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Autophosphorylation of αCaMKII Is Required for Ocular Dominance Plasticity

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Summary

Experience is a powerful sculptor of developing neural connections. In the primary visual cortex (V1), cortical connections are particularly susceptible to the effects of sensory manipulation during a postnatal critical period. At the molecular level, this activity-dependent plasticity requires the transformation of synaptic depolarization into changes in synaptic weight. The molecule α calcium-calmodulin kinase type II (αCaMKII) is known to play a central role in this transformation. Importantly, αCaMKII function is modulated by autophosphorylation, which promotes Ca^{2+}-independent kinase activity. Here we show that mice possessing a mutant form of αCaMKII that is unable to autophosphorylate show impairments in ocular dominance plasticity. These results confirm the importance of αCaMKII in visual cortical plasticity and suggest that synaptic changes induced by monocular deprivation are stored specifically in glutamatergic synapses made onto excitatory neurons.

Introduction

During a critical period in early postnatal development, sensory experience potently shapes cortical connectivity. Subjecting an animal to brief periods of monocular deprivation profoundly alters the relative strength of the two eyes' input to V1, such that the drive of the nondeprived, open eye to the cortex is greatly amplified at the expense of connections subserving the deprived closed eye (Freeman and Olson, 1982). Although the physiological and anatomical dimensions of this plasticity are understood in some detail, our understanding of the molecular substrates of this synaptic competition remains incomplete.

The molecule αCaMKII is a signal transduction molecule that is known to be a fundamental player in synaptic plasticity mechanisms (Lisman et al., 2002; Soderling, 2000). It is abundant in the postsynaptic density (PSD) of excitatory synapses, making it well positioned to detect synaptic Ca^{2+} influx there (Kennedy et al., 1983). Activation of αCaMKII occurs through binding of Ca^{2+} and the cofactor calmodulin, and initiates signaling which flows in at least two directions: toward activated dendrites, where αCaMKII substrates include the α-amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid receptor (Barria et al., 1997), and toward the nucleus, where αCaMKII activity can lead to phosphorylation of transcription factors such as the cAMP/Ca^{2+} response element binding protein (CREB), which is itself known to be important for plasticity (Braun and Schulman, 1995; Dash et al., 1991). The multiplicity of αCaMKII signaling targets makes the molecule a potent control element in regulating plasticity mechanisms.

Autophosphorylation of αCaMKII provides an added layer of control over plasticity processes, as this renders the molecule Ca^{2+} autonomous, such that its activation can outlast fleeting Ca^{2+} transients (Miller and Kennedy, 1986a). This Ca^{2+}-autonomous state can be maintained for at least an hour in vitro (Barria et al., 1997) and potentially much longer in vivo. This sustained plasticity induction signal plays a necessary role in rapid induction of a number of forms of synaptic change, including long-term potentiation (Giese et al., 1998) and barrel cortex plasticity (Glazewski et al., 2000).

While these and other experiments suggest that αCaMKII is necessary for the induction of synaptic plasticity (Malinow et al., 1989), it may play a second, more enduring role in maintaining existing synaptic changes. Supported by modeling work and some experimental evidence (Feng, 1995), Lisman and others have proposed that autophosphorylated αCaMKII could itself serve as a kind of molecular memory, such that the strength of a given synapse could be encoded by the size of a local population of autophosphorylated αCaMKII (Lisman, 1994; Miller and Kennedy, 1986b).

In V1, αCaMKII is expressed in all laminae except layer I, with highest expression levels in the supragranular layers (Hendry and Kennedy, 1986; Tighilet et al., 1998). Both transcription and translation (Tighilet et al., 1998; Wu et al., 1998) of the molecule can be driven by visual experience, and the developmental time course of the molecule's expression plateaus near the peak of the critical period (Burgin et al., 1990; Kelly et al., 1987). One previous study has directly examined the role of αCaMKII in developmental plasticity in V1 by examining the effects of monocular deprivation on αCaMKII knockout mice (Gordon et al., 1996). Homozygous knockout animals, but not heterozygote or wild-type littermates, show a partial plasticity defect, such that about half of all homozygous mutants fail to show measurable plasticity in response to monocular deprivation. The same mice show a similar bimodal distribution of scores on a water maze task, with performance impairments apparent in roughly half of the homozygous mice tested (Gordon et al., 1996).

Here we revisit the question of αCaMKII function in ocular dominance plasticity. Using transgenic mice possessing a mutant form of αCaMKII (alanine substituted for threonine at position 286: T286A) which cannot autophosphorylate, we show that the Ca^{2+}-autonomous, autophosphorylated form of αCaMKII is necessary for nor-

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mal ocular dominance plasticity. Studies in vitro suggest that the biochemical effects of the T286A mutation are confined to preventing autophosphorylation: the mutant molecule possesses the same Ca^{2+}-dependent activity (Giese et al., 1998), and, as far as is known, the same substrate specificity as the wild-type form of the molecule (Fong et al., 1989; Hanson et al., 1989; Waxham et al., 1990). These results confirm the importance of αCaMKII in ocular dominance plasticity, demonstrating that the Ca^{2+}-autonomous state of the molecule is required, and suggest that excitatory synapses are the primary locus of storage for plasticity induced by monocular deprivation.

Results

Biochemical and Immunohistochemical Analysis

Previous biochemical analyses of $\alpha CaMKII^{T286A}$ mutants used only whole-brain extracts (Giese et al., 1998), and subsequent investigations of cortical function in these mice have not characterized the biochemical effects of the introduced point mutation upon cortical tissue (Glazewski et al., 2000). To specifically examine the effects of the point mutation upon the visual cortex, we examined levels of phospho-αCaMKII and total αCaMKII in protein extracted from homogenates of the visual cortex. We also used immunohistochemical techniques to investigate the localization of these molecules within V1.

$\alpha CaMKII^{T286A}$ heterozygotes possessed the expected ~50% reduction in phospho-αCaMKII relative to wild-type littermates (Figure 1B). Phospho-αCaMKII signal was never detectable in protein extracts from $\alpha CaMKII^{T286A}$ homozygotes (see example in Figure 1A), even when blots were overexposed (data not shown). Surprisingly, total levels of αCaMKII were substantially reduced in both $\alpha CaMKII^{T286A}$ homozygotes and heterozygotes, to approximately 60% of the levels seen in wild-type littermates (Figure 1C).

