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Genetic Demonstration of a Role for PKA in the Late Phase of LTP and in Hippocampus-Based Long-Term Memory

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Summary

To explore the role of protein kinase A (PKA) in the late phase of long-term potentiation (L-LTP) and memory, we generated transgenic mice that express R(AB), an inhibitory form of the regulatory subunit of PKA, only in the hippocampus and other forebrain regions by using the promoter from the gene encoding Ca^{2+}/calmodulin protein kinase IIα. In these *R(AB)* transgenic mice, hippocampal PKA activity was reduced, and L-LTP was significantly decreased in area CA1, without affecting basal synaptic transmission or the early phase of LTP. Moreover, the L-LTP deficit was paralleled by behavioral deficits in spatial memory and in long-term but not short-term memory for contextual fear conditioning. These deficits in long-term memory were similar to those produced by protein synthesis inhibition. Thus, PKA plays a critical role in the consolidation of long-term memory.

Introduction

Genetically modified mice have provided a powerful new tool to explore the role of specific signal transduction pathways in neuronal physiology and behavior, and to examine the correlation between explicit memory storage and hippocampal long-term potentiation (LTP), an activity-dependent form of synaptic plasticity (Bliss and Collingridge, 1993). LTP in the Schaffer collateral pathway appears to be critical for learning and memory (Mayford et al., 1995; Tsien et al., 1996). Thus far, most of these studies have focused on an early, transient phase of LTP (E-LTP) that lasts about an hour. They have shown that genetic manipulation of any one of several kinases, including Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) and the tyrosine kinase Fyn, interferes with not only E-LTP, but also short-term memory (Mayford et al., 1995).

The study of amnestic patients and experimental animals has revealed, however, that the role of the hippocampus in memory storage extends from weeks to months (Squire and Alvarez, 1995), suggesting that longer lasting forms of hippocampal synaptic plasticity may be required. In contrast to E-LTP, the late phase of LTP (L-LTP) lasts for up to 8 hr in hippocampal slices (Frey et al., 1993) and for days in the intact animal (Abraham et al., 1993). However, it remains unclear how this phase of LTP correlates with memory storage. Long-term memory storage is sensitive to disruption by inhibitors of protein synthesis (Davis and Squire, 1984), and L-LTP in the CA1 region of hippocampal slices, unlike E-LTP, shares with long-term memory a requirement for translation and transcription (Frey et al., 1988, 1993; Huang and Kandel, 1994; Nguyen et al., 1994).

Pharmacological experiments have suggested that cAMP-dependent protein kinase A (PKA) plays a critical role in the L-LTP (Frey et al., 1993; Huang and Kandel, 1994). One of the nuclear targets of PKA is the cyclic AMP response element–binding protein (CREB), and CRE-mediated gene expression is induced in response to stimuli that generate L-LTP (Impey et al., 1996). Behavioral studies of mice lacking the α and δ isoforms of CREB have suggested that this transcription factor plays a role in long-term memory storage, but the relationship between these memory deficits and L-LTP is unclear, because a deficit in LTP is observed during E-LTP following a single stimulus train (Bourtchouladze et al., 1994). Moreover, because CREB is a multifunctional transcription factor that can be activated by second messenger systems other than PKA, including CaM kinases and the MAP kinase pathway (Sassone-Corsi, 1995; Deisseroth et al., 1996; Xing et al., 1996), these data on *CREB* knockout mice do not define a role for PKA in long-term memory.

By analyzing knockout mice lacking individual PKA subunit isoforms, it has not yet been possible to obtain compelling genetic data linking PKA to L-LTP on the one hand and to memory on the other (Brandon et al., 1995; Huang et al., 1995; Wu et al., 1995; Qi et al., 1996). We have taken a transgenic approach to reduce PKA activity in the hippocampus by using R(AB), a dominant negative form of the regulatory subunit of PKA. R(AB) carries mutations in both cAMP binding sites and acts as a dominant inhibitor of both types of PKA catalytic subunits (Clegg et al., 1987; Ginty et al., 1991). Because conventional knockout mice lacking the RIβ or $C\beta_1$ subunits of PKA did not show alterations in PKA activity, perhaps due to compensatory changes in the level of other PKA subunits (Brandon et al., 1995; Qi et al., 1996), we have used the promoter from the *CaMKIIα* gene to limit expression of R(AB) to the postnatal forebrain (Mayford et al., 1996). In *R(AB)* transgenic mice, PKA activity was reduced, and L-LTP was decreased in area CA1. Behaviorally, the *R(AB)* transgenics exhibited normal short-term memory but deficient long-term memory. The time course of the memory deficit of *R(AB)* transgenics in contextual fear conditioning parallels that of wild-type animals treated with the protein synthesis inhibitor anisomycin. Thus, the PKA pathway is critically important for the consolidation of short-term memory into protein synthesis dependent long-term memory, perhaps because PKA induces the transcription of genes encoding proteins required for long-lasting synaptic potentiation.

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Results

Generation of *R(AB)* Transgenic Mice

To reduce PKA activity selectively within the hippocampus and other forebrain regions, we have used the *CaMKII*α promoter to express R(AB), an inhibitory form of the RIα subunit of PKA (Clegg et al., 1987; Ginty et al., 1991). An 8.5 kb portion of the *CaMKII*α promoter, containing upstream control regions and the transcriptional initiation site, drives expression postnatally in the hippocampus, neocortex, amygdala, and striatum (Mayford et al., 1996). The *R(AB)* cDNA was flanked by a human growth hormone polyadenylation signal at the 3′ end and by a 5′ untranslated leader containing a heterologous intron, elements which have been shown to enhance expression of transgenes (Choi et al., 1991). This hybrid *R(AB)* construct was then placed under the control of the *CaMKII*α promoter (Figure 1D), and transgenic mice were generated by pronuclear injection. Four independent founder animals were obtained, three of which bred successfully and transmitted the transgene. These lines, designated *R(AB)-1*, *R(AB)-2*, and *R(AB)-3*, carried 7 copies, 16 copies, and 1 copy of the transgene, respectively, as determined by Southern blot analysis (data not shown). Two of these lines, *R(AB)-1* and *R(AB)-2*, have been analyzed in detail in this study. Northern blot analysis of forebrain RNA, using a probe specific for the 3′ end of the transgene, revealed that a transcript of about 1.5 kb is expressed at similar levels in each transgenic line (data not shown).

Expression of the *R(AB)* Transgene in the Forebrain

To determine the distribution of transgene expression in the brain, we used in situ hybridization using a transgene-specific oligonucleotide. In both lines, the transgene was expressed in the neocortex, olfactory bulb, hippocampus, striatum, and amygdala (Figure 1). In the hippocampus, the *R(AB)* transgene is present throughout hippocampal areas CA1, CA3, and the dentate gyrus. To explore potential neuroanatomical alterations in transgenic animals, we compared Nissl-stained sagittal sections of brains from transgenic and wild-type mice and observed no gross anatomical abnormalities within the hippocampus or other regions of transgenic animals (data not shown).

