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Deficient Plasticity in the Primary Visual Cortex of α-Calcium/Calmodulin-Dependent Protein Kinase II Mutant Mice

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Summary

The recent characterization of plasticity in the mouse visual cortex permits the use of mutant mice to investigate the cellular mechanisms underlying activity-dependent development. As calcium-dependent signaling pathways have been implicated in neuronal plasticity, we examined visual cortical plasticity in mice lacking the α-isoform of calcium/calmodulin-dependent protein kinase II (αCaMKII). In wild-type mice, brief occlusion of vision in one eye during a critical period reduces responses in the visual cortex. In half of the αCaMKII-deficient mice, visual cortical responses developed normally, but visual cortical plasticity was greatly diminished. After intensive training, spatial learning in the Morris water maze was severely impaired in a similar fraction of mutant animals. These data indicate that loss of αCaMKII results in a severe but variable defect in neuronal plasticity.

Introduction

During a critical period early in postnatal life, neuronal activity shapes the patterns of connections between neurons in the developing visual system. This process was first described in the primary visual cortex of the cat, where activity drives the segregation of inputs from the two eyes in the first few weeks after eye opening (LeVay et al., 1978; Stryker and Harris, 1986). Depriving one eye of vision during this period leads to a loss of inputs from the deprived eye (Wiesel and Hubel, 1963; Shatz and Stryker, 1978). These and other manipulations of visual experience during this critical period have revealed that these processes require activity and depend on a correlation-based competition between inputs to primary visual cortex (Shatz, 1990).

In contrast, little is known about the molecular basis underlying experience-dependent plasticity. Pharmacological interventions have provided evidence for a role for neurotrophins, neuromodulators, and the N-methyl-D-aspartate (NMDA) receptor (Bear and Singer, 1986; Bear et al., 1990; Maffei et al., 1992), and evidence against a role for nitric oxide synthesis and the metabotropic glutamate receptor (Hensch and Stryker, 1996; Reid et al., 1996; Ruthazer et al., 1996). The utility of a pharmacological approach, however, is limited by the availability of effective and specific reagents that target particular molecules and by the general inability to verify pharmacological effects in vivo.

Some of the limitations of pharmacological experiments can be circumvented by using genetic interventions in genetically accessible organisms such as mice. We have recently shown that a correlation-based competitive plasticity similar to that described in other animals exists during a critical period in the developing mouse visual cortex (Gordon and Stryker, 1996). Brief periods of monocular visual deprivation at this time decrease neuronal responses to stimuli presented to the deprived eye.

The α-subunit of calcium/calmodulin-dependent protein kinase II (αCaMKII) is well situated to play a role in ocular dominance plasticity. Studies of long-term potentiation (LTP) in slices of hippocampus and visual cortex have suggested a role for calcium/calmodulin-dependent protein kinases in these in vitro models of synaptic plasticity (Malinow et al., 1989; Funauchi et al., 1992; Lledo et al., 1995). Expression of αCaMKII, one of at least four isoforms (Braun and Schulman, 1995), increases postnatally to high levels in the neocortex (Kelly et al., 1987; Burgin et al., 1990), and can be altered by manipulations of visual activity (Hendry and Kennedy, 1986). Mice deficient in αCaMKII show deficits in both hippocampal and neocortical LTP (Silva et al., 1992a; A. Kirkwood, A. J. S., and M. Bear, submitted), specifically implicating the α-subunit in these in vitro plasticity models.

Studies of spatial learning, seizure susceptibility, and barrel cortex plasticity have confirmed a role for αCaMKII-dependent processes in neuronal function in vivo (Silva et al., 1992b; Butler et al., 1995; Glazewski et al., 1996). Interestingly, although all αCaMKII-deficient (αCaMKII$^-$) mice showed abnormalities in their susceptibility to seizure induction, there was a striking variability in their individual responses (Butler et al., 1995), raising the possibility that a lack of αCaMKII leads to variable deficits in some aspects of neuronal function. We therefore examined visual cortical responses and plasticity and reexamined spatial learning in mice with a targeted deletion of the gene encoding αCaMKII. We found that although all αCaMKII$^-$ mice developed normal visual cortical responses, half of the mutant animals had dramatically reduced plasticity following monocular deprivation. Furthermore, although all mutants demonstrated impaired spatial learning on the Morris water maze, approximately half of them showed evidence of learning after intensive extended training. These results demonstrate that the loss of αCaMKII leads to severe plasticity deficits in about 50% of animals with the genetic background studied.

Results

Visual Cortical Development

We first determined whether αCaMKII$^-$ mice could develop normal visual cortical responses. Standard electrophysiological techniques were used to record