This reduction in αCaMKII levels did not affect the molecule's laminar distribution in V1 of heterozygous and homozygous animals (Figures 2E and 2F). As for wild-type mice (Figure 2D), αCaMKII staining in heterozygotes and homozygotes was heaviest in the supragranular layers, with moderate signal apparent in thalamorecipient layer IV and the subgranular layers. In individual neurons, staining was robust in the cytoplasm, and in some cases was accompanied by immunoreactivity extending into dendrites; this was particularly true of the primary apical dendrite of layer II/III pyramidal neurons. In addition, portions of the neuropil were heavily stained, with individual axons distinguishable in the white matter below layer VI.

The distribution of phospho-CaMKII staining was similar in wild-type and heterozygous mice, with the most intense staining in upper layers II/III (Figures 2A and 2B). Signal was largely absent from $\alpha CaMKII^{T286A}$ homozygotes (Figure 2C); a faint band of immunoreactivity is visible in the most dorsal portions of the supragranular layer, presumably due to crossreactivity of the antibody with βCaMKII. No differences in the gross morphology of laminae were apparent in Nissl stains (Figures 2G–2I).

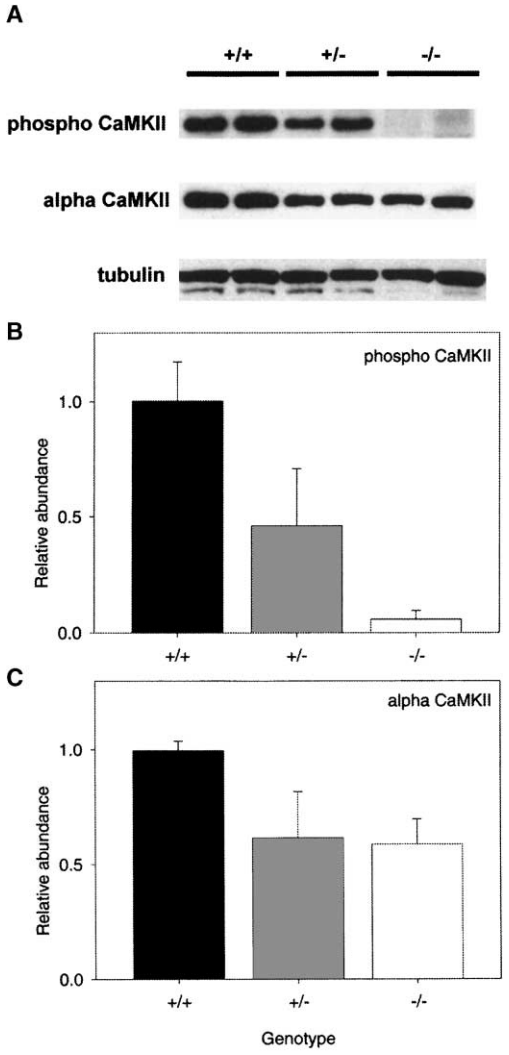

Figure 1. Western Blots of Phospho- and Total αCaMKII in Homogenates of Visual Cortex

(A) A typical example blot shows results from three littermates; duplicate lanes contain homogenates from each hemisphere of the animal of that genotype. Tubulin controls show that loading was equal for each lane.

(B) Phospho-αCaMKII abundance faithfully reflects genotype. Mean values ± SEM, normalized to wild-type levels: wild-type (+/+) = 1.00 ± 0.09; heterozygote (+/−) = 0.46 ± 0.12; homozygote (−/−) = 0.06 ± 0.02. All values are significantly different (all $p << 0.05$, t test).

(C) αCaMKII levels are significantly reduced in heterozygous (mean ± SEM = 0.61 ± 0.10) and homozgyous mutants (mean ± SE = 0.59 ± 0.05) relative to wild-type levels (mean ± SEM = 1.00 ± 0.02; both $p < 0.01$, t test), but are not significantly different from each other ($p = 0.82$, t test).

Visual Cortical Plasticity

When initiated during the normal murine critical period for plasticity, brief (4 day) periods of monocular deprivation can induce a rapid and robust shift in the balance of the two eyes' input to V1. To test the effects of the T286A mutation, we subjected $\alpha CaMKII^{T286A}$ mutants to 4 day monocular deprivation during the peak of the critical period. Single-unit extracellular recordings were

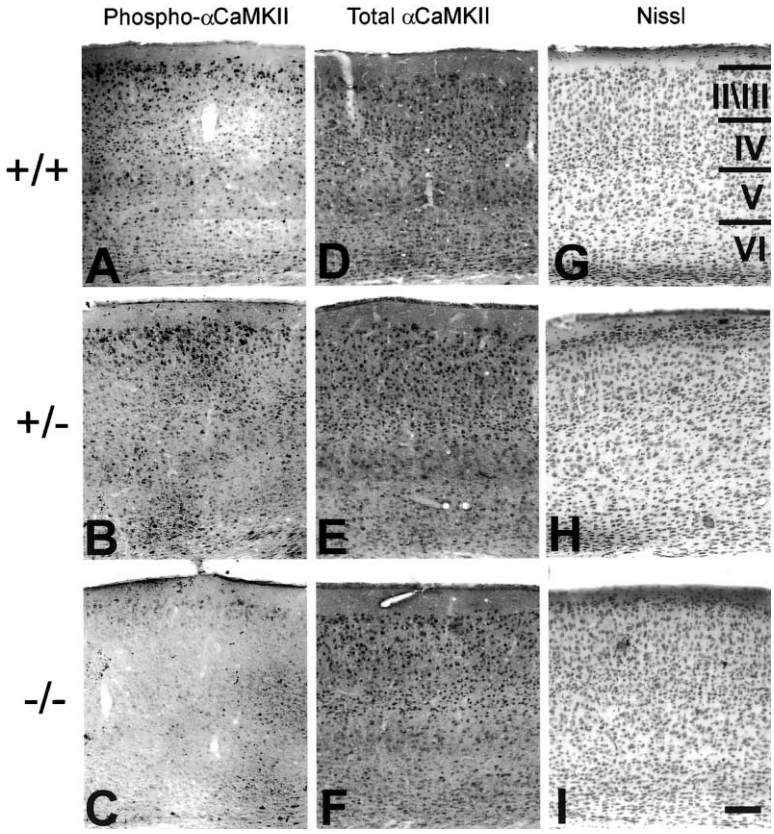

Figure 2. Immunohistochemistry of Phospho- and Total αCaMKII in V1

(A–C) Phospho-αCaMKII distribution in wild-type and heterozygote animals is similar, with heaviest staining apparent in pyramidal neurons of upper layer II/III. Staining is moderate in thalamorecipient layer IV, weak in subgranular layers V and VI, and absent in layer I. Very faint staining of layer II/III is evident in homozygous mutants (C), perhaps caused by crossreactivity to βCaMKII.