Hippocampal PKA Activity Is Reduced in *R(AB)* Transgenics

To determine whether the *R(AB)* transgene functioned in an inhibitory manner, we measured PKA activity in hippocampal extracts (Figure 2). The transgenic animals showed a 50% reduction in basal PKA activity (wild type [WT]: 499 $\pm$ 44 pmol/min/mg, n = 6; *R(AB)-1*: 239 $\pm$ 6 pmol/min/mg, n = 3, $p < 0.002$; *R(AB)-2*: 117 $\pm$ 16 pmol/min/mg, n = 3, $p < 0.0002$). When the kinase was activated by 5 μM cAMP, PKA activity was also significantly reduced by 25% in the mutant mice (WT: 4077 $\pm$ 360 pmol/min/mg, n = 6; *R(AB)-1*: 3223 $\pm$ 171 pmol/min/mg, n = 3, $p < 0.02$; *R(AB)-2*: 2933 $\pm$ 296 pmol/min/mg, n = 3, $p < 0.04$).

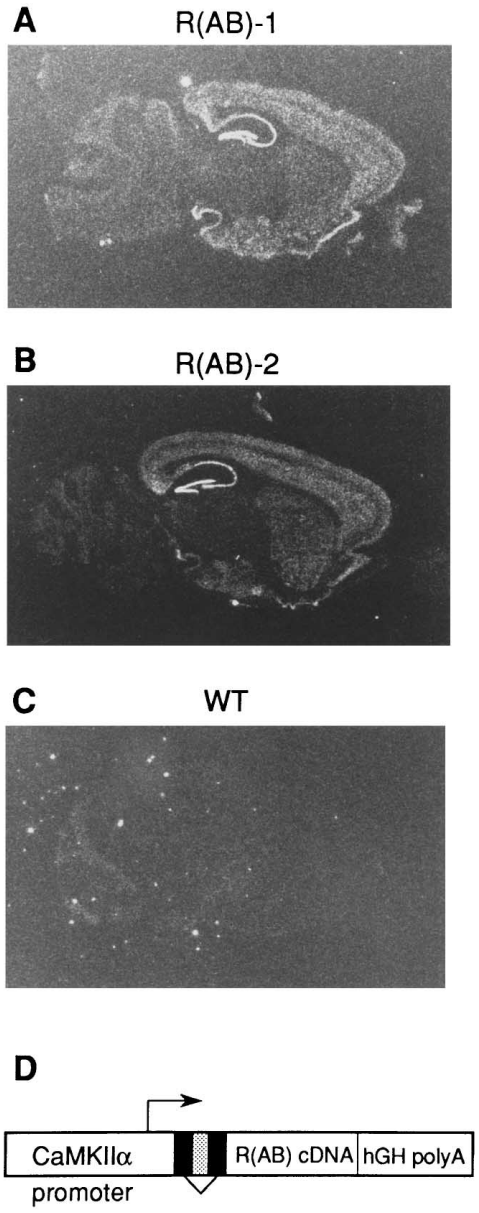

Figure 1. Regional Distribution of *R(AB)* Transgene Expression

Sagittal sections from the brains of *R(AB)* transgenic mice (A and B) and a wild-type littermate control animal (C) were hybridized with a probe specific to the *CaMKII-R(AB)* transgene. Expression of the transgene is seen throughout the hippocampus and in the cortex, olfactory bulb, amygdala, and striatum. (D) The *CaMKII-R(AB)* transgene contains an 8.5 kb *CaMKII*α gene promoter, a hybrid intron in a 5′ untranslated leader (closed boxes), the coding region of *R(AB)*, and a human growth hormone (hGH) polyadenylation signal.

Basal Synaptic Physiology and Posttetanic Potentiation Are Unaffected in *R(AB)* Transgenic Mice

As an initial step toward the electrophysiological analysis of *R(AB)* transgenic mice, we examined basal synaptic function in hippocampal area CA1. Slices obtained from wild-type and mutant animals did not show any marked difference in their input–output coupling during extracellular stimulation of Schaffer collaterals (Figure

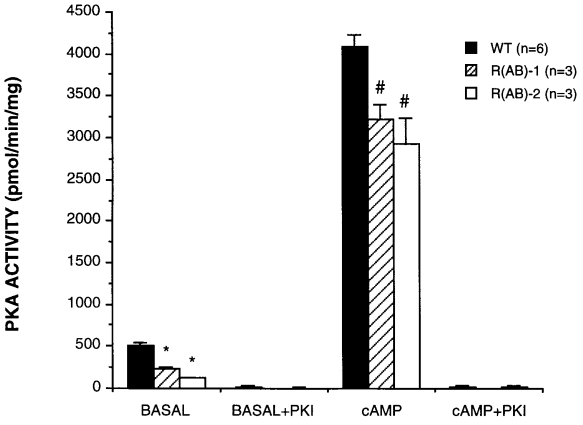

Figure 2. PKA Activity Is Reduced in Hippocampal Extracts Prepared from *R(AB)* Transgenic Mice

PKA activity was measured in extracts prepared from hippocampi of *R(AB)* transgenic mice (*R(AB)-1*, n = 3; *R(AB)-2*, n = 3) and wild-type littermate controls (n = 6). Twenty micrograms of total protein was used in each assay, and the phosphorylation of the Kemptide substrate was determined in the absence (basal) or presence (cAMP) of 5 μm cAMP. Stars, $p < 0.01$; pound signs, $p < 0.05$.

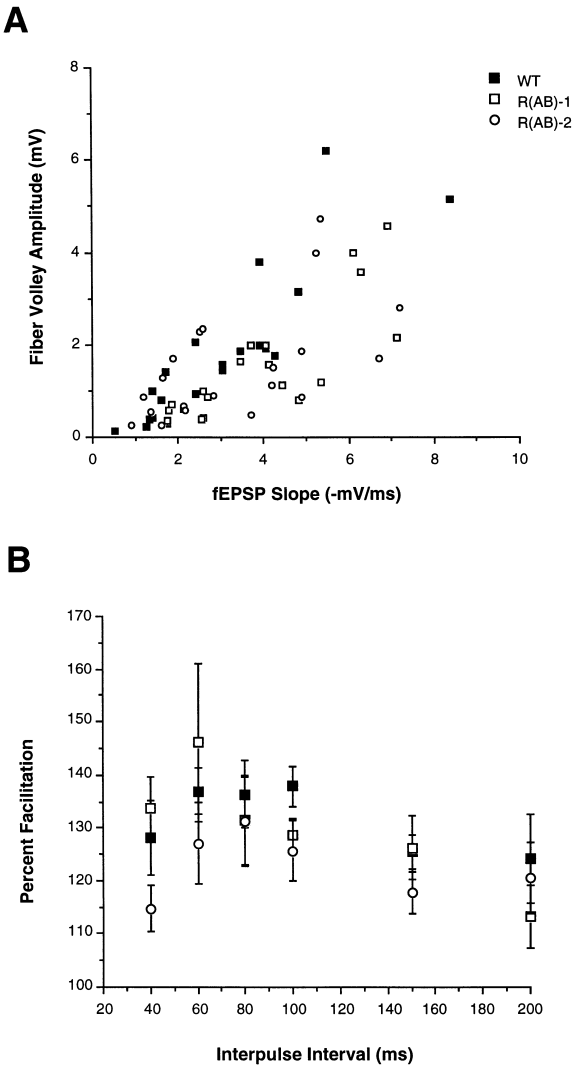

Figure 3. Basal Synaptic Transmission and Paired-Pulse Facilitation Are Normal in *R(AB)* Transgenic Mice

(A) Scatter plot of fEPSP slopes and their corresponding presynaptic fiber potentials shows no marked difference or trend in the distribution of values obtained from mutant (*R(AB)-1*, n = 6 mice, 17 slices; *R(AB)-2*, n = 5 mice, 20 slices) and wild-type mice (n = 8 mice, 22 slices).