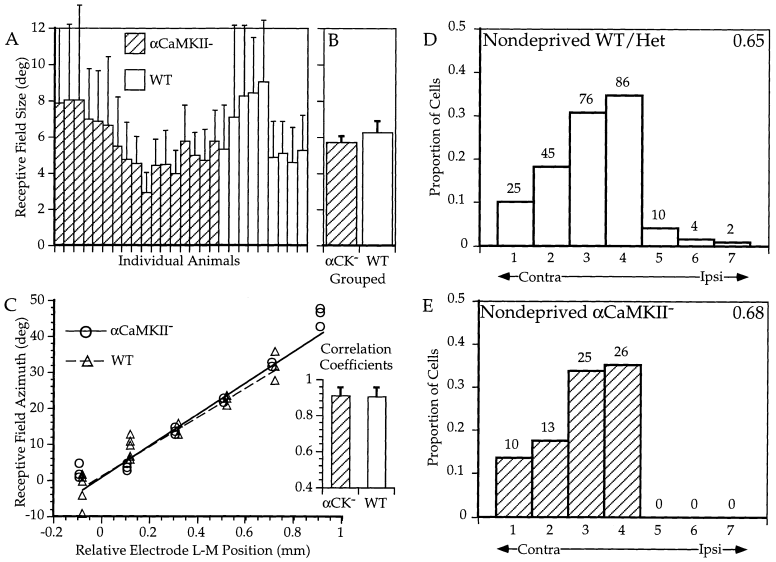

Figure 1. Visual Cortical Responses Develop Normally in αCaMKII-Deficient Mice

(A) Receptive field size in individual αCaMKII⁻ (hatched bars) and wild-type (WT) mice (open bars). Mean ± SD shown (n = 23–49 receptive fields/animal).

(B) Mean ± SEM receptive field size for 17 αCaMKII⁻ and 9 wild-type mice. (n = 521 and 327 receptive fields for αCaMKII⁻ and wild-type mice, respectively).

(C) Receptive field center azimuths of cells encountered in a series of penetrations spaced across the lateromedial extent of V1 in one αCaMKII⁻ (circles) and one wild-type (triangles) mouse are plotted versus electrode position. Lines are linear regressions of azimuth on position. Inset, mean ± SD correlation coefficients of ten and five such regressions from αCaMKII⁻ (hatched bar) and wild-type (open bar) mice, respectively.

(D) and (E) ocular dominance distributions of nondeprived control (D) and αCaMKII⁻ (E) mice. n = 247 and 74 neurons from six (wild-type/heterozygous) and three (αCaMKII⁻) mice, respectively. CBIs (see Experimental Procedures) are shown in the upper right hand corner of each graph.

single-unit activity in the primary visual cortex (V1) of homozygous αCaMKII⁻ mice and heterozygous and wild-type littermate control mice. All recordings were performed blind to genotype. Each neuron was evaluated for the following response properties: receptive field size and location, preference for stimuli of a given orientation, maximum response strength, and ocular dominance (the degree to which the neuron was driven better by inputs from one eye versus the other). Qualitatively, neuronal response properties in αCaMKII⁻ and control mice were normal.

Quantitative comparison of receptive field size, retinotopy, and ocular dominance also failed to reveal differences between mutant and control mice. The distributions of receptive field sizes in 17 αCaMKII⁻ and 9 wild-type mice were overlapping (Figure 1A). There was no significant difference between the mean receptive field sizes of neurons recorded from αCaMKII⁻ mice and wild-type animals (*t* test, $p > 0.25$; Figure 1B). The retinotopic relationship was quantified by plotting the receptive fields of neurons encountered in successive penetrations as the electrode was moved across the lateromedial extent of the cortex. Regressions of receptive field azimuth versus electrode position revealed a linear relationship between these parameters in both mutant and control animals (Figure 1C). This relationship was equally precise in the αCaMKII⁻ and wild-type animals, as demonstrated by the equally high mean correlation coefficients from several such regressions (n = 10 and 5 regressions from eight αCaMKII⁻ and four wild-type animals, respectively; Figure 1C, inset).

Most of mouse V1 is monocular, but the lateral 600 μm, which represent approximately the frontal 30°–40° of the upper portion of each hemifield, receive geniculocortical inputs representing both eyes (Dräger, 1975; Wagor et al., 1980). Within this binocular zone, we found cells responding to input from both eyes; each cell was then evaluated for ocular dominance on a 7-point scale according to the methods of Hubel and Wiesel (1962). The distributions of ocular dominance scores of cells in αCaMKII⁻ and control binocular zones showed the normal bias toward dominance by the contralateral eye and were not significantly different from each other (chi-square test, $p > 0.2$; Figures 1D and 1E). The distributions were quantified and represented as a single parameter, the contralateral bias index (CBI), which is a measure of the degree to which the contralateral eye dominates cortical responses (see Experimental Procedures, and Gordon and Stryker, 1996). The CBIs for each grouped distribution are shown in Figure 1. CBIs were also calculated for each hemisphere individually; the mean and individual CBIs for nondeprived αCaMKII⁻ and control mice were within the previously described normal range (0.6–0.7; Gordon and Stryker, 1996) and are shown in Figure 2.

Visual Cortical Plasticity

Brief periods of monocular deprivation during a critical period shift the responses of neurons recorded from the binocular zone of wild-type mice toward the open eye. As an initial attempt to determine whether this plasticity occurred in these mutants, we monocularly deprived seven αCaMKII⁻ mice for 4–7 days starting between P26 and P33, during the critical period defined for wild-type mice (Gordon and Stryker, 1996). Each deprived mutant was paired with an identically deprived control animal. The CBIs from ocular dominance distributions of binocular zone neurons recorded from each of these animals are shown in Table 1; CBIs below 0.6 are not seen in nondeprived mice and are indicative of a shift in the ocular dominance distribution toward the open ipsilateral eye (Gordon and Stryker, 1996). Monocular deprivation induced a shift in all 7 control mice, but in only 4 of the 7 mutant animals. Moreover, in 6 out of the 7 pairs, the αCaMKII⁻ member of the pair shifted less (Table 1). For these seven pairs, the αCaMKII⁻ animals