(D–F) Total αCaMKII staining is similar in all three genotypes, with the most intense signal in the supragranular layers, followed by moderate staining in granular layers and subgranular layers.

(G–I) Nissl stains show that the gross organization of cortical laminae in all three αCaMKII^{T286A} genotypes are similar. Scale bar in (I), 100 μm.

used to quantify the strength of the two eyes' input to individual neurons. Units from each animal were used to calculate a single contralateral bias index (CBI) (see Experimental Procedures), a weighted measure of the relative input strength of the contralateral eye's cortical drive.

In normal mice, input from the contralateral eye predominates in V1, apparent in the left-shifted distribution of ocular dominance scores (Figure 3A). These single-unit responses show that contralateral eye input is evident in all cortical neurons, and approximately 20% of cortical neurons are driven exclusively by the contralateral eye. In wild-type mice, this balance is exquisitely sensitive to changes in input activity: 4 days of contralateral eyelid suture induce a dramatic rearrangement of connections in the cortex, such that the balance of input is altered to favor the ipsilateral eye (Figure 3B, black bars).

The distribution of ocular dominance scores seen in nondeprived αCaMKII^{T286A} homozygotes (Figure 3C) is nearly identical to that seen in wild-type littermates. In contrast, however, 4 day monocular deprivation fails to induce robust plasticity in the homozygous animals (Figure 3D), and the distribution of ocular dominance scores remains heavily weighted toward the deprived, contralateral eye. Following monocular deprivation, neurons driven predominantly by the open, ipsilateral eye account for more than 40% of all the neural responses in wild-type animals (Figure 3B, ocular dominance categories 5–7); the same response categories account for less than 10% of the units recorded in homozygous mutant mice (Figure 3D).

Figure 4A shows the distribution of CBI scores for individual animals. Before monocular deprivation ("No MD"), wild-type (closed symbols; mean ± SEM = 0.74 ± 0.04, n = 6 mice) and αCaMKII^{T286A} homozygotes (open symbols; mean ± SEM = 0.74 ± 0.03, n = 6) show very similar mean CBI values ($p = 0.97$, t test). While 4 days of monocular deprivation induce a large shift in the mean CBI value of wild-type mice (mean ± SEM = 0.46 ± 0.04, n = 4), plasticity is impaired in αCaMKII^{T286A} homozygotes subjected to the same deprivation protocol (mean ± SEM = 0.64 ± 0.03, n = 6; significantly different from wild-type littermates after 4 day monocular deprivation, $p < 0.01$, t test). Nevertheless, the effect of monocular deprivation on αCaMKII^{T286A} homozygotes, though small, was not insignificant ($p = 0.03$ relative to baseline CBI value; $p = 0.05$ relative to wild-type baseline CBI value). Ocular dominance histograms are shown for each αCaMKII^{T286A} homozygote studied to demonstrate the full range of response variability measured in nondeprived (Figure 4B) and monocularly deprived (Figure 4C) animals.

The distribution of αCaMKII shows laminar specificity, raising the possibility that the effects of the T286A mutation might also vary by cortical layer. Analysis of single units as a function of the cortical depth at which each was recorded (see Experimental Procedures) shows that the T286A mutation significantly impaired plasticity in all cortical layers (data not shown; mean ocular dominance

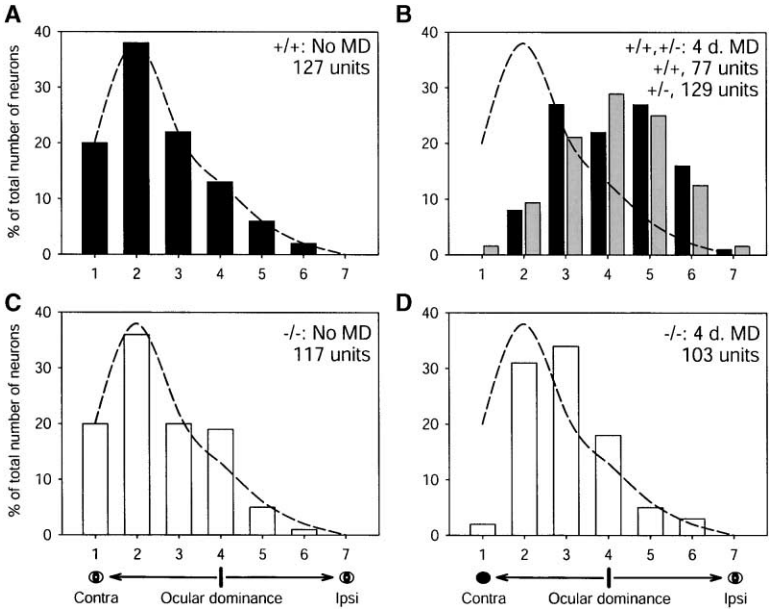

Figure 3. Plasticity Is Impaired in αCaMKII^{T286A} Homozygous Mice

(A) Prior to monocular deprivation, wild-type mice show a typical left-shifted distribution of scores in ocular dominance histograms, reflecting predominantly contralateral eye input. The broken line in all panels is a smoothed fit to the distribution of units recorded in nondeprived, wild-type animals.

(B) Following 4 day monocular deprivation, wild-type (black bars) and heterozygous animals (gray bars) show robust plasticity, such that most units are preferentially driven by the open ipsilateral eye. Plasticity following monocular deprivation is evident in these animals by the pronounced shift in the distribution of units to the right, relative to the distribution in nondeprived, wild-type mice.

(C) Prior to monocular deprivation, αCaMKII^{T286A} homozygous mice show a distribution of ocular dominance scores very similar to that seen in wild-type animals.

(D) Following 4 day monocular deprivation, the majority of units in homozygous mutant mice continue to be driven by the deprived contralateral eye.

scores significantly different from wild-type littermates at all cortical depths; $p < 0.01$, Mann-Whitney). Comparison of units recorded in nondeprived wild-type and homozygous mutant mice showed that mean ocular dominance scores prior to monocular deprivation did not differ (data not shown; $p > 0.10$ for all cortical depths).