(B) Percent facilitation, calculated from the ratio of the second fEPSP slope to the first fEPSP slope, is shown at interpulse intervals ranging from 40 ms to 200 ms. At all interpulse intervals, no significant differences in paired-pulse facilitation were observed between mutants and wild-type controls (at 200 ms, $p > 0.30$; at 150 ms, $p > 0.15$; at 100 ms, $p > 0.08$; at 80 ms, $p > 0.63$; at 60 ms, $p > 0.30$). At 40 ms interpulse interval, however, the two lines of transgenic mice are significantly different from each other ($p < 0.05$), although not significantly different than wild-type ($p > 0.1$). *R(AB)-1*, n = 6 mice, 17 slices; *R(AB)-2*, n = 6 mice, 10 slices; WT, n = 10 mice, 23 slices.

3A). Similarly, we found no significant differences between the mean ratios of fEPSP slopes to presynaptic fiber volley sizes in wild-type and transgenic mice: mean ratios were -2.60 ± 0.33 ms^{-1} for wild-types (n = 8 mice, 22 slices), -3.17 ± 0.36 ms^{-1} for *R(AB)-1* (n = 6 mice, 17 slices), and -2.99 ± 0.42 ms^{-1} for *R(AB)-2* (n = 5 mice, 20 slices) ($p > 0.2$).

We next examined two forms of short-term synaptic plasticity: paired-pulse facilitation (PPF) and posttetanic potentiation (PTP). PPF, a presynaptic enhancement of transmission in the second of two closely spaced stimuli (Katz and Miledi, 1968), was not altered in the transgenic mice relative to wild-type controls (Figure 3B). We also compared PTP in wild-type and *R(AB)* transgenic mice. In the presence of 100 μM 2-amino-5-phosphonovaleric acid (APV), an antagonist of NMDA receptors, a single 100 Hz train (1 s duration) elicited a transient enhancement of fEPSP slopes immediately following tetanization. There was no significant difference between the mean PTPs observed in wild-type mice (171% ± 9%; n = 4 mice, 11 slices) and those seen in the two mutant lines (*R(AB)-1*: 184% ± 22%, n = 4 mice, 10 slices; *R(AB)-2*: 170% ± 7%, n = 4 mice, 12 slices; $p > 0.5$). Thus, our tests of basal synaptic function and short-term forms of synaptic plasticity suggest that overexpression of an inhibitory form of the regulatory subunit of PKA did not significantly perturb the ability of CA1 neurons to respond to controlled presynaptic stimulation.

LTP Induced by One or Two Trains of Stimulation Is Normal in *R(AB)* Transgenic Mice

We next explored the role of PKA in LTP induction by applying one or two 100 Hz trains of stimulation to the Schaffer collateral-commissural pathway. With a single train, there were no significant differences between the mean fEPSP slopes measured from wild-type and transgenic mice immediately following or 30 min posttetanus (Figure 4A). Immediately following tetanus, the percentage potentiation was 177% ± 10% for wild-type (n = 8 mice, 19 slices), 168% ± 8% for *R(AB)-1* (n = 8 mice, 15 slices), and 156% ± 9% for *R(AB)-2* (n = 6 mice, 11 slices, $p > 0.1$). At 30 min posttetanus, no

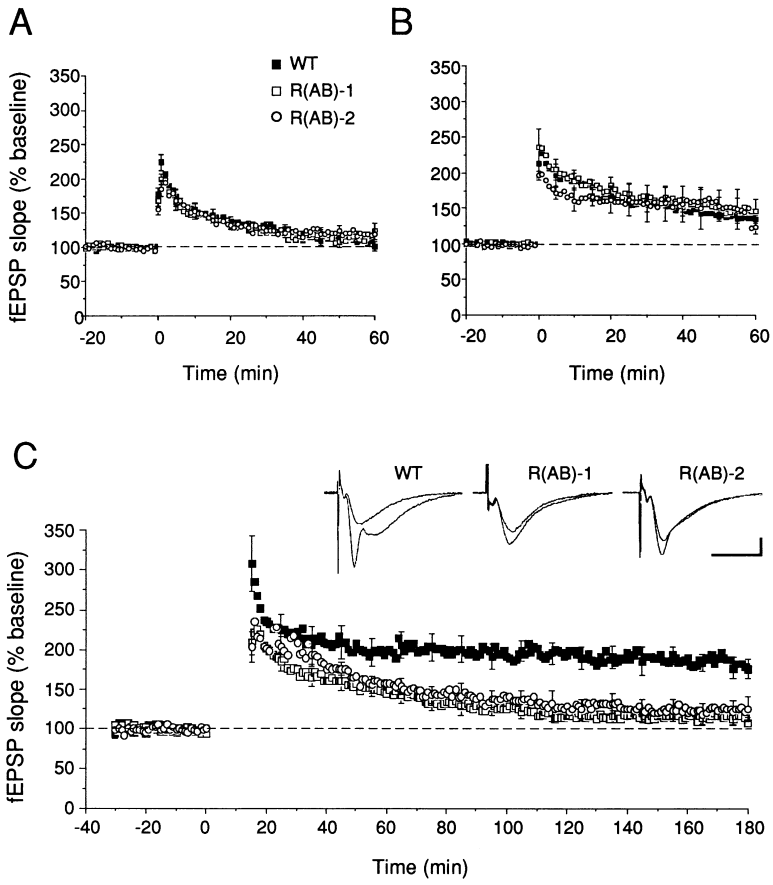

Figure 4. Genetic Reduction of PKA Activity Selectively Eliminates the Late Phase of LTP in Area CA1 of *R(AB)* Transgenic Mice

(A) Mean fEPSP slopes are plotted as a percent of pretetanus baseline values. Synaptic facilitation induced by a single train of stimulation (100 Hz, 1 s) decayed to pretetanus baseline values in less than 60 min in both wild-types and mutants. There was no significant difference between the mean fEPSP slopes measured in these animals at all time points tested.

(B) LTP induced by two 100 Hz trains (1 s duration) decayed more slowly than the potentiation elicited by a single train. There was no significant difference between the mean fEPSP slopes measured in wild-type and transgenic mice 60 min after tetanization.