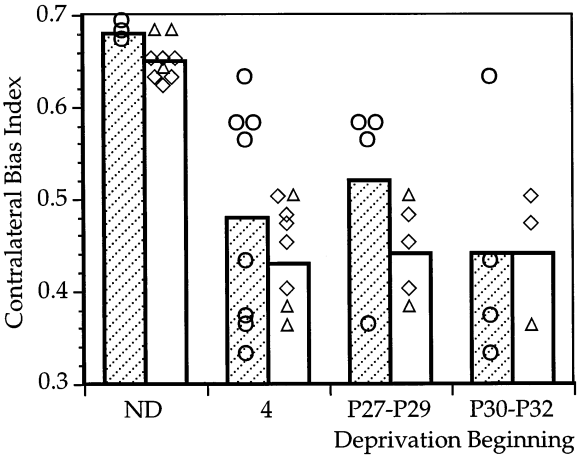

Figure 2. Summary of the Effects of 4 Days of Monocular Deprivation in αCaMKII⁻ Mice

Means (bars) and individual CBIs (symbols) are shown for αCaMKII⁻ (hatched bars, circles), wild-type (WT) (diamonds), and heterozygous (Het, triangles) mice. Wild types and heterozygotes were grouped together for the control means (open bars). ND, nondeprived animals. For P27–P29, and P30–P32, the deprived animals are divided into two groups based on the age at which the deprivation was started. Recordings were made from the hemisphere contralateral to the deprived eye. n = 21–35 cells/animal.

were on average significantly less shifted than controls (paired *t* test, $p < 0.04$). These data suggest that a loss of αCaMKII leads to a deficit in ocular dominance plasticity.

To quantify more carefully the degree of the plasticity deficit, we examined the effects of 4 days of monocular deprivation starting at the peak of the critical period, P27–P32. The minimum time required for a maximal ocular dominance shift in wild-type mice of this age is 4 days; deprivations of this length should be most sensitive to the effects of a mutation affecting plasticity (Gordon and Stryker, 1996). Recordings were made from an additional four αCaMKII⁻ and four control mice, making a total of eight mutant and eight control animals deprived for 4 days at the peak of the critical period. The mean and individual CBIs for these animals are shown in Figure 2. Once again, all control animals shifted in response to monocular deprivation. In contrast, the αCaMKII⁻ animals demonstrated a markedly variable response to 4 days of monocular deprivation. Although the mean CBIs for control and αCaMKII⁻ mice were not statistically different (0.44 and 0.48, respectively; *t* test, $p = 0.22$), the variability of the mutants was significantly greater than that of controls ($p < 0.05$, Siegel–Tukey test). This increased variability was primarily due to a failure of four of the αCaMKII⁻ mice to shift fully in response to monocular deprivation. CBIs from these four mice were outside the range obtained from wild-type animals; mutants deprived at both younger (P27–P29) and older (P29–P32) ages failed to shift fully in response to monocular deprivation (Figure 2). A Monte Carlo analysis of ocular dominance distributions obtained in the mutant animals confirmed that the CBIs were bimodally distributed by rejecting the hypothesis that the results could have been obtained from a single distribution by chance ($p < 0.00001$). The same analysis on the data from the eight control animals was nonsignificant ($p > 0.1$). The decreased shift in half of the mutant mice is also evident in the individually compiled ocular dominance histograms, shown in Figure 3. Grouped ocular dominance histograms from deprived wild-type and heterozygous animals are shown for comparison.

Table 1. CBIs from Identically Deprived αCaMKII⁻ and Control Mice

Deprivation Period	αCaMKII⁻	WT/Het
p26–p32	0.69	0.51
p27–p31	0.57	0.44
p28–p32	0.36	0.49
p29–p33	0.56	0.36
p32–p36	0.63	0.45
p33–p37	0.67	0.58
p30–p37	0.52	0.38

Abbreviations: WT, wild-type; Het, heterozygous.

The demonstration of a variable effect of 4 days of monocular deprivation on binocular responses in the mutants relies on the accuracy of the estimates of the population distribution of ocular dominance scores. An artifactual finding of variable shifts might have been obtained if the sample size of binocular neurons used to calculate CBIs was too small. To rule out this possibility, we divided our samples from each of the eight αCaMKII⁻ mice deprived for 4 days into two populations recorded alternately, and calculated CBIs separately. The CBIs from the odd-sampled neurons are plotted against those of the even-sampled neurons in Figure 4A. Reducing the sample size by half increases the scatter in the CBIs only slightly compared with the distance between the partially and fully shifted groups. These results are consistent with observations from 33 wild-type C57Bl/6 mice, from which we estimate that the degree of variability in the CBI calculated from samples of 20–30 neurons is ~0.05 units (Gordon, 1995). These data demonstrate that the variability seen in the αCaMKII⁻ animals cannot be accounted for by variability in the CBIs as an estimate of the true degree of shift.