The impaired plasticity observed in αCaMKII^{T286A} homozygotes also cannot be attributed to reduced levels of total αCaMKII (Figure 1C). Despite a similar reduction in overall levels of αCaMKII, αCaMKII^{T286A} heterozygotes show plasticity in response to monocular deprivation which is indistinguishable from that measured in wild-type animals (Figures 3B, gray bars, and 4, gray symbols; mean ± SEM = 0.48 ± 0.04; $p = 0.70$, t test) and significantly greater than that seen in αCaMKII^{T286A} homozygotes ($p < 0.01$, t test).

Single-Unit Responses

To ensure that the phenotype observed in αCaMKII^{T286A} homozygotes was not the consequence of impaired cortical responsiveness or disorganized receptive fields, we examined the retinotopic organization of V1 and the single-unit responses of individual cortical neurons.

The vigor of single-unit responses was not altered in αCaMKII^{T286A} homozygotes, and measures of both responsivity and habituation were similar to those found in wild-type animals (Figures 5C and 5D; $p >> 0.05$ for both, t test). Similarly, the size of receptive fields in central V1 was not altered (Figure 5B; $p = 0.19$, t test) nor did the retinotopic mapping of receptive fields differ between αCaMKII^{T286A} homozygotes and wild-type animals (Figure 5A; $p = 0.63$, t test).

Timing of the Critical Period

Recent studies of other transgenic mice have highlighted the possibility that apparent defects in plasticity can arise from changes in the timing of the critical period (Fagiolini and Hensch, 2000; Hanover et al., 1999). The developmental time course of αCaMKII expression in the mouse visual cortex is consistent with a role in regulating the timing of cortical plasticity (Burgin et al., 1990; Kelly et al., 1987), and functional studies in other experimental systems indicate that the molecule may exert control over developmental plasticity (Wu et al., 1996; Wu and Cline, 1998; Zou and Cline, 1999).

To investigate this possibility, we studied the effects of monocular deprivation initiated before and after the normal peak of the critical period. Plasticity of wild-type mice peaked near postnatal day 26 (Figure 6), consistent with findings from previous studies (Gordon and Stryker, 1996; Hanover et al., 1999). Although the magnitude of plasticity in αCaMKII^{T286A} homozygotes was much reduced relative to wild-type animals, the greatest plasticity occurred at similar ages. Thus, the impairment observed in αCaMKII^{T286A} homozygous animals cannot be accounted for by a precocious or delayed critical period for ocular dominance plasticity.

Discussion

A previous study from this laboratory has implicated the molecule αCaMKII in ocular dominance plasticity (Gordon et al., 1996). Homozygous αCaMKII knockout mice possess impairments in both ocular dominance plasticity and water maze performance, but this phenotype is only partially penetrant, occurring in ~50% of the mice tested. The results reported here confirm a requirement for αCaMKII in ocular dominance plasticity following brief monocular deprivation. Because the effects of the T286A mutation are confined to preventing autophosphorylation and do not interfere with Ca^{2+}-dependent αCaMKII activity (Giese et al., 1998), these results extend our understanding of the molecule's role in ocular dominance plasticity by specifically demonstrating the importance of the Ca^{2+}-autonomous state of αCaMKII in this process. The plasticity defect in the αCaMKII^{T286A} homozygotes is more penetrant than that of the knockout, presumably because the absence of

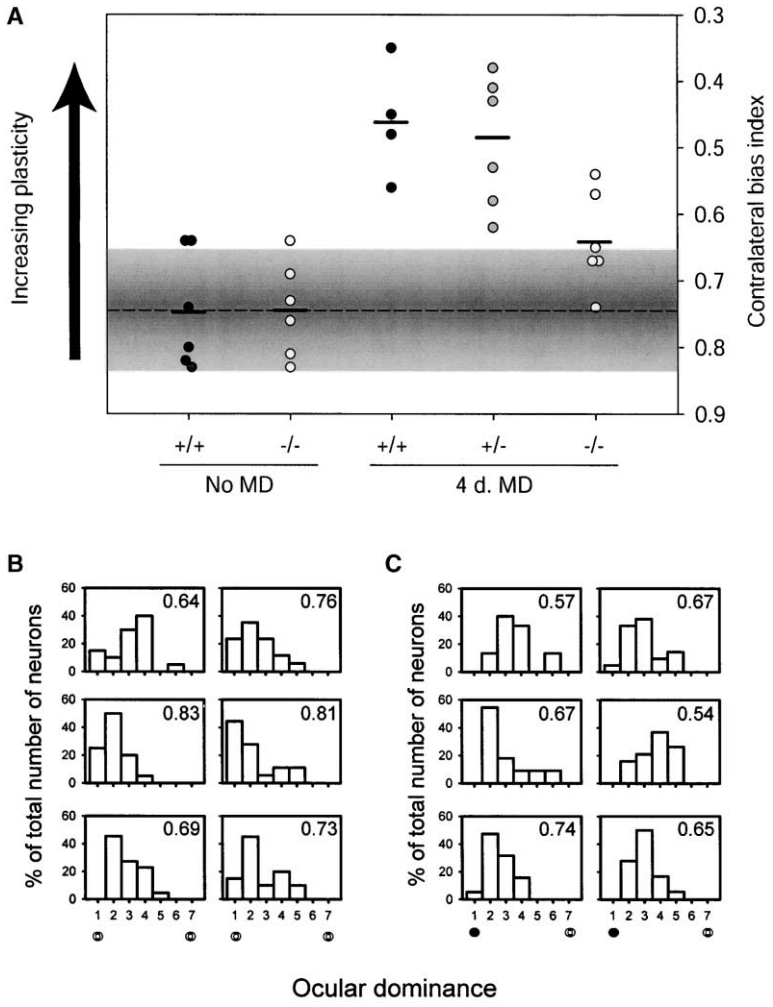

Figure 4. Contralateral Bias Index Scores for Individual Animals, and Ocular Dominance Histograms for All αCaMKII^{T286A} Homozygous Mice

(A) Prior to monocular deprivation ("No MD" columns), ocular dominance scores for wild-type (closed symbols; mean ± SEM = 0.74 ± 0.04, n = 6 mice) and homozygous mice (open symbols; mean ± SEM = 0.74 ± 0.03, n = 6 mice) are similar (p = 0.97), with the majority of cortical input driven by the contralateral eye, as is normal for mice. Four day monocular deprivation induces robust plasticity in wild-type and heterozygous mice, evident in lower CBI values (mean ± SEM: wild-type, 0.46 ± 0.04, n = 4 mice; heterozygote, gray symbols, 0.48 ± 0.04, n = 6 mice) which indicate a relative increase in the strength of the open ipsilateral eye's input to V1. In contrast, plasticity following monocular deprivation is markedly impaired in homozygous mice (mean ± SEM = 0.64 ± 0.03, n = 6 mice), such that cortical input from the deprived contralateral eye continues to predominate. Following 4 day monocular deprivation, the mean CBI of homozygous mice is significantly higher than that of wild-type and heterozygote animals ($p < 0.01$ for both, t test). Each symbol represents the CBI value for a single mouse. Mean values are shown by horizontal bars. The dotted line shows the mean baseline CBI value, and the gray box extends plus or minus a single standard deviation. Note inverted *y* axis values.