(C) In two different lines of transgenic mice, L-LTP was reduced following four 100 Hz trains (1 s duration, 5 min apart) of stimulation. A stable level of robust potentiation was observed in wild-type controls, while a marked decline of potentiation back to near baseline values was measured in both lines of transgenic mice. Sample fEPSP traces measured from area CA1 in wild-type *R(AB)-1* and *R(AB)-2* slices at 15 min before and 180 min after four tetanic trains. Each superimposed pair of sweeps was measured from a single slice. Scale bars: 2 mV, 10 ms.

significant differences were observed between wild-types (130% ± 7%) and the *R(AB)-1* (124% ± 7%, $p > 0.5$) or the *R(AB)-2* (123% ± 4%) transgenics ($p > 0.4$). We also compared LTP elicited by two successive trains (100 Hz, 1 s) applied 20 s apart (Figure 4B). With this induction protocol, we observed a more lasting form of potentiation: mean fEPSP slopes at 60 min posttetanization were 135% ± 6% in wild-types (n = 8 mice, 20 slices), 146% ± 17% in *R(AB)* line 1 (n = 5 mice, 10 slices), and 124% ± 11% in *R(AB)* line 2 (n = 5 mice, 10 slices) (Figure 4B). These values were not significantly different from each other ($p > 0.5$). Thus, LTP induced by one or two trains of high-frequency stimulation was not affected by overexpression of an inhibitory form of the RIα subunit of PKA.

Genetic Attenuation of PKA Activity Eliminates the Late Phase of LTP in Hippocampal Area CA1

Because pharmacological studies suggest that PKA plays a role in more long-lasting forms of LTP produced by three or more trains of stimulation (Frey et al., 1993; Huang and Kandel, 1994), we next explored the effects of repeated tetanization of the Schaffer collateral pathway in *R(AB)* transgenics. With four 100 Hz trains (1 s duration, spaced 5 min apart; Figure 4C), wild-type animals (n = 7 mice, 14 slices) showed long-lasting and robust L-LTP: mean fEPSP slopes were 236% ± 19%, 199% ± 19%, and 176% ± 12% at 20 min, 60 min, and 180 min posttetanization, respectively. In contrast, the same induction regimen, when applied to slices obtained from mutant mice, elicited a continually decaying potentiation. The corresponding values for *R(AB)-1* mutants (n = 7 mice, 14 slices) were 202% ± 15% ($p > 0.3$), 144% ± 11% ($p > 0.06$), and 107% ± 7% ($p < 0.001$). In the second transgenic line, *R(AB)-2* (n = 5 mice, 10 slices) values were 201% ± 11% ($p > 0.1$), 157% ± 14% ($p > 0.1$), and 125% ± 16% ($p < 0.03$). The potentiation observed in hippocampal slices prepared from transgenic animals is significantly below that observed in wild-types at 180 min, and this significant difference first appears at about 70 min posttetanization. The observation that E-LTP induced by one or two stimulus trains is unchanged in the *R(AB)* transgenics while the early component of L-LTP is reduced suggests that L-LTP, unlike E-LTP, requires PKA and recruits distinct signaling pathways immediately following tetanization.

R(AB) Transgenics Show a Deficit in Spatial Memory

Given that *R(AB)* transgenic mice have a defect in Schaffer collateral L-LTP as well as decreased PKA activity in the hippocampus, we began to explore behavioral deficits in these transgenics. We first trained mice on the hidden platform version of the Morris water maze task, a hippocampus-dependent task which depends on the ability of the animal to learn and remember the

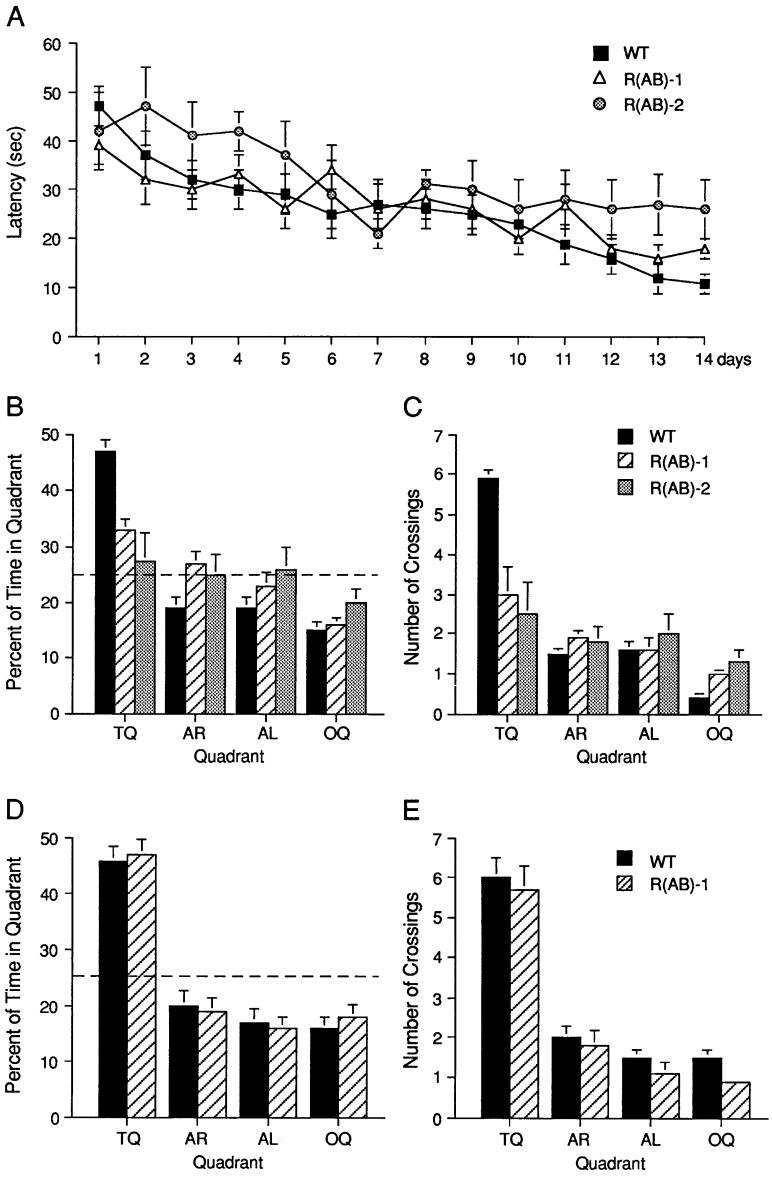

Figure 5. Performance of *R(AB)* Mice in the Morris Water Maze Task

(A) The average time to reach the platform (latency) was plotted versus trial day. Overall performance of *R(AB)-1* (n = 14) and *R(AB)-2* mice (n = 6) was not significantly different from that of controls (n = 20). $F(1,32) = 0.02$, $p = 0.87$ for controls versus *R(AB)-1* and $F(1,24) = 4.34$, $p = 0.05$ for controls versus *R(AB)-2*. However, planned comparisons of latency on each individual day reveal a significant difference between *R(AB)-1* mice and controls on day 14 ($p < 0.05$) and between *R(AB)-2* mice and controls on days 11 and 14 ($p < 0.05$).

(B) The graph shows the results of a probe trial given on day 15 of training. Controls searched significantly more in the quadrant where platform was located during training than *R(AB)-1* mice ($F(3,124) = 21.00$, $p < 0.0001$), or *R(AB)-2* mice ($F(3,96) = 25.05$, $p < 0.0001$). Planned comparisons confirmed that controls spent significantly more time in the training (TQ) than in adjacent quadrant to the left of T (AL), in adjacent quadrant to the right of T (AR), or in the quadrant opposite to T (OQ), (TQ versus AR, AL, OQ, $p < 0.0001$). Planned comparisons showed that *R(AB)-1* mice spent significantly more time in the TQ than in AL, or OQ, but not in AR (T versus AL, $p < 0.05$; T versus OQ, $p < 0.01$; T versus AR, $p = 0.24$). No difference was found for *R(AB)-2* mice (T versus AR, AL, OQ, $p > 0.05$).