An apparently smaller shift might have been obtained artifactually if recordings were made from neurons with receptive fields located more peripherally in the visual field in a subset of the αCaMKII⁻ mice. This possibility follows from the finding that neurons with more centrally located receptive fields tend to shift more toward the open ipsilateral eye (Gordon and Stryker, 1996). To rule out this possibility, we examined the relationship between ocular dominance score and receptive field location in partially and fully shifted αCaMKII⁻ animals deprived for 4 days (Figure 4B). The difference between the fully shifted and partially shifted groups was consistent across all azimuths within the binocular zone, demonstrating that differences in receptive field location of recorded neurons cannot account for the variability of monocular deprivation effects in αCaMKII⁻ mice.

Similarly, differences in the effects of monocular deprivation between animals could have been artifactually generated by recording neurons from different cortical layers in each animal, as the degree of shift depends on laminar location (Gordon and Stryker, 1996). We guarded against this possibility by recording primarily

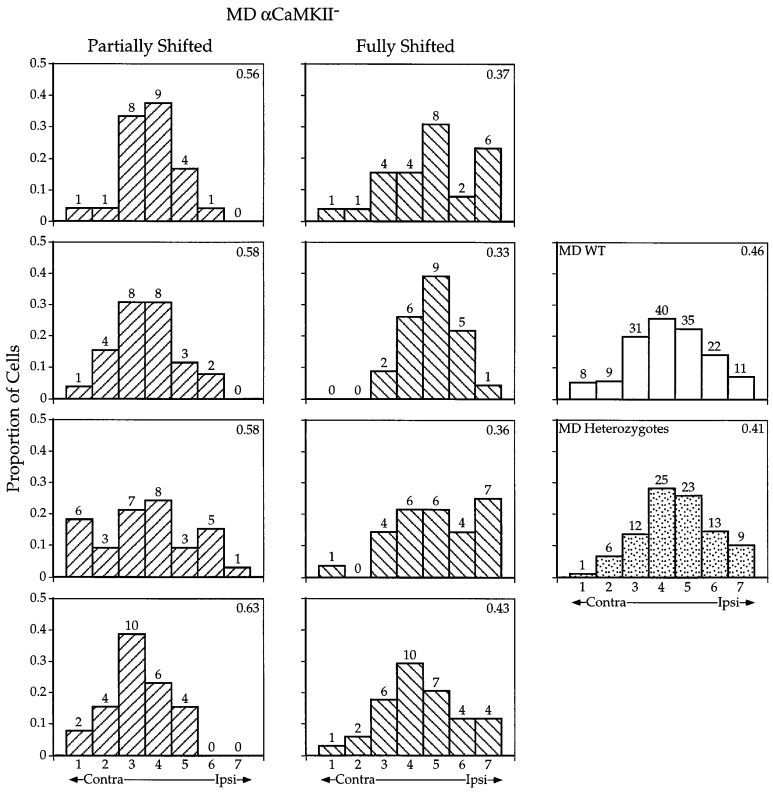

Figure 3. Individual Ocular Dominance Distributions of Eight αCaMKII⁻ Mice Deprived for 4 Days Starting between P27 and P32

Animals that demonstrated a shift toward the open eye equal to that seen in the control mice are shown in the center column (fully shifted). Animals that shifted less are shown in the left column (partially shifted). n = 22–34 neurons/distribution. Right column, grouped ocular dominance distributions from five wild-type (WT, upper graph) and three heterozygous (Het, lower graph) mice deprived for 4 days beginning between P27 and P32. n = 158 and 84 neurons for wild-type and heterozygous distributions, respectively. All recordings were made contralateral to the deprived eye. CBIs are shown in the upper right hand corner of each graph. Note that the most striking differences between fully and partially shifted animals are found in the percentage of cells of ocular dominance class 6 or 7, which are common only among fully shifted animals.

from the supragranular layers in all animals; 85% of recorded cells were within 400 μm of the pial surface. This distribution of depths of neurons recorded from partially and fully shifted animals were not significantly different (chi-square test, $p > 0.9$), and neither were the mean depths (mean ± SEM = 229 ± 16 and 225 ± 17 for partially shifted and fully shifted animals, respectively; *t* test, $p = 0.86$). Histological analysis of electrolytic lesions in at least one penetration per animal confirmed that the great majority of cells were within layers II/III. In the eight αCaMKII⁻ mice deprived for 4 days, 40 of 41 cells localized in this manner were found to be in layers II/III; the remaining cell was in layer IV.

The αCaMKII mutation was originally derived in the 129Ola genetic background (Silva et al., 1992a), and then transferred into the C57Bl/6 genetic background. Importantly, we observed similar variable effects of monocular deprivation in mutants derived from four or seven crosses into the C57Bl/6 background. Additionally, previous studies have shown that mice of the 129 genetic background have normal ocular dominance plasticity (Gordon et al., in press). These results suggest that 129-derived mutations linked to the αCaMKII locus are not responsible for the abnormal effects of monocular deprivation in αCaMKII mutants.