(B and C) Ocular dominance histograms for each nondeprived (B) and monocularly deprived (C) αCaMKII^{T286A} homozygote shows the full range of response variability encountered in these mice. CBI scores are indicated in the upper right corner of each histogram.

αCaMKII in the knockout mouse elicits upregulation of the β isoform of the molecule (A. Silva, personal communication). The impairment we see in αCaMKII^{T286A} homozygotes is unlikely to be the consequence of changes in neuronal excitability, as blind measurements of neuronal response properties showed no differences between homozygous and wild-type animals. Furthermore, deficient plasticity in αCaMKII^{T286A} homozygotes does not result from altered timing of the critical period.

Our results are consistent with growing evidence of

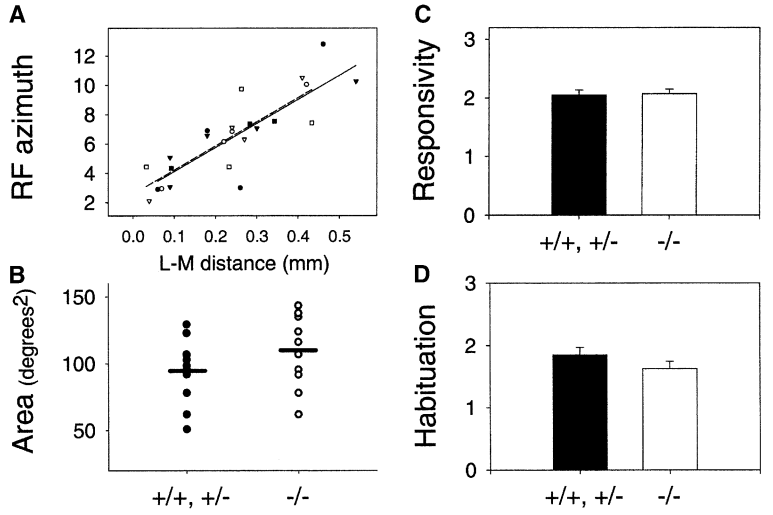

Figure 5. Response Properties Are Unchanged in αCaMKII^{T286A} Homozygous Mice

Many single-unit response properties are not altered in homozygous mice relative to wild-types littermates (all $p >> 0.05$, t test), including the following. (A) retinotopy (wild-type/heterozygote n = 12 units in 3 mice; homozygote n = 12 units in 3 animals); (B) receptive field area (wild-type/heterozygote n = 11; homozygote n = 12 units); (C) responsivity of single units; (D) single-unit habituation (wild-type/heterozygote, n = 57; homozygote, n = 59 units for [C] and [D]).

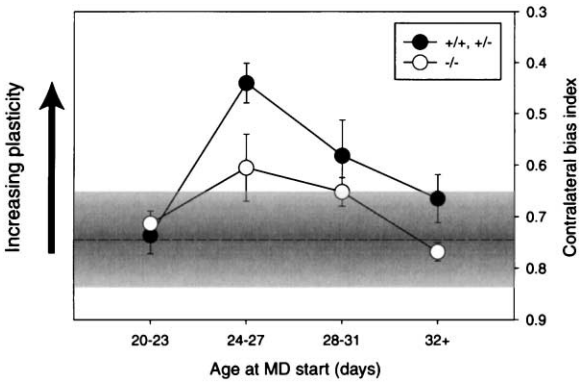

Figure 6. Timing of the Critical Period Is Similar in αCaMKII^{T286A} Homozygotes and Wild-Type Littermates

Apparent defects in plasticity are not the consequence of a shift in the timing of the critical period of CaMKII^{T286A} homozygous mice, as 4 day monocular deprivation initiated before and after the peak of the normal critical period (postnatal day 24–27 in wild-type littermates) does not induce plasticity. Each symbol represents the mean ± SEM CBI value after 4 day monocular deprivation beginning at the age ranges indicated. Filled symbols show wild-type (n = 13) and heterozygous mice (n = 9); open symbols indicate homozygous mice (n = 16).

a central role for Ca^{2+}-dependent signaling in ocular dominance plasticity. Ramoa and colleagues have shown, using innovative techniques, that the N-methyl-D-aspartate (NMDA) receptor is required for ocular dominance plasticity, and that this role can be separated from its role in basal synaptic transmission (Roberts et al., 1998). The NMDA receptor provides a means for Ca^{2+} entry into cortical neurons, triggered by coincident depolarizing input: our results suggest that this Ca^{2+} influx may signal via αCaMKII activation. Downstream targets of αCaMKII may include CREB, which is necessary for ocular dominance plasticity (Mower et al., 2002) and is transcriptionally regulated by monocular deprivation exclusively during the critical period for ocular dominance plasticity (Pham et al., 1999). Ca^{2+}-mediated signaling is also likely to drive protein translation, which is necessary for ocular dominance plasticity (Taha and Stryker, 2002).

Experiments in vivo and in vitro have closely linked synapses' potential for plasticity with αCaMKII signaling. For example, postsynaptic expression of constitutively active αCaMKII in hippocampal neurons enhances synaptic transmission and precludes subsequent tetanizing stimuli from eliciting additional potentiation (Pettit et al., 1994). Similar manipulations in the *Xenopus* tectum drive maturation of retinotectal synapses and, presumably, preclude further plasticity at these synapses (Wu et al., 1996; Wu and Cline, 1998). We sought to test whether similar mechanisms underlie the timing of the critical period for ocular dominance plasticity: namely, whether the accumulation of αCaMKII-mediated synaptic changes to some threshold level accounts for closure of the critical period. The attenuated plasticity observed in αCaMKII^{T286A} homozygotes, however, is confined to the normal critical period for ocular dominance plasticity; impaired αCaMKII signaling does not prolong the susceptibility of V1 neurons to activity-dependent plasticity. These results suggest signaling pathways independent of αCaMKII bring the critical period to a close.