(C) Controls crossed the exact site where the platform was located during training significantly more times than *R(AB)-1* mice ($F(3,124) = 27.27$, $p < 0.0001$) or *R(AB)-2* mice ($F(3,96) = 30.05$, $p < 0.0001$). Planned comparisons confirmed that controls crossed the platform site significantly more times in TQ than they crossed the equivalent sites in the other three quadrants (TQ versus AR, AL, OQ, $p < 0.0001$). Planned comparisons showed that *R(AB)-1* mice crossed the platform site more often in the TQ than in AL, or OQ, but not in AR (T versus AL, $p < 0.05$; T versus OQ, $p < 0.01$; T versus AR, $p = 0.19$). No difference was found for *R(AB)-2* mice (T versus AR, AL, OQ, $p > 0.05$).

(D) The graphs shows the results of a probe trial given on day 15 of the one distal cue experiment. Both *R(AB)-1* mice (n = 7) and controls (n = 7) searched selectively in the quadrant in which the platform was located during training ($F(3,44) = 20.22$, $p < 0.0001$). No significant difference in genotype × quadrant interaction was observed ($F(3,44) = 1.10$, $p = 0.36$).

(E) Both *R(AB)-1* mice and controls crossed the exact site where the platform was located during training significantly more times than they crossed equivalent sites in the other three quadrants ($F(3,44) = 19.65$, $p < 0.0001$). No significant difference in genotype × quadrant interaction was observed [$F(3,44) = 1.69$, $p = 0.18$).

relationships between multiple distal cues and the platform (Morris et al., 1982). The overall performance of *R(AB)* transgenics during training was not significantly different than controls (Figure 5). However, escape latency is a poor measure of spatial memory capacity (Schenk and Morris, 1985). To assess spatial memory more directly, we tested the mice in a probe trial in which the platform was removed and the mice were allowed to search for 60 s. The time spent in each quadrant measures the spatial bias of an animal's search pattern and is thought to reflect explicit aspects of long-term spatial memory (Schenk and Morris, 1985). The mutants searched less selectively than wild-type controls for the absent platform and spent significantly less time than wild-type controls in the target quadrant (Figure 5; $p < 0.001$ for controls versus *R(AB)-1* or *R(AB)-2*). Mutants also crossed the exact site of the former position of the platform significantly less frequently than did wild-type controls (Figure 5).

These data suggest that mice with reduced hippocampal PKA activity have impaired spatial memory. To rule out a deficit due to poor vision, motor coordination, or motivation, we designed an experiment in which the hidden platform was associated with a single distal cue. We removed all but one distal cue and placed the submerged platform such that mice always had to swim toward that one distal cue to find the hidden platform. Here, as in the "visible" platform task (Morris et al., 1982),

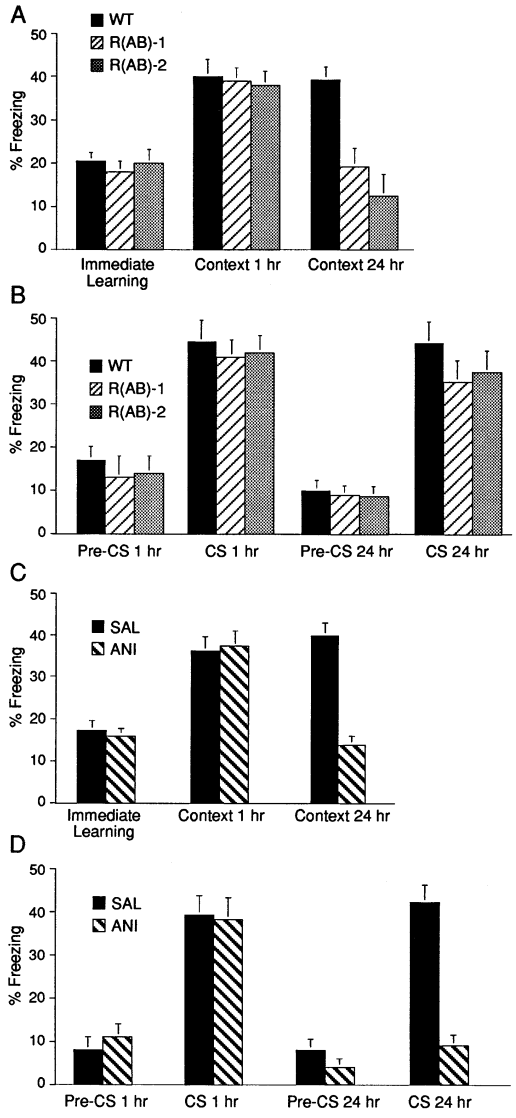

Figure 6. Fear Conditioning in *R(AB)* Transgenics and Anisomycin-Injected Wild-Type Mice

(A) *R(AB)* transgenics show a deficit in long-term but not short-term memory for contextual fear conditioning. *R(AB)* mice and controls were given one CS/US pairing. No significant difference between mutants and controls in freezing responses immediately following the US was observed [F(1,95) = 0.74, p = 0.41 for controls versus *R(AB)-1*; F(1,75) = 0.14, p = 0.89 for controls versus *R(AB)-2*]. Mice were tested for contextual conditioning 1 hr after training and no difference in freezing responses was observed for *R(AB)-1* (n = 10) and *R(AB)-2* mice (n = 7) relative to that of controls (n = 15) [F(1,23) = 0.54, p = 0.38 for controls versus *R(AB)-1* and F(1,20) = 0.62, p = 0.27 for controls versus *R(AB)-2*]. Mice were tested for contextual conditioning 24 hr after training. Freezing responses of *R(AB)-1* (n = 11) and *R(AB)-2* mice (n = 5) were significantly less from that of controls (n = 14) [F(1,23) = 3.90, p = 0.0008 for controls versus *R(AB)-1*; F(1,17) = 4.53, p = 0.0003 for controls versus *R(AB)-2*)].

(B) *R(AB)* transgenics show no significant impairment in cued conditioning. On the pre-CS test 1 hr after training, the freezing of *R(AB)-1* (n = 10) and *R(AB)-2* mice (n = 7) was not statistically different from that of controls (n = 15) [F(1,23) = 0.81, p = 0.42 for controls versus *R(AB)-1*; F(1,20) = 0.73, p = 0.79 for controls versus *R(AB)-2*]. No difference was found on CS test [F(1,23) = 0.63, p = 0.43 for controls versus *R(AB)-1*; F(1,20) = 2.53, p = 0.15 for controls versus *R(AB)-2*]. Mice were tested for cued conditioning 24 hr after training. On the pre-CS test, the freezing of *R(AB)-1* (n = 10) and *R(AB)-2* mice (n = 9) was not statistically different from that of controls (n = 16). [F(1,24) = 0.54, p = 0.62 for controls versus *R(AB)-1*; F(1,23) = 1.16, p = 0.26 for controls versus *R(AB)-2*]. No difference was found on the CS test [F(1,23) = 1.38, p = 0.18 for controls versus *R(AB)-1*; F(1,23) = 1.46, p = 0.16 for controls versus *R(AB)-2*].

