α-CaMKII–dependent plasticity in the cortex is required for permanent memory

Paul W. Frankland†, Cara O’Brien†, Masuo Ohno†, Alfredo Kirkwood† & Alcino J. Silva*

†Departments of Neurobiology, Psychiatry and Psychology, Brain Research Institute, University of California, Los Angeles, California 90095-1761, USA
‡Department of Neuroscience and Mid/Brain Institute, Johns Hopkins University, Baltimore, Maryland 21218, USA

Cortical plasticity seems to be critical for the establishment of permanent memory traces1–3. Little is known, however, about the molecular and cellular processes that support consolidation of memories in cortical networks4,5. Here we show that mice heterozygous for null mutation of α-calcium-calmodulin kinase II (α-CaMKII−/−) show normal learning and memory 1–3 days after training in two hippocampus-dependent tasks. However, their memory is severely impaired at longer retention delays (10–50 days). Consistent with this, we found that α-CaMKII−/− mice have impaired cortical, but not hippocampal, long-term potentiation. Our results represent a first step in unveiling the molecular and cellular mechanisms underlying the establishment of permanent memories, and they indicate that α-CaMKII may modulate the synaptic events required for the consolidation of memory traces in cortical networks.

A homozygous null mutation of α-CaMKII blocks hippocampal long-term potentiation (LTP) and learning6,7, and disrupts experience-dependent plasticity in the sensory cortex without affecting cortical topography8,9. In contrast, α-CaMKII heterozygous mice learn normally in hippocampus-dependent tasks under moderately intensive training conditions10. We were, therefore, able to study the impact of reduced levels of α-CaMKII on the establishment of permanent memories. We trained α-CaMKII−/− mutants and their wild-type littermate controls in contextual fear conditioning. In contextual fear conditioning, an association is formed between a distinctive context and an aversive event that takes place in that context. When placed back in the context, mice exhibit a range of conditioned fear responses, including freezing (the absence of all but respiratory movement). This is a particularly robust and long-lasting form of learning that is dependent on the hippocampus11,12. Importantly, post-training lesions of the hippocampus disrupt recent, but not remote, contextual fear memories10.

After training with three shocks, α-CaMKII−/− mutants and wild-type mice were placed back in the context at different times (1, 3, 10, 17 or 50 days), and freezing behaviour was recorded using automated procedures12. To avoid the confounding effects of extinction, separate groups of mice were tested at each retention delay. Whereas wild-type mice showed stable contextual freezing at all test delays, contextual freezing declined sharply at longer test delays in α-CaMKII−/− mutants (genotype × delay interaction, F(4, 102) = 17.6, P < 0.01) (Fig. 1a).

During training, α-CaMKII−/− mutants were around 20% more active than the wild-type mice before shock delivery (F(1, 102) = 14.8, P < 0.05) (data not shown). We therefore assessed contextual memory with a different measure that is not affected by differences in basal activity. In the same mice, we calculated activity suppression by comparing activity levels before a shock delivery during training with activity levels during the test, thus normalizing for initial differences in activity12. Although wild-type and α-CaMKII−/− mice showed indistinguishable levels of activity suppression one day after training, at longer delays, activity
Suppression was impaired in α-CaMKII+/– mutants but not in wild-type controls (genotype × delay interaction, \( F(4,102) = 28.5, P < 0.05 \)) (Fig. 1b). Post-hoc analyses confirmed that levels of activity suppression were greater in wild-type controls than in α-CaMKII+/– mutants at all retention delays except at day one (\( P < 0.05 \)).

We next tested whether intense training could compensate for the memory deficits of the α-CaMKII+/– mutants. We trained mice with eight shocks and tested separate groups 1, 3, or 17 days after training. Consistent with our previous experiment, freezing declined in mutants, but not in wild-type controls, at longer test delays (genotype × delay interaction, \( F(2,41) = 9.1, P < 0.05 \)) (Fig. 1c). Post-hoc analyses confirmed that freezing was lower in α-CaMKII+/– mutants only in the 3- and 17-day tests (\( P < 0.05 \)). A similar pattern was observed when activity suppression was used as an index of contextual fear (Fig. 1d). Activity suppression was impaired in α-CaMKII+/– mutants, but not wild-type mice, at longer test delays (genotype × delay interaction, \( F(2,41) = 11.0, P < 0.05 \)). Therefore, after either moderate (three shocks) or intense (eight shocks) training, memory loss in α-CaMKII+/– mutants is specific to longer retention delays. Protein-synthesis blockers impair contextual memory 3–5 hours after training. The similar levels of contextual fear in the mutant and wild-type mice 24 h after training indicate that protein synthesis-dependent processes underlying the initial formation of hippocampus-dependent memories are unaffected in these mutant mice. Notably, the time course of memory decay in α-CaMKII+/– mutants corresponds to the shift from hippocampus-dependent to hippocampus-independent retrieval inferred from imaging and lesion studies in mice and rats. For example, the genetic disruption of NMDA (N-methyl-D-aspartate) receptors in the CA1 region of the hippocampus during the 0–14-day period after training blocks contextual memories in mice. In contrast, similar disruption three weeks after training leaves these memories intact.