The relative contribution of inhibitory or excitatory circuits in mediating the effects of monocular deprivation has long been debated. Mower and others, based on investigations in which iontophoresed bicuculline was used to locally disinhibit inputs to V1 neurons, have

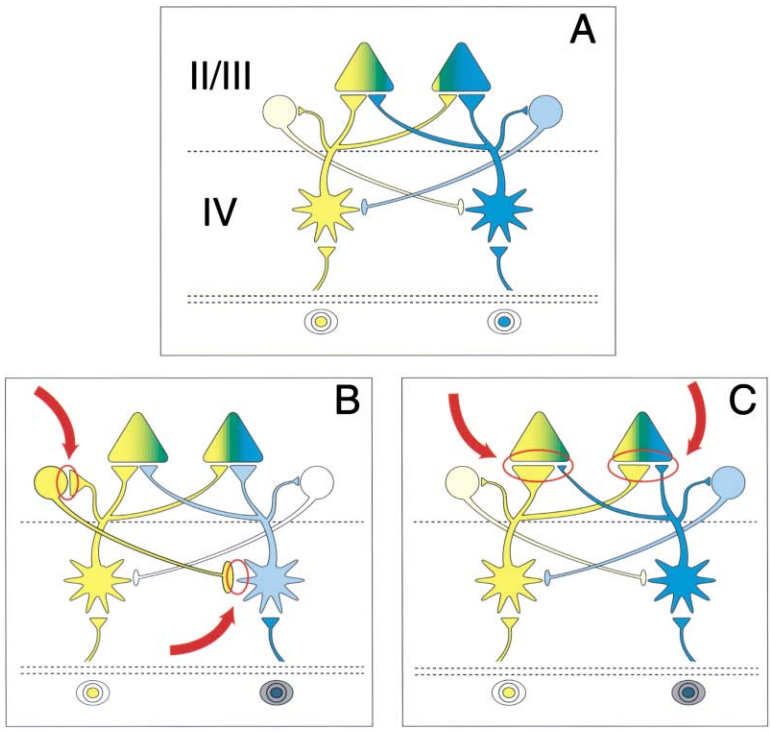

Figure 7. Glutamatergic Synapses Made onto Excitatory Neurons Are the Likely Site of Synaptic Change Induced by Monocular Deprivation

(A) Schematic diagram of cortical circuitry underlying ocular dominance plasticity. Spiny stellate neurons in layer IV receive input from thalamic afferents, and project to supragranular pyramidal neurons, as well as interneurons (round cell bodies) which provide cross-inhibition to layer IV neurons driven by the opposite eye. Depolarizing input from each eye is color coded, with firing rate indicated by color saturation.

(B) Monocular deprivation could induce increased inhibition by acting through the highlighted synapses (red ovals), which would have the effect of reducing the deprived eye input (blue pathways) to pyramidal neurons. However, αCaMKII is not expressed at either of the highlighted synapses.

(C) In V1, αCaMKII occurs exclusively in excitatory neurons and is the most abundant protein in the PSD of excitatory synapses. If these synapses (red ovals) are the primary locus of storage for plasticity induced by monocular deprivation, reduced αCaMKII function would result in impairment of ocular dominance plasticity, as we have shown here.

argued that the network substrate of plasticity induced by monocular deprivation lies in increased inhibition of deprived eye circuits, driven by nondeprived eye inputs (Kratz and Spear, 1976; Mower and Christen, 1989). But the effects of bicuculline application are difficult to interpret, as they could arise either through enhanced inhibition of deprived eye pathways, or simply through unmasking of a reduced excitatory input in these pathways, allowing both eyes sufficient drive to produce saturating responses (Sillito et al., 1981).

Our investigation brings a new tool to this question. Despite its abundance, αCaMKII is not ubiquitous—in the visual cortex it occurs specifically in excitatory neurons, and where localized to synapses, occurs postsynaptically only in the PSD of asymmetric (presumed) excitatory synapses (Liu and Jones, 1996). This specificity, combined with our demonstration of a requirement for normal αCaMKII function in ocular dominance plasticity, suggests that synaptic changes induced by monocular deprivation occur primarily in excitatory synapses made onto glutamatergic neurons.

Figure 7 shows a schematized representation of the cortical network in V1 in which spiny stellate neurons in layer IV receive thalamic input and make excitatory projections onto layer II/III pyramidal neurons, as well as onto inhibitory interneurons (round cell bodies). Interneurons provide crossinhibition through inhibitory synapses onto layer IV neurons subserving the opposite eye. The layer II/III pyramidal neurons shown in this schematic are both binocular but receive the bulk of their depolarizing drive (indicated by color) from a single eye.

In principle, changes in either inhibition or excitation could account for plasticity induced by monocular deprivation. Increased crossinhibition of deprived eye pathways (Figure 7B), potentially the consequence of potentiation induced by monocular deprivation in the highlighted synapses (red ovals), would suppress activity in deprived eye pathways (blue). Results presented in this paper, however, show that plasticity is impaired through mutation of αCaMKII, which does not occur at either of the highlighted synapses. The molecule is present neither in inhibitory neurons nor localized to the postsynaptic element of inhibitory synapses made onto excitatory neurons and therefore is unlikely to participate in plasticity at these synapses.

In the visual cortex, αCaMKII occurs exclusively in excitatory neurons and is an abundant protein in the PSD of excitatory synapses. Thus, mutation of αCaMKII is most likely to affect plasticity at excitatory synapses made onto excitatory neurons (Figure 7C). If these synapses (red ovals) are the primary locus of plasticity induced by monocular deprivation, reduced αCaMKII function would result in an impairment of ocular dominance plasticity, as we have shown here.

It remains possible that ocular dominance plasticity is composed of an obligatory progression of steps in which sequential stages might require synaptic change in excitatory synapses followed by plasticity in inhibitory synapses, or vice versa. Interrupting any step in this progression would block the effects of monocular deprivation. Thus plasticity in excitatory synapses might not be the only synaptic change necessary for ocular dominance plasticity; our results indicate, however, that it is required.

The specific nature of the defect caused by mutant $\alpha CaMKII^{T286A}$ remains unresolved. Autophosphorylation of αCaMKII undoubtedly acts as a facilitatory step toward inducing plasticity by sustaining the otherwise transient activation of the molecule. The sustained signaling initiated by the Ca^{2+}-autonomous state of αCaMKII is clearly an important aspect of the molecule's function—in ocular dominance plasticity, as in other forms of synaptic plasticity, it is necessary for normal synaptic change. However, it remains possible that autophosphorylated αCaMKII is also necessary for the maintenance of existing synaptic changes, as has been suggested (Lisman et al., 2002). Pharmacological or genetic techniques that allow temporally circumscribed manipulation of αCaMKII are likely to shed light on this issue.