(C) Long-term memory for contextual fear is anisomycin sensitive. No significant difference in freezing responses between ANI- and SAL-injected mice was found immediately after the US [F(1,62) = 0.02, p = 0.89]. No significant difference in freezing responses between ANI- and SAL-injected mice (n = 8 in each group) was found 1 hr after training [F(1,14) = 0.33, p = 0.56]. Freezing of ANI-injected mice (n = 8) was significantly less from that of SAL-injected animals (n = 8) [F(1,14) = 4.89, p < 0.0001] 24 hr after training.

(D) Long-term memory for cued conditioning is anisomycin sensitive. No significant difference in freezing responses between ANI- and SAL-injected mice (n = 8 in each group) was found 1 hr after training in the pre-CS test [F(1,14) = 1.23 p = 0.13] or CS test [F(1,14) = 1.12, p = 0.28]. No significant difference in freezing responses between ANI- and SAL-injected mice was found in the pre-CS test [F(1,14) = 1.24 p = 0.08] 24 hr after training. In the CS test, freezing of ANI-injected mice (n = 8) was significantly less than that of SAL-injected animals (n = 8) [F(1,14) = 4.98, p < 0.0001].

mice have to associate only one cue with an escape platform; but unlike the visible platform task, the cue is distal and the platform is hidden. This test provides a good measure of distal vision as well as swimming abilities and motivation. Here again, we trained mice with 2 trials per day. In the probe trial (day 15), *R(AB)-1* mice were as good as control mice in searching for the absent platform and in crossing the exact site of the former position of the platform (Figure 5). *R(AB)* mice also were not different from control mice in latencies to find the platform in the frequently used "visible" platform task: 40.5 ± 4.2 s, 39 ± 3.4 s, and 40.2 ± 3 s ($p > 0.05$) for a block of 4 trials (30 s intertrial interval) on the first day of training versus 6.6 ± 2.2 s, 7 ± 2.6, and 6.8 ± 2.1 ($p > 0.05$) for a block of 4 trials on the third (last) day of training, for *R(AB)-1* (n = 6), *R(AB)-2* (n = 7) and wild-type controls (n = 7), respectively. Taken together, the water maze experiments reveal that the *R(AB)* mutation specifically impairs spatial memory but not visual acuity, motivation to escape the water, or motor coordination.

R(AB) Transgenic Mice Are Deficient in Long-Term but Not Short-Term Memory for Context-Dependent Fear Conditioning

Because the Morris Maze task requires repeated training over several days, this task does not provide the temporal resolution necessary to distinguish the different phases of memory storage. Because L-LTP and long-term memory share a requirement for protein synthesis, one might predict that the *R(AB)* transgenics, which have an L-LTP deficit, would have normal short-term but defective long-term memory. We therefore turned to contextual and cued conditioning tasks in which robust learning can be triggered by a single trial.

In these tasks, animals learn to fear a new environment or an emotionally neutral conditioned stimulus (CS), such as a tone, because of its temporal association with an aversive unconditioned stimulus (US), usually foot shock. When exposed to the same context or the same CS, conditioned animals show freezing behavior (Phillips and LeDoux, 1992; Kim et al., 1993). In addition to

allowing good temporal resolution, these two forms of fear conditioning involve distinct neuroanatomical substrates: contextual conditioning is dependent on both hippocampus and amygdala function, whereas cued conditioning requires only the amygdala.

To assess memory for contextual conditioning, we tested mice in the same context at 1 hr and 24 hr after 1 CS/US training (Figure 6A). *R(AB)* transgenic mice and controls showed similar levels of freezing immediately after training (20.7% ± 2.4%, 18% ± 2.4%, and 20% ± 3.6% for controls, *R(AB)-1*, and *R(AB)-2*, respectively; $p = 0.41$ for controls versus *R(AB)-1*, and $p = 0.89$ for controls versus *R(AB)-2*) and 1 hr after training (controls, 40.2% ± 4%; *R(AB)-1*, 39% ± 3%; *R(AB)-2*, 38.1% ± 3.2%; $p = 0.38$ for controls versus *R(AB)-1*, and $p = 0.27$ for controls versus *R(AB)-2*). This indicates that *R(AB)* mice learn normally and have normal short-term memory at 1 hr. By contrast, *R(AB)* mutants showed a dramatic reduction in long-term memory for contextual fear responses relative to wild-type controls when tested 24 hr after training (39.2% ± 3%, 19.3% ± 4.3%, and 12.4% ± 5% for controls, *R(AB)-1*, and *R(AB)-2*, respectively; $p < 0.001$ for controls versus *R(AB)-1* or *R(AB)-2*).

To test for deficits in cued conditioning, we measured freezing in response to a tone that had previously been paired with a foot shock. To avoid confounding effects of contextual conditioning, mice were tested for cued conditioning in a novel context. As Figure 6B shows, *R(AB)* mice displayed normal memory for cued conditioning at 1 hr (47% ± 4%, 42% ± 5%, and 42% ± 3.8% for controls, *R(AB)-1*, and *R(AB)-2*, respectively; $p = 0.43$ for controls versus *R(AB)-1*, and $p = 0.38$ for controls versus *R(AB)-2*) or 24 hr after training (44.2% ± 5%, 35.3% ± 3.6%, and 37.6% ± 5% for controls, *R(AB)-1*, and *R(AB)-2*, respectively; $p = 0.18$ for controls versus *R(AB)-1*, and $p = 0.16$ for controls versus *R(AB)-2*).

To determine if R(AB) expression affects shock sensitivity, we measured the minimal amount of current required to elicit three stereotypical behaviors: flinching/running, jumping, and vocalizing. We found that each of these behaviors was elicited with similar current intensities in *R(AB)* transgenic mice ($n = 8$) and controls ($n = 7$; $p = 0.97$ for flinching/running, $p = 0.86$ for jumping, and $p = 0.70$ for vocalizing; data not shown).

The Inhibition of Contextual Memory by Anisomycin Parallels That Observed in *R(AB)* Transgenics, Suggesting That PKA Is Essential for Memory Consolidation

Long-term storage for both implicit and explicit memory differs from short-term processes in being sensitive to disruption by inhibitors of protein synthesis (Davis and Squire, 1984). Protein synthesis inhibitors also selectively block L-LTP in hippocampal area CA1 (Frey et al., 1988, 1993; Huang and Kandel, 1994). Because *R(AB)* transgenics are deficient in both L-LTP and long-term contextual memory, we asked if the time window for long-term memory formation in *R(AB)* mice correlated with the time window for protein synthesis–dependent memory in wild-type mice. To test this, mice were injected with anisomycin (ANI; 150 mg/kg) 30 min before Pavlovian fear conditioning training. We found that initial acquisition (immediate freezing responses) and retention at 1 hr after training were independent of protein synthesis for both contextual and cued conditioning tasks (Figures 6C and 6D). By contrast, ANI-injected mice showed a dramatic deficit in freezing responses when tested for contextual (38.4% ± 3.4% and 12.4% ± 2.3%, control and ANI mice, respectively; $p < 0.0001$) or cued memory tasks 24 hr after training (43% ± 3.3% and 8.4% ± 2%; control and ANI mice, respectively; $p < 0.0001$; Figures 6C and 6D). In addition, ANI was also effective when given immediately after training. Again, ANI-injected animals showed significantly less contextual conditioning than saline (SAL)-injected mice when tested 24 hr after training (40.5% ± 6% and 8.7% ± 3%, control and ANI mice, respectively; $p < 0.001$; $n = 7$ in each group). However, ANI was not effective when given at 1 hr, 3 hr, 4.5 hr, 6 hr, 8 hr, or 23.5 hr after training ($n = 14$ for each time point; R. B. et al., unpublished data), indicating that only a single wave of protein synthesis during or shortly after training is required for contextual fear conditioning over 24 hr. Moreover, the inhibition of contextual memory by anisomycin parallels that observed in *R(AB)* transgenics, suggesting that PKA is essential for contextual memory consolidation.

