to the extracellular solution. Recordings were made at room temperature (20°C–23°C).

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LTD at Thalamocortical Synapses

Immediately before the stimulus artifact, the magnitude of LTD was defined as (mean EPSC amplitude of 50 sweeps starting 5 min after pairing) – (mean EPSC amplitude during final 50 sweeps of baseline period)/(mean EPSC amplitude during final 50 sweeps of baseline period). By this definition, a reduction in mean EPSC size from 10 pA to 7 pA would equal a –30% LTD. LTD was considered significant for a given cell if a two-tailed t test revealed a significant difference (p < 0.05) between the amplitude of the 50 consecutive EPSCs beginning 5 min after pairing with that of the 50 consecutive EPSCs immediately before pairing. For all cells in which LTD was induced, the mean series resistance was 21 ± 1 MΩ before pairing and 22 ± 1 MΩ after pairing.

For measurement of the NMDAR current-voltage relationship, NMDA receptor-mediated thalamocortical EPSCs were measured in the presence of 10 μM CNQX, 100 μM picrotoxin, 300 μM saclofen, and 1 μM glycine. Blocks of 20 NMDAR EPSCs were recorded as the holding potential was stepped in pseudorandom order in 10 mV increments between −90 mV and +30 mV. The amplitude of isolated NMDA receptor currents was calculated using a fixed 5 ms window at the peak of the current and subtracting the current during a similar window immediately before the stimulus artifact. Conductance was calculated as (current amplitude)/ (holding potential – reversal potential), and the reversal potential was determined by linear interpolation. To average conductance-membrane potential relationships between cells, conductance was first normalized to the conductance observed at +10 mV for each cell.

For minimal stimulation experiments, the stimulation intensity was initially set low so that no responses were recorded. Stimulation intensity was then increased slowly until the lowest intensity that elicited a mixture of failures and responses was found. At least 50 stimuli were collected for failure rate analysis in the baseline period, and at least 120 in the postpairing period. The failure rate was determined by visual inspection of individual sweeps: sweeps containing an EPSC whose onset occurred within 2 ms of the mean latency were classified as successes, and all other sweeps were classified as failures. This method produced the same results as measuring the number of responses with amplitude > 0 pA and then doubling this number to provide the number of failures (Liao et al., 1995; Isaac et al., 1997). The success rate was defined as (1 – failure rate), and the success rate ratio was defined as (success rate after pairing)/ (success rate during the baseline period). Potency was defined as the mean amplitude of the EPSC divided by the success rate (Stevens and Wang, 1994). For depotentiation experiments, LTP was induced by pairing at 0 to −10 mV for 50 stimuli with no change in stimulation rate. Twelve to fifty minutes after LTP induction, LTD (depotentiation) was induced by pairing at −50 mV for 100 stimuli. Data are expressed as mean ± SEM. Statistical significance was assessed by two-tailed t test or by ANOVA, and the criterion for significance was p < 0.05.

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