Experimental Procedures

Visual Cortex Dissection

Mice were anesthetized with isofluorane and decapitated. V1 was dissected from each hemisphere by removing a $\sim 2 \times 2$ mm block of cortical tissue (3 mm lateral relative to the midline). Each sample was flash frozen in liquid N_2.

Tissue Preparation

Cortical tissue was homogenized at 4°C in a buffer containing the following: 50 mM Tris-HCl, pH 7.5, 10 mM EGTA, 10 mM EDTA, 50 mM NaCl, 2 mM sodium pyrophosphate, 4 mM para-nitrophenylphosphate, 1 mM sodium orthovanadate, 1 mM PMSF, 25 mM NaF, 2 mM DTT, 20 μg/ml leupeptin, and 10 μg/ml aprotinin. Brain homogenates were sonicated and immediately placed in protein loading buffer. A small volume of each homogenate was retained for estimating protein concentration using the Bradford assay.

Western Blots

Protein homogenates were run on 10% SDS/polyacrylamide gels and transferred to PSVD membrane. Blots were blocked for in 3% bovine sterile albumin and incubated serially in primary antibody; 3× TBST + 3% milk; biotinylated secondary antibody; and 3× TBST. Primary antibodies used were anti-phospho-αCaMKII (1:5000; Promega, Madison, WI) and anti-αCaMKII (1:1000; Chemicon, Temecula, CA). Protein concentrations were visualized using ECL bioluminescent detection (Amersham Biosciences, Piscataway, NJ). On each blot, dilution series of the sample containing the most abundant signal were used to establish the relative intensity of other bands on the same blot. Samples from four hemispheres (two mice) were used for each genotype.

Immunohistochemistry

Phospho-αCaMKII and αCaMKII were detected using standard immunohistochemistry techniques. Anti-phospho-αCaMKII (1:200; Promega) and anti-αCaMKII (1:500; Chemicon) primary antibodies were used in conjunction with immunoperoxidase detection kits (Vectastain ABC; Vector, Burlingame, CA).

Monocular Deprivation

Monocular deprivation was performed according to published protocols (Gordon and Stryker, 1996), except 3% isofluorane (Abbott, North Chicago, IL) in oxygen was used for anesthesia. In every case, monocular deprivation lasted for 4 days. Experiments assessing plasticity during the normal critical period for ocular dominance plasticity were initiated between postnatal days 26 and 30. The timing of the critical period in $\alpha CaMKII^{T286A}$ mice was probed by initiating monocular deprivation before (as early as p21) and after (as late as p38) the normal critical period. A total of 38 mice were used for critical period timing studies: 13 wild-type, 9 heterozygous, and 16 homozygous mice.

Single-Unit Recording

In all experiments, recordings were performed blind to the genotype of the animal studied. Mice were anesthetized for electrophysiological recording with a combination of Nembutal (50 mg/kg; Abbott) and chlorprothixene (0.2 mg; Sigma) using standard protocols (Gordon and Stryker, 1996). In each mouse, single units were isolated at intervals of 60 μm or more, using lacquer-coated tungsten electrodes in the binocular region of V1 contralateral to the deprived eye. A handlamp was used to project moving bars or squares onto a tangent screen to drive neuronal responses. The balance of the two eyes' input to each unit was scored on the 1–7 ocular dominance scale of Hubel and Wiesel (Hubel and Wiesel, 1962), where a value of 1 indicates complete domination by the contralateral eye, and 7 indicates input arises from the ipsilateral eye only. For each mouse, these ocular dominance scores were used to compute a single contralateral bias index (CBI) according to the formula:

$$CBI = [(n_1 - n_7) + (2/3)(n_2 - n_6) + (1/3)(n_3 - n_5) + N]/2N,$$

where N = total number of cells, and n_x = number of cells with ocular dominance scores equal to x. Most units were also scored for habituation and for responsivity on a 0 (no habituation/sluggish responsivity) to 3 (prolonged and rapid habituation/brisk firing) scale.

In order to assess potential laminar effects of the T286A mutation upon cortical plasticity, electrophysiological data was analyzed as a function of the cortical depth at which each unit was encountered. Units were divided into those recorded $<$350 μm, 350–550 μm (presumed layer IV) (S.T. and M.P.S., unpublished data), and $>$550 μm below the cortical surface. Mean ocular dominance scores for each of these categories was calculated for both wild-type and homozygous mutant mice before and after monocular deprivation.

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References

Barria, A., Muller, D., Derkach, V., Griffith, L.C., and Soderling, T.R. (1997). Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. Science *276*, 2042–2045.

Braun, A.P., and Schulman, H. (1995). The multifunctional calcium/calmodulin-dependent protein kinase: from form to function. Annu. Rev. Physiol. *57*, 417–445.

Burgin, K.E., Waxham, M.N., Rickling, S., Westgate, S.A., Mobley, W.C., and Kelly, P.T. (1990). In situ hybridization histochemistry of Ca^{2+}/calmodulin-dependent protein kinase in developing rat brain. J. Neurosci. *10*, 1788–1798.

Dash, P.K., Karl, K.A., Colicos, M.A., Prywes, R., and Kandel, E.R. (1991). cAMP response element-binding protein is activated by Ca^{2+}/calmodulin- as well as cAMP-dependent kinase. Proc. Natl. Acad. Sci. USA *88*, 5061–5065.

Fagiolini, M., and Hensch, T.K. (2000). Inhibitory threshold for critical-period activation in primary visual cortex. Nature *404*, 183–186.

Feng, T.P. (1995). The involvement of PKC and multifunctional CaM kinase II of the postsynaptic neuron in induction and maintenance of long-term potentiation. Prog. Brain Res. *105*, 55–63.

Fong, Y.L., Taylor, W.L., Means, A.R., and Soderling, T.R. (1989). Studies of the regulatory mechanism of Ca^{2+}/calmodulin-dependent protein kinase II. Mutation of threonine 286 to alanine and aspartate. J. Biol. Chem. *264*, 16759–16763.

Freeman, R.D., and Olson, C. (1982). Brief periods of monocular deprivation in kittens: effects of delay prior to physiological study. J. Neurophysiol. *47*, 139–150.