R(AB) Transgenics Exhibit Normal Long-Term Memory in Amygdala-Dependent Conditioned Taste Aversion Task

To study another amygdala-dependent task in the *R(AB)* transgenic mice, we turned to conditioned taste aversion (CTA; Yamamoto et al., 1994). Tastes are of great ecological significance, and memories governing food ingestion are crucial for the well-being of an animal. On occasions when food causes a toxic effect, animals must be able to remember the taste stimuli associated with that particular food to avoid repeated intake. CTA is easily demonstrated by administering to animals a novel gustatory stimulus (e.g., saccharin) followed by intraperitoneal injection of LiCl that elicits transient malaise. Strong CTA to novel taste stimuli can be established after a single CS (taste)/US (malaise) pairing. In contrast to conventional classical and instrumental conditioning, the association between the taste and the negative reinforcer in CTA can develop with delays of several hours. CTA is a form of implicit memory, and lesion studies have shown that the basolateral nucleus of the amygdala is essential for CTA (Yamamoto et al., 1994).

A single trial of saccharin sampling followed 45 min later by intraperitoneal LiCl injection resulted in a marked aversion for both *R(AB)* transgenic and wild-type mice when tested 24 hr after conditioning. Both *R(AB)* transgenics (*R(AB)-1*, $n = 6$; *R(AB)-2*, $n = 8$) and wild-type controls ($n = 12$) consumed significantly more water than saccharin on a choice test (1.1 ± 0.1 ml water and 0.6 ± 0.1 ml saccharin for *R(AB)* mice, $p < 0.001$; and 1.2 ± 0.1 ml water and 0.6 ± 0.1 ml saccharin for controls, $p < 0.001$). Normally, mice prefer saccharin to water, and nonconditioned *R(AB)* transgenics ($n = 6$) and wild-type mice ($n = 6$) preferred saccharin to water (0.9 ± 0.1 ml saccharin and 0.7 ± 0.2 ml water for *R(AB)*

mice and 1.1 ± 0.1 ml saccharin and 0.6 ± 0.1 ml water for controls). Thus, the *R(AB)* transgenics are normal in CTA and cued conditioning, amygdala-based memory tasks thought to resemble implicit memory in humans. By contrast, the *R(AB)* transgenics exhibit deficits in the Morris water maze and contextual fear conditioning, hippocampus-dependent memory tasks thought to reflect properties of explicit memory in humans.

Discussion

Beginning with the neuropsychological study of patient H. M., a consensus has developed that memory is not a single process but consists of several forms, including explicit memory, the conscious recall of facts and events, and implicit memory, the unconscious recollection of learned skills and habits (Graf and Schacter, 1985; Squire, 1992). These two forms use different brain systems, but each has distinct stages of memory storage. Moreover, in both cases, the long-term process requires new protein synthesis, whereas the short-term process does not. Studies of implicit memory in *Aplysia* and Drosophila suggest that the cAMP/PKA signaling pathway is involved in both short-term and long-term processes (Abel et al., 1995; Davis, 1996). To extend this genetic analysis to explicit forms of memory storage in mammals, we explored the role of PKA in synaptic plasticity and spatial and contextual memory by generating *R(AB)* transgenic mice. We found that PKA plays an important role in mice in converting short-term to long-term explicit memory storage.

A Molecular Dissociation of Explicit and Implicit Memory Storage

In mammals, the hippocampus is essential for the initial consolidation of explicit, or declarative, memory (Squire, 1992). Our analysis of hippocampal function in the *R(AB)* transgenic animals began with the Morris water maze. Transgenic animals improved during training, but when tested for memory in a probe trial, transgenic animals exhibited spatial memory deficits. These spatial memory deficits, which were not due to visual, motivational, or motor coordination difficulties, likely result from impaired hippocampal function.

To define more precisely the time course of the memory deficit in *R(AB)* transgenics, we used contextual and cued fear conditioning tasks, in which learning occurs after a single trial. Because the *R(AB)* transgenics are deficient in a form of LTP that requires new protein synthesis (Frey et al., 1988, 1993; Huang and Kandel, 1994), we asked whether the memory deficit produced by inhibiting protein synthesis parallels that produced by expression of the *R(AB)* transgene. Long-term contextual memory storage requires both PKA activity and protein synthesis. By contrast, the long-term memory for cued conditioning, a task which is mediated by the amygdala (Phillips and LeDoux, 1992; Kim et al., 1993), is also anisomycin sensitive, but is not disrupted in *R(AB)* mice. This may be due to a general requirement for protein synthesis in both explicit and implicit memory and perhaps lower levels of *R(AB)* transgene expression in the amygdala. Importantly, *R(AB)* transgenic mice also showed normal long-term memory in CTA, a task which is highly sensitive to amygdala lesions (Yamamoto et al., 1994). Together, our findings strengthen the idea of multiple memory systems and show that genetic inhibition of PKA can differentially affect them.

PKA, L-LTP, and Long-Term Memory

Our findings show that the PKA signaling pathway is critical for the expression of L-LTP in hippocampal area CA1 and that the genetic manipulation of this pathway disrupts only L-LTP without affecting E-LTP or basal synaptic transmission. The effect of the *R(AB)* transgene on L-LTP has an early onset that parallels closely that observed in area CA1 in hippocampal slices treated with inhibitors of PKA (Frey et al., 1993; Huang and Kandel, 1994; Blitzer et al., 1995) and is more severe than that observed after treatment with inhibitors of transcription or translation (Huang and Kandel, 1994; Nguyen et al., 1994). These results suggest that repeated spaced trains of tetanic stimuli, but not a single train or two closely spaced trains, recruit PKA shortly following tetanization, consistent with biochemical experiments that showed increases in cAMP levels soon after spaced tetanization protocols (Chetkovich et al., 1991; Frey et al., 1993).