Spatial Learning

The finding of a phenotype that was evident in only half of the mutant mice prompted us to look for evidence of variability of effect in other known deficits in these animals. αCaMKII⁻ mice have severe deficits in hippocampal-dependent learning tasks, including the Morris water maze (Silva et al., 1992b). Training these mutant mice with 12 trials/day for 3 days failed to produce learning in all of the mutants (N = 7), but not in the wild-type animals tested (N = 9; data not shown). To determine whether the αCaMKII⁻ animals might learn with more extended training, we continued to train the mice in the hidden platform version of the water maze for an additional 24 trials (12 trials/day). All control animals learned the task, as measured by both time-to-platform during the final four trials and by time spent in the platform quadrant during a probe trial without a platform (Figure 5). In contrast, the αCaMKII⁻ animals learned on average poorly by both measures, in agreement with our earlier findings (Silva et al., 1992b). However, the mice separate into two groups on the time-to-platform task: 3 of the 7 αCaMKII⁻ mice eventually learned to reach the platform within the same time as controls, whereas 4 showed no evidence of learning (Figure 5A). During a probe trial, the same three mice also focused their searching in the quadrant where the platform had been during training, revealing that they had learned its location (Figure 5B). These data show that intensive training allows about half the αCaMKII⁻ mice to learn the water maze.

Interestingly, we also found variable water maze performance in heterozygous αCaMKII mutants that were progeny from a cross between heterozygotes (four crosses into the C57Bl/6 background) and pure C57Bl/6 mice. Of the 24 animals tested, 10 were unable to learn the hidden platform version of the water maze after 3 days of training (12 trials/day), while only 2 out of 20 of the littermate controls showed a similar impairment (data not shown). Additionally, F1 progeny between 129 and C57Bl/6 mice (n = 10) show normal learning and memory in the water maze (data not shown). All together, these results indicate that 129-derived mutations linked

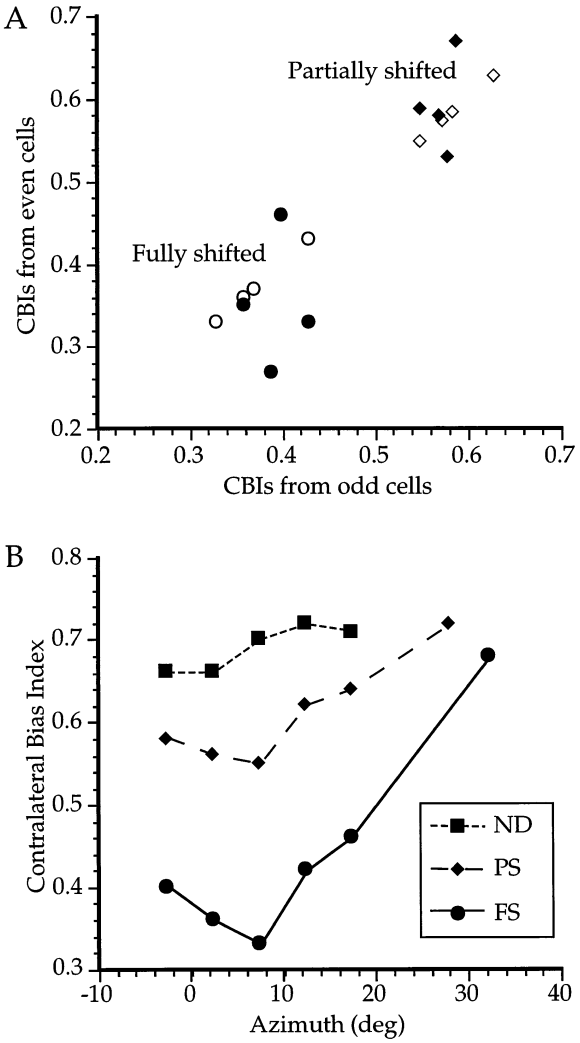

Figure 4. Neither CBI Variability nor Recording Location Can Account for Variability Seen in αCaMKII⁻ Mice

(A) CBI variability. CBIs were calculated independently for alternately encountered neurons in each of the αCaMKII⁻ animals deprived for 4 days beginning between P27 and P32. The graph plots the CBI calculated from the even-numbered cells versus the CBI plotted from the odd-numbered cells for each of the fully shifted (filled circles) and partially shifted (filled diamonds) animals. For comparison, the CBI from the entire sample is plotted against itself for each of the fully shifted (open circles) and partially shifted animals (open diamonds).

(B) Ocular dominance as a function of receptive field position in αCaMKII⁻ mice. CBIs were calculated separately for cells with receptive field center azimuths in the following ranges: −5–0, 1–5, 6–10, 11–15, 15–20, and greater than 20. Squares, nondeprived animals. Diamonds, partially shifted animals. Circles, fully shifted animals.

to the αCaMKII locus are not responsible for the learning phenotype of these mutants.

Discussion

We examined neuronal responses and plasticity in the visual cortex of mice lacking αCaMKII and compared them with littermate controls in experiments blind to genotype. Development appeared to have taken place normally, in that V1 neurons had retinotopically organized receptive fields of normal size. In addition, most neurons in the binocular zone responded to stimuli presented to both eyes, and the distribution of ocular dominance scores reflected the normal bias toward dominance by the contralateral eye. A severe but variable deficit in plasticity, however, was revealed in three different experiments. Pairwise analysis of αCaMKII⁻ and control animals deprived for identical periods of time starting on the exact same postnatal day revealed that αCaMKII⁻ mice shift significantly less for a given deprivation. Furthermore, following brief monocular deprivation, half of the αCaMKII⁻ animals shifted considerably less than any control animals. The bimodal nature of ocular dominance plasticity in the αCaMKII⁻ animals was statistically highly significant. Finally, a similar fraction of αCaMKII⁻ animals were markedly deficient in performance on a spatial learning task even after intensive training.