Under less intensive training conditions, α-CaMKII+/– mutants exhibit learning impairments that are affected by genetic background (data not shown). Thus, despite equivalent levels of freezing in the retention test at day one, the initial underlying memory trace might be weaker in α-CaMKII+/– mutants, and hence less durable. To address this, we compared contextual memory in wild-type mice after weak training (one shock) with contextual memory in α-CaMKII+/– mutants that had been trained intensively (eight shocks). Wild-type mice, trained at the same time as the α-CaMKII+/– mutants, were tested 1, 3 or 17 days after training. WT mice exhibited much lower freezing levels than α-CaMKII+/– mutants at the one-day retention delay. However, unlike the mutants, their levels of freezing were similar at all retention intervals (genotype × delay interaction, \( F(2,54) = 13.7, P < 0.05 \)) (Fig. 1e), indicating that lower initial levels of freezing do not necessarily result in unstable memory. Post-hoc analyses revealed that α-CaMKII+/– mutants froze more than wild-type controls one day after training, but significantly less than wild-type controls 17 days after training (\( P < 0.05 \)).

To assess the severity of memory loss in mutants at long delays, we compared the acquisition of contextual fear conditioning in naive (non-trained) α-CaMKII+/– mutants and α-CaMKII+/– mutants that had been trained 30 days before with three shocks. Both naive and previously trained mutants showed similar rates of acquisition of contextual freezing when trained with one shock per day over four days (Fig. 1f); this was supported by an absence of a condition (trained versus naive) × day (1–4) interaction (\( F(3,30) = 0.20, P = 0.89 \)); the absence of accelerated acquisition (or behavioural savings) in the previously trained mutants is consistent with the hypothesis that memory loss at long retention delays is substantial.

To test whether our initial findings applied to another form of hippocampal learning, we trained α-CaMKII+/– mutants in the Morris water maze. In this spatial learning task, mice learn to navigate to a submerged platform by using extra-maze cues. Mice were trained with 12 trials per day over 5 days, as under these conditions α-CaMKII+/– mutants performed this task as well as wild-type controls. At the completion of training, spatial learning was assessed in a 60-s probe test where the platform was removed from the pool (Fig. 2a–c). To assess spatial memory at different retention delays, separate groups of mice were tested 3, 10 or 17 days after completing the training (Fig. 2d–f). No other training was given between the probe test at the end of training and the memory probe test. In probe tests at the completion of training, wild-type and α-CaMKII+/– mice spent equivalent amounts of time in the target quadrant of the pool (\( F(1,49) = 0.93, P = 0.34 \)). Similarly, α-CaMKII+/– mutants exhibited normal spatial memory when tested three days after completing the training (\( F(1,16) = 0.37, P = 0.55 \)). Therefore, consistent with the fear conditioning data, α-CaMKII+/– mutants showed normal acquisition and normal