Giese, K.P., Fedorov, N.B., Filipkowski, R.K., and Silva, A.J. (1998). Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science *279*, 870–873.

Glazewski, S., Giese, K.P., Silva, A., and Fox, K. (2000). The role of alpha-CaMKII autophosphorylation in neocortical experience-dependent plasticity. Nat. Neurosci. *3*, 911–918.

Gordon, J.A., and Stryker, M.P. (1996). Experience-dependent plasticity of binocular responses in the primary visual cortex of the mouse. J. Neurosci. *16*, 3274–3286.

Gordon, J.A., Cioffi, D., Silva, A.J., and Stryker, M.P. (1996). Deficient plasticity in the primary visual cortex of alpha-calcium/calmodulin-dependent protein kinase II mutant mice. Neuron *17*, 491–499.

Hanover, J.L., Huang, Z.J., Tonegawa, S., and Stryker, M.P. (1999). Brain-derived neurotrophic factor overexpression induces precocious critical period in mouse visual cortex. J. Neurosci. *19*, RC40.

Hanson, P.I., Kapiloff, M.S., Lou, L.L., Rosenfeld, M.G., and Schulman, H. (1989). Expression of a multifunctional Ca^{2+}/calmodulin-dependent protein kinase and mutational analysis of its autoregulation. Neuron *3*, 59–70.

Hendry, S.H., and Kennedy, M.B. (1986). Immunoreactivity for a calmodulin-dependent protein kinase is selectively increased in macaque striate cortex after monocular deprivation. Proc. Natl. Acad. Sci. USA *83*, 1536–1541.

Hubel, D. and Wiesel, T. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J. Physiol. *160*, 106–154.

Kelly, P.T., Shields, S., Conway, K., Yip, R., and Burgin, K. (1987). Developmental changes in calmodulin-kinase II activity at brain synaptic junctions: alterations in holoenzyme composition. J. Neurochem. *49*, 1927–1940.

Kennedy, M.B., Bennett, M.K., and Erondu, N.E. (1983). Biochemical and immunochemical evidence that the "major postsynaptic density protein" is a subunit of a calmodulin-dependent protein kinase. Proc. Natl. Acad. Sci. USA *80*, 7357–7361.

Kratz, K.E., and Spear, P.D. (1976). Postcritical-period reversal of effects of monocular deprivation on striate cortex cells in the cat. J. Neurophysiol. *39*, 501–511.

Lisman, J.E. (1994). The CaM kinase II hypothesis for the storage of synaptic memory. Trends Neurosci. *17*, 406–412.

Lisman, J., Schulman, H., and Cline, H. (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. Nat. Rev. Neurosci. *3*, 175–190.

Liu, X.B., and Jones, E.G. (1996). Localization of alpha type II calcium calmodulin-dependent protein kinase at glutamatergic but not gamma-aminobutyric acid (GABAergic) synapses in thalamus and cerebral cortex. Proc. Natl. Acad. Sci. USA *93*, 7332–7336.

Malinow, R., Schulman, H., and Tsien, R.W. (1989). Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science *245*, 862–866.

Miller, S.G., and Kennedy, M.B. (1986a). Regulation of brain type II Ca^{2+}/calmodulin-dependent kinase by autophosphorylation: a Ca^{2+}-triggered molecular switch. Cell *44*, 861–870.

Miller, S.G., and Kennedy, M.B. (1986b). Regulation of brain type II Ca^{2+}/calmodulin-dependent protein kinase by autophosphorylation: a Ca^{2+}-triggered molecular switch. Cell *44*, 861–870.

Mower, G.D., and Christen, W.G. (1989). Evidence for an enhanced role of GABA inhibition in visual cortical ocular dominance of cats reared with abnormal monocular experience. Brain Res. Dev. Brain Res. *45*, 211–218.

Mower, A.F., Liao, D.S., Nestler, E.J., Neve, R.L., and Ramoa, A.S. (2002). cAMP/Ca^{2+} response element-binding protein function is essential for ocular dominance plasticity. J. Neurosci. *22*, 2237–2245.

Pettit, D.L., Perlman, S., and Malinow, R. (1994). Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons. Science *266*, 1881–1885.

Pham, T.A., Impey, S., Storm, D.R., and Stryker, M.P. (1999). CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period. Neuron *22*, 63–72.

Roberts, E.B., Meredith, M.A., and Ramoa, A.S. (1998). Suppression of NMDA receptor function using antisense DNA blocks ocular dominance plasticity while preserving visual responses. J. Neurophysiol. *80*, 1021–1032.

Sillito, A.M., Kemp, J.A., and Blakemore, C. (1981). The role of GABAergic inhibition in the cortical effects of monocular deprivation. Nature *291*, 318–320.

Soderling, T.R. (2000). CaM-kinases: modulators of synaptic plasticity. Curr. Opin. Neurobiol. *10*, 375–380.

Taha, S., and Stryker, M.P. (2002). Rapid ocular dominance plasticity requires cortical but not geniculate protein synthesis. Neuron *34*, 425–436.

Tighilet, B., Hashikawa, T., and Jones, E.G. (1998). Cell- and lamina-specific expression and activity-dependent regulation of type II calcium/calmodulin-dependent protein kinase isoforms in monkey visual cortex. J. Neurosci. *18*, 2129–2146.

Waxham, M.N., Aronowski, J., Westgate, S.A., and Kelly, P.T. (1990). Mutagenesis of Thr-286 in monomeric Ca^{2+}/calmodulin-dependent protein kinase II eliminates Ca^{2+}/calmodulin-independent activity. Proc. Natl. Acad. Sci. USA *87*, 1273–1277.

Wu, G.Y., and Cline, H.T. (1998). Stabilization of dendritic arbor structure in vivo by CaMKII. Science *279*, 222–226.

Wu, G., Malinow, R., and Cline, H.T. (1996). Maturation of a central glutamatergic synapse. Science *274*, 972–976.

Wu, L., Wells, D., Tay, J., Mendis, D., Abbott, M., Barnitt, A., Quinlan, E., Heynen, A., Fallon, J.R., and Richter, J.D. (1998). CPEB-mediated cytoplasmic polyadenylation and the regulation of experience-dependent translation of a-CaMKII mRNA at synapses. Neuron *21*, 1129–1139.

Zou, D.J., and Cline, H.T. (1999). Postsynaptic calcium/calmodulin-dependent protein kinase II is required to limit elaboration of presynaptic and postsynaptic neuronal arbors. J. Neurosci. *19*, 8909–8918.