αCaMKII Deficiency Causes a Variable Defect in Plasticity

Several potential artifactual explanations for the finding of decreased shifts in some of the αCaMKII⁻ animals were considered and rejected. The effects of monocular deprivation in P26–P33 mice are particularly sensitive to age (Gordon and Stryker, 1996), raising the possibility that the decreased shift seen in some αCaMKII⁻ animals was due to the time at which these animals were deprived. Two lines of evidence argue strongly against this possibility. First, examining the effects of 4 days of deprivation separately for those mice deprived starting in earlier (P27–P29) and later (P30–P32) epochs reveals that at least one αCaMKII⁻ animal in each epoch failed to shift completely (Figure 2). Second, the paired analysis of αCaMKII⁻ and control mice deprived at identical times argues that age differences do not account for the effects of the mutation.

Artifactual variability was not induced by inadequate neuronal sampling. Recordings were obtained from a sufficiently large sample of neurons in each animal to rule out the possibility that our estimates of the ocular dominance distributions of the whole population were inaccurate owing to inadequate sample size. Neurons with receptive fields of all eccentricities within the binocular zone were consistently less shifted in those animals with blunted plasticity. Recordings were made primarily from supragranular neurons to rule out the possibility of laminar differences playing a role. Other possible sources of variability investigated included receptive field size, visual responsivity, weight, and gender. There were no consistent differences between fully and partially shifted animals across these parameters. Notably, partially shifted animals developed fully normal visual cortical responses.

A variable deficit was also seen in spatial learning. An earlier report (Silva et al., 1992b) described a severe disruption of spatial learning in the Morris water maze in αCaMKII⁻ mice trained with two trials/day. After intensive extended training (12 trials/day), however, we found that about half of the mutants were able to perform as well as controls. These results clarify the variable nature of the deficit, and strongly argue that an underlying

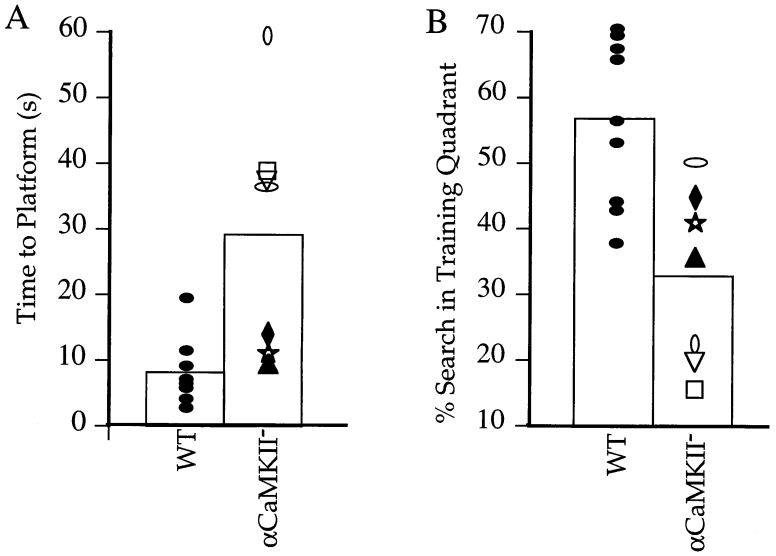

Figure 5. Bimodal Performance in the Water Maze

(A) Time to platform. αCaMKII⁻ mice (n = 7) and controls (n = 9) were trained in the hidden platform version of the water maze for 5 days (12 trials/day). The graph shows the average performance of each control and αCaMKII⁻ on the last block of four trials.

(B) Probe test. After 5 days of training, the escape platform was removed, and the mice were allowed to search for it for 60 s. The graph shows the percent of time each mouse spent searching for the platform in the quadrant where the platform had been during training. Symbols show the performance of an individual animal. Bars show the average of each group. Each αCaMKII⁻ mouse is identified by a different symbol, so that performances during the last four training trials (A) can be compared with performances in the probe trial (B). Interestingly, the three αCaMKII⁻ mice that found the platform as fast as controls during the last four trials of training (1–3) also searched as selectively as controls during the probe trial.

physiological variability, rather than an assay-specific artifact, explains the variable phenotypes. The cortical plasticity and spatial learning findings together support the hypothesis that a lack of αCaMKII leads to a generalized but variable neuronal plasticity deficit. Studying these two phenomena in the same mice, an approach which is not currently technically feasible, could confirm this hypothesis.

Genetic Background and the Effects of the αCaMKII Mutation

The αCaMKII mutation was originally derived in the 129Ola genetic background (Silva et al., 1992a) and then transferred into the C57Bl/6 genetic background by crossing the mutants with C57Bl/6 mice. The complete transfer of the mutation can be difficult because the 129 genomic region immediately around the targeted gene cannot easily be exchanged by C57Bl/6 sequences. While the rest of the genome is freely exchanged during crosses into the C57Bl/6 line, DNA around the targeted locus is not, because the presence of the mutation is selected for. Thus, there may be 129 mutations carried over with the αCaMKII mutation that could affect the phenotype of the mutants. In contrast, the control mice do not preserve this region since it is not selected for during breeding. Our results, however, suggest that this does not confound either the ocular dominance plasticity or the water maze studies described above. Previous studies have shown that mice of the 129 genetic background have normal ocular dominance plasticity (Gordon et al., in press), which indicates that the 129 genetic background left over in the mutants studied does not account for the deficits after monocular deprivation. Albeit less profound, the heterozygous αCaMKII mutants have a similar learning deficit to that described for the homozygotes. This result shows that 129-derived recessive mutations linked to the αCaMKII locus are not responsible for the learning deficits of the homozygotes. Additionally, F1 progeny between 129 and C57Bl/6 mice have normal learning, suggesting that 129-derived mutations linked to the αCaMKII locus could also, in the absence of balancer effects, not account for the learning phenotype of this mutation.