Figure 1 Contextual memory in α-CaMKII+/– mutants. a, b, Freezing and activity suppression for wild-type (WT) and α-CaMKII+/– mice trained with three shocks and tested at different retention delays. c, d, Freezing and activity suppression for WT and α-CaMKII+/– mice trained with eight shocks and tested at different retention delays. e, Freezing at different retention delays in WT mice after weak training (one shock) and α-CaMKII+/– mice after intensive training (eight shocks). f, Acquisition of contextual freezing in previously trained and naive α-CaMKII+/– mutants.
memory at short retention delays, indicating that motivation, vision and motor skills required for the task were not affected. However, $\alpha$-CaMKII$^{-/-}$ mutants spent significantly less time searching the target quadrant when tested 10 days (F(1, 15) = 15.2, $P < 0.05$) and 17 days (F(1, 18) = 10.1, $P < 0.05$) after the completion of training. Notably, there was a significant genotype $\times$ delay interaction (F(2, 49) = 3.23, $P < 0.05$), reflecting poorer spatial memory in $\alpha$-CaMKII$^{-/-}$ mutants only at long retention delays (Fig. 2g). Retrograde amnesia after hippocampal lesions is typically not graded in water maze studies$^{1–3}$, possibly because the hippocampus is required for some aspects of performance during navigation, such as path integration$^{15–16}$. However, under the training conditions used, $\alpha$-CaMKII$^{-/-}$ mutants learned the water maze normally, indicating that the processing required for performance of the task, such as path integration, is not affected under these conditions in these mice. Rather, the poor performance at long retention delays is likely to reflect loss of spatial memory.

These data show that $\alpha$-CaMKII$^{-/-}$ mutants have deficits in memory at a time when memories become more dependent on cortical function, and less on the hippocampus$^{10,14}$. Whereas hippocampal plasticity is required for initial learning and memory formation, cortical plasticity is thought to be critical for the establishment of permanent memory traces$^{1–3,14}$. Therefore, hippocampal and cortical synaptic plasticity may be differentially affected in $\alpha$-CaMKII$^{-/-}$ mutants$^{2–3}$. To investigate this possibility, we examined LTP induced by five $\theta$-burst stimulations (TBS) in the hippocampus and cortex in the same slices from $\alpha$-CaMKII$^{-/-}$ mutants and wild-type mice (Fig. 3). This strong induction protocol saturates LTP in the cortex (A.K., unpublished observations). Memory is likely to be widely distributed within cortical networks$, so we chose to study the visual cortex as a representative of primary sensory cortex and the temporal cortex as a representative of associational cortex. Stimulation of Schaffer collaterals produced stable LTP in the CA1 region of the hippocampus of wild-type (t(10) = 7.17, $P < 0.05$) and of $\alpha$-CaMKII$^{-/-}$ mice (t(8) = 4.54, $P < 0.05$), and the magnitude of LTP did not differ between the two (F(1, 18) = 1.83, $P = 0.19$). In addition, protein-synthesis-
dependent late LTP, induced by three trains of ten TBS, was normal in α-CaMKIIΔ mutants (F(1, 11) = 2.87, P > 0.05). Post-tetanic potentiation was reduced in α-CaMKIIΔ mutants. This is consistent with our behavioural data showing that memory is normal in α-CaMKIIΔ mutants up to three days after training. In contrast, stimulation of layer IV failed to produce LTP in visual cortex (t(10) = 1.30, P > 0.05) and temporal cortex (t(22) = 1.82, P > 0.05) in α-CaMKIIΔ mutants. Similar stimulation produced stable LTP in visual cortex (t(18) = 4.23, P < 0.05) and temporal cortex (t(21) = 7.00, P < 0.05) in wild-type mice, and these levels of potentiation were significantly higher than those in α-CaMKIIΔ mutants (visual cortex: F(1, 28) = 5.87, P < 0.05; temporal cortex: F(1, 43) = 15.3, P < 0.05). Consistent with previous data, these results indicate that LTP impairments in α-CaMKIIΔ mutants are restricted to the cortex22. Both hippocampal and cortical LTP are impaired in α-CaMKII homozygous mice25. The α-CaMKII protein is expressed more abundantly in the hippocampus than in the cortex, so it is likely that the loss of half the normal levels of this kinase affects cortical plasticity more severely.