The reduction in hippocampal PKA activity observed in the *R(AB)* transgenic mice is associated with deficits in L-LTP and long-term memory, suggesting L-LTP in area CA1 plays a role in long-term memory storage. To affect behavioral memory, substantial reductions in basal PKA levels may be required. Knockout mice lacking the genes encoding the RIβ or $C\beta_1$ subunits of PKA did not have reductions in PKA activity, had mild L-LTP deficits, and did not show abnormalities in behavioral memory (Huang et al., 1995). By contrast, we found a significant reduction in hippocampal PKA activity in the *R(AB)* transgenics. In *R(AB)* transgenics, L-LTP is reduced immediately following tetanization and significantly impaired by 70 min posttetanization, and spatial memory is impaired. In contrast, L-LTP is not significantly impaired until 2.5 hr after tetanization in the $C\beta_1$ knockouts (Qi et al., 1996), and their spatial memory is normal (Huang et al., 1995). This quantitative difference in the early component of L-LTP may be important and may account for the behavioral deficit observed in the *R(AB)* transgenics. Alternatively, although PKA is clearly important for both L-LTP and long-term memory, the relationship between the L-LTP and long-term memory may be more complex.

This correlation between deficits in L-LTP and impaired behavioral long-term memory is not absolute and may be overridden by other factors. Thus, although the mice lacking the gene for tissue-type plasminogen activator (tPA) have a defect in L-LTP similar to that observed in the *R(AB)* transgenics, behavioral studies revealed only minor memory deficits in these knockout mice (Huang et al., 1996). However, factor analysis has revealed that noncognitive factors masked a deficiency in spatial memory in the water maze in the tPA knockouts (Hans-Peter Lipp, personal communication). Additionally, behavioral studies of tPA knockout mice were carried out using more intense training paradigms than in our behavioral studies, and this may partially overcome

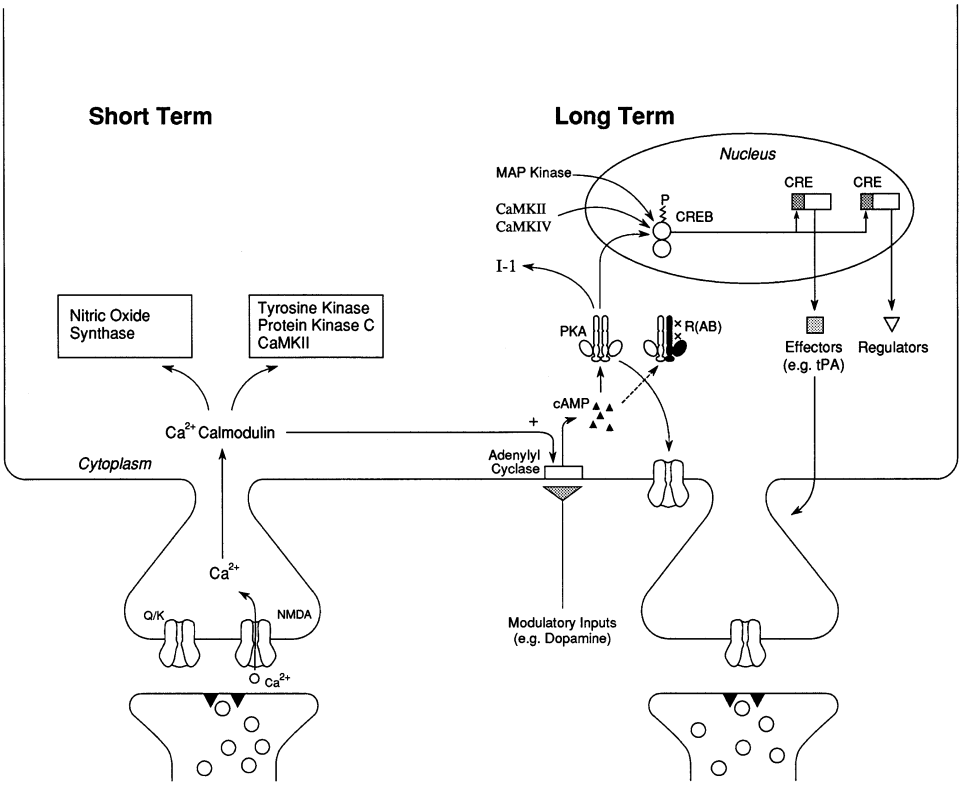

Figure 7. A Molecular Model for the Consolidation of the Late Phase of LTP and Hippocampus-Based Long-Term Memory

Calcium influx through NMDA receptors activates a variety of kinases, including CaMKII, and other enzymes such as nitric oxide synthase, to produce E-LTP (Short Term). L-LTP (Long Term) is produced when adenylyl cyclase is activated by Ca^{2+} increases or by neuromodulatory transmitters such as dopamine. The rise in cAMP activates PKA, which can then phosphorylate a variety of targets such as protein phosphatase inhibitor-1 (I-1), ion channels, and CREB. In the *R(AB)* transgenic mice, the expression of an inhibitory form of the regulatory subunit of PKA leads to reduced PKA levels and deficits in the L-LTP and in hippocampus-based long-term memory.

memory impairments (Bourtchouladze et al., 1994). Finally, our transgenic experiment targets expression of a dominantly acting inhibitor to postnatal neurons, which is a much more restricted and selective approach than the conventional knockout approach that was used to generate the tPA mutant animals.

A Molecular Model for the Late Phase of LTP and Explicit Forms of Long-Term Memory Storage

Our results allow us to outline a preliminary molecular model for L-LTP in area CA1 (Figure 7). According to this view, there are at least two components of L-LTP that are induced by multiple trains: an early component and a late component. The early component of L-LTP requires PKA activity, but is independent of protein and RNA synthesis (Frey et al., 1988, 1993; Huang and Kandel, 1994; Nguyen et al., 1994). While our results have emphasized the role of stimulus-induced PKA activity in long-term changes in synaptic strength, it is important to note that the *R(AB)* transgenics showed reductions in both basal and induced PKA activity. The early component of L-LTP may be mediated by the action of PKA on preexisting substrates at the synapse, including ion channels (Raymond et al., 1993), synaptic vesicle proteins (Greengard et al., 1993) and protein phosphatase inhibitor–1 (Blitzer et al., 1995). By inhibiting phosphatase activity, PKA could play a "gating" role, promoting the action of other serine-threonine kinases. Support for this idea comes from our observations that okadaic acid, a phosphatase inhibitor, rescues the L-LTP deficit observed in the *R(AB)* transgenics (T. A. et al., unpublished data). The late component, which begins 1–2 hr after tetanic stimulation, is blocked by inhibitors of RNA and protein synthesis and may result from the action of PKA on transcriptional regulatory processes, perhaps via CREB.

Our experiments define a role for PKA in L-LTP and long-term memory, and they provide a framework for a molecular understanding of the consolidation of long-term explicit memory in mice. Pioneering work on verbal learning in humans by Müller and Pilzecker (1900) established that memory is particularly sensitive to disruption during the transition from short-term memory to long-term memory. This transition, called the consolidation period, was given a molecular basis by the observation that long-term memory storage requires new protein and RNA synthesis (Davis and Squire, 1984). Indeed, our results demonstrate that anisomycin, when administered around the time of training, selectively impairs long-term memory for conditioned fear. Thus, the consolidation period is a critical period during which genes

are induced that encode proteins essential for stable long-term memory. The long-term memory deficits in *R(AB)* transgenic mice demonstrate that PKA plays a role in the hippocampus in initiating the molecular events leading to the consolidation of short-term changes in neuronal activity into long-term memory.

Experimental Procedures

Expression Vector

A mutant cDNA of the mouse RIα regulatory subunit (*R(AB)*, kindly provided by Dr. G. S. McKnight, University of Washington) was subcloned from