Potential Sources of Phenotypic Variability

The source of the variability seen in the αCaMKII⁻ mice deserves further attention. Since the mutation studied has not been bred into a pure C57Bl/6 background, one possibility is that another gene at an independently segregating locus controls the phenotypic consequences of αCaMKII deficiency. Alternatively, variably activated compensatory mechanisms might fully rescue the deficit in some but not all animals. There are many potential candidate molecules that might interact in such a fashion with αCaMKII; several other isoforms of CaMKII and other multifunctional calcium/calmodulin-dependent protein kinases, as well as other calcium-modulated kinases such as protein kinase C and protein kinase A, are expressed in visual cortex during the critical period (Brandt et al., 1987; Huang et al., 1987, 1988; Cadd and McKnight, 1989; Tsujino et al., 1990; Braun and Schulman, 1995). Genetic experiments, such as attempting to selectively breed plasticity-deficient and plasticity-competent strains of αCaMKII⁻ animals, and biochemical experiments, such as examining the regulation of other kinases in the mutants and correlating these data with plasticity measures, might shed further light on the causes of the phenotypic variability.

Alternatively, some of the αCaMKII⁻ animals might be nonspecifically impaired, owing to some variable developmental abnormality or environmental insult to which the mutants are more sensitive. The visual cortex and learning studies, however, reveal no evidence for this hypothesis, in that nonspecific differences between mutants could not be identified. The observation that seizures occur spontaneously in these mutants raises the possibility that different levels of seizure activity might affect plasticity and learning differently in individual animals (Butler et al., 1995). Seizure activity in the fully

plastic heterozygotes is similarly increased, however (Butler et al., 1995), arguing against this explanation. Furthermore, seizures do not prevent ocular dominance shifts in kittens (Videen et al., 1986).

The Role of αCaMKII in Visual Cortical Plasticity

Regardless of the cause of the variability, the effects of monocular deprivation in αCaMKII⁻ mice, as compared with littermate controls, prove at least a contributory role for CaMKII-dependent processes in ocular dominance plasticity. These data fit well with models of correlation-based plasticity relying on calcium influx through the NMDA receptor as a coincidence detector (Collingridge and Bliss, 1987), and with the more controversial evidence for a specific role of the NMDA receptor in ocular dominance plasticity (Bear et al., 1990; but see Miller et al., 1989). They also are consistent with a role for in vitro models of plasticity such as LTP, some forms of which have been shown to require CaMKII activity (Malinow et al., 1989; Funauchi et al., 1992).

These results considered in concert with recent in vitro studies on the same mice provide some additional guidance regarding the relevance of in vitro models. Kirkwood et al. (A. Kirkwood, A. J. S., and M. Bear, submitted) recently found a severe but incomplete deficit in one form of LTP in visual cortical slices from 5- to 7-week-old αCaMKII⁻ mice, slightly older than the critical period for the effects of monocular deprivation (Gordon and Stryker, 1996). Recording field potentials in layer II/III evoked by stimulation of layer IV, they found a reduction in both the likelihood of obtaining LTP and the magnitude of potentiation. Nonetheless, in young animals, a statistically significant potentiation was obtained in most slices. These data raise the possibility that the quantitative reduction in LTP may be responsible for the reduction in ocular dominance plasticity. To explore further the mechanistic relationship between these two phenomena, one could study LTP in visual cortical slices from the very same mice in which the effects of monocular deprivation were measured. An animal-by-animal correlation between the two deficits would support the notion that the mechanisms responsible for LTP are also responsible for ocular dominance plasticity.

The fact that there is residual plasticity both in vivo and in vitro in these animals raises the following question: what is more relevant to the biology of the system, the quantitative deficit or the residual plasticity? The finding that αCaMKII⁻ mice develop normal visual cortical responses strongly argues that the residual capability for plasticity, evident both in vivo and in vitro, is sufficient. If LTP or ocular dominance plasticity really are involved in development, the system is robust enough to withstand severe deficits. These findings are consistent with many earlier demonstrations that the development of the visual cortex is robust to surprisingly severe quantitative alterations of visual experience (Stryker et al., 1978) and neuronal activity (T. K. Hensch and M. P. S., unpublished data).

Glazewski et al. (1996) recently demonstrated that experience-dependent plasticity of the adult barrel cortex is disrupted in αCaMKII⁻ mice. In contrast, plasticity in the juvenile barrel cortex is wholly intact. These findings suggest that plasticity in the developing barrel and visual cortices utilize different molecular mechanisms.