In humans and animals, damage limited to the hippocampal formation produces a transient amnesia for recently acquired information that is declarative or episodic in nature, but spares memory for similar remotely acquired information10,22–24. The opposite pattern is observed in cases of semantic dementia associated with atrophy of the temporal cortex, which leads to impairments of remote, rather than recent, memory2. Consistent with this, retrieval of recently acquired spatial information is associated with activation of the hippocampus, whereas retrieval of remotely acquired spatial information is associated with activation of the cortex in mice24. This has led to the idea that storage and retrieval of recent memories is dependent on hippocampal networks, whereas storage and retrieval of remote memories depends on cortical1–3 or cortical–hippocampal25 networks. Although hippocampal lesions preferentially disrupt recent but not remote (or permanent) memories, we found that a functional disruption of cortical plasticity preferentially disrupts the establishment of permanent memories. Furthermore, the time course of the memory decay in the α-CaMKIIΔ mutants corresponds to that predicted by imaging and lesion studies in mice and rats10,14,15,26. In addition, we found that mice that are heterozygous for a point mutation that substitutes threonine 286 for alanine in α-CaMKII (α-CaMKIIΔT286/E)27,28 exhibit normal contextual and spatial memory for up to 50 days (data not shown). Because cortical plasticity is not impaired in these mice28, this finding strengthens the conclusion that normal cortical plasticity is required for the establishment of permanent memory traces. The consolidation of memory traces in cortical networks is likely to depend on the recurrent activation of cortical traces during, for example, sleep1. Our results indicate that repetitive LTP-like events, which are dependent on α-CaMKII, are required for the consolidation of memory traces in cortical networks, and they represent a first step in unveiling the molecular and cellular mechanisms underlying cortical memory consolidation. They further indicate that the molecular processes underlying the establishment of permanent cortical memory traces may share some of the same mechanisms as those underlying initial encoding of hippocampal memories.

Methods

Mice

The α-CaMKIIΔ mice used in the fear conditioning and electrophysiological experiments were the F1 progeny from a cross between α-CaMKIIΔ mice in the C57Bl/6 background (> 99%) and 129/SvEms mice. For the water maze studies, mice were tested from two different genetic backgrounds with similar findings. The two backgrounds tested included mice that were the offspring from a cross between α-CaMKIIΔ mice in the C57Bl/6 background (> 99%) and 129/SvEms mice, and mice resulting from a cross between chimeras having the α-CaMKIIΔ mutation in the 129/Ola background and C57Bl/6 mice. The C57Bl/6 background exacerbates the effects of the α-CaMKIIΔ heterozygous mutation (data not shown). Mice were housed in groups and maintained on a 12 h light/dark cycle.
A new role for cryptochrome in a Drosophila circadian oscillator

Balaji Krishnan†‡, Joel D. Levine†‡, M. Kathleen S. Lynch†, Harold B. Douvès§, Pablo Funes, Jeffrey C. Hall†, Paul E. Hardin† & Stuart E. Dryer†

† Department of Biology and Biochemistry and Biological Clocks Program, University of Houston, Houston, Texas 77204, USA
‡ Department of Biology and NSF Center for Biological Timing, Brandeis University, Waltham, Massachusetts 02454, USA
§ Department of Biological Sciences, University of Maine, Orono, Maine 04469, USA

* These authors contributed equally to this work

Cryptochromes are flavin/pterin-containing proteins that are involved in circadian clock function in Drosophila and mice. In mice, the cryptochromes Cry1 and Cry2 are integral components of the circadian oscillator within the brain and contribute to circadian photoreception in the retina. In Drosophila, cryptochrome (CRY) acts as a photoreceptor that mediates light input to circadian oscillators in both brain and peripheral tissue. A Drosophila cry mutant, cryB, leaves circadian oscillator function intact in central circadian pacemaker neurons but renders peripheral circadian oscillators largely arrhythmic. Although this arrhythmicity could be caused by a loss of light entrainment, it is also consistent with a role for CRY in the oscillator. A peripheral oscillator drives circadian olfactory responses in Drosophila antennae. Here we show that CRY contributes to oscillator function and physiological output rhythms in the antenna during and after entrainment to light–dark cycles and after photic input is eliminated by entraining flies to temperature cycles. These results demonstrate a photoreceptor-independent role for CRY in the periphery and imply fundamental differences between central and peripheral oscillator mechanisms in Drosophila.

We initially established that the cryB mutant severely reduces or eliminates circadian rhythms in olfactory responses and molecular rhythms in oscillator components during and after entrainment to light–dark cycles. Electroantennogram (EAG) responses to the food odorant ethyl acetate were measured in wild-type and cryB flies under 12 h:12 h light–dark cycles (LD) and on the second day of constant darkness (DD) after LD entrainment. In cryB mutants, we observed EAG responses of similar magnitude throughout the day except for a weak but statistically significant peak at Zeitgeber time 17 (5 h after lights off; Fig. 1a), indicating that the cryB allele is nearly a loss-of-function mutant. This small peak was lost when mutant antennae were recorded in DD (Fig. 1b). Thus, cryB is required for a...