The finding of variable plasticity in the visual cortex of αCaMKII⁻ mice suggests that αCaMKII plays a role, albeit nonessential, in ocular dominance plasticity. The nature of that role requires further investigation. The mouse model for ocular dominance plasticity will permit one to ask more sophisticated questions as genetic technology improves (Mayford et al., 1995). Inducible and tissue-specific expression technology will soon allow one to ask when, and in which cells, αCaMKII expression is required. These experiments, coupled with an investigation of the role of other components of the CaMKII signaling pathway, promise to help elucidate the cellular mechanisms underlying plasticity in the developing visual system.

Experimental Procedures

Mice

A total of 34 mice were used for the neurophysiological studies of visual cortical plasticity: 14 homozygous αCaMKII⁻ animals, 10 wild-type, and 10 heterozygous littermate controls. Spatial learning was studied in an additional seven homozygous αCaMKII⁻ animals and eight wild-type controls as described previously (Silva et al., 1992b). αCaMKII⁻ mice were generated as described (Silva et al., 1992a, 1992b). The αCaMKII mutation was generated in a 129SvOla genetic background, and then crossed into a C57Bl/6 background. The behavioral studies described were done in animals with four such crosses into the C57Bl/6 background. The cortical plasticity experiments were done in animals with four to seven crosses into the C57Bl/6 background. All experiments were conducted blind to genotype.

Monocular and Binocular Deprivation

Surgical procedures were as described in detail elsewhere (Gordon and Stryker, 1996). In brief, mice of various ages were anesthetized with 3% halothane in a 3:2 mixture of nitrous oxide and oxygen. Lid margins were trimmed and the eye was flushed with saline. Two mattress sutures were placed using 7-0 silk, opposing the full extent of the trimmed lids. Animals were returned to their cages when fully alert. Sutures were checked daily to make sure the eyes remained closed and uninfected. Recordings were made from the binocular zone contralateral to the deprived eye immediately at the conclusion of the deprivation period.

Electrophysiological Recordings

Electrophysiological procedures were adapted from those of Dräger (1975) and Wagor et al. (1980) and were described in detail elsewhere (Gordon and Stryker, 1996). Mice were anesthetized with 50 mg/kg nembutal (Abott) supplemented with 0.2 mg chlorprothixene (Sigma); additional doses of 0.15–0.25 mg of nembutal were given when necessary. Lidocaine (2% anthocaine, Anpro) was applied locally to all incisions. Atropine (0.3 mg, Butler) and dexamethasone (0.05 mg, Anpro) were injected subcutaneously. The temperature of the animal was maintained at 36.5°C and the heart rate was monitored throughout the experiment. The animal was intubated and breathed a mixture of 100% oxygen and room air. The animal was then secured in a custom built stereotaxic device and a large (5 mm by 5 mm) section of bone was removed. The dura was left intact, and the exposure was covered with warm agarose (2.8% in saline). The eyes were then opened, and lids trimmed. The corneas were protected by covering them with silicone oil, and the optic disks projected onto a tangent screen.

Resin-coated tungsten microelectrodes (Hubel, 1957) with tip resistances of 2–4 MOhms were used to record single units from the primary visual cortex. Electrodes were positioned under visual guidance, and correspondence between receptive field locations and published maps (Dräger, 1975; Wagor et al., 1980) was used to locate the binocular zone. In most animals, lesions were located within V1 using Nissl stained sections according to established cytoarchitectonic criteria (Caviness, 1975). Receptive fields of isolated

single units were plotted using a hand-held projection lamp on a tangent screen. Bars of light were varied in size and orientation, where appropriate, to obtain a maximal response; for those cells with orientation selectivity, the preferred orientation was noted. We conservatively defined the binocular zone to be the central most 50° of the upper visual field (25° of each hemifield). The vertical meridian was defined as the intersection of the midline of the animal with the tangent screen. Cells within the binocular zone were assigned ocular dominance scores according to the methods of Hubel and Wiesel (1962). Optimal stimuli were presented to each eye alternately, and the relative strength of the response was determined. Cells were assigned an ocular dominance score of 1 if they responded only to stimuli presented to the contralateral eye, and 7 if they responded only to stimuli presented to the ipsilateral eye. Cells responding equally well to stimuli presented independently to either eye were assigned an ocular dominance score of 4. Ocular dominance scores of 2 or 3 and 5 or 6 were assigned if the cell responded better or was dominated by response to stimuli presented to the contralateral and ipsilateral eye, respectively. The CBI was calculated 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.

Monte Carlo Analysis

Monte Carlo analysis of the bimodality of the ocular dominance distributions obtained from knockout and control animals was performed by first pooling the ocular dominance histograms from each group and then constructing 1×10^6 groups of simulated animals in which the same number of cells as were recorded in each real animal were randomly drawn with equal probability from the pooled distribution of the group. The standard deviations and interquartile ranges of the real groups were compared with the distributions of these values from the simulated groups to evaluate the probability that the real values within each group were obtained from the pooled distribution of the group.

Histology

Electrolytic lesions (4.5 μA for 4.5 s) were placed in one to two penetrations per animal. At the end of each experiment the animal was given an overdose of nembutal and perfused transcardially with 0.5 M phosphate-buffered saline (PBS) followed by 10% formaldehyde in PBS. After postfixation, the brain was removed, cryoprotected in 30% sucrose–10% formaldehyde, and cut into 40-μm sections. Sections were mounted on slides, defatted, and stained with cresylecht violet. Cytoarchitectonic borders of Area 17 were determined as described in Caviness (1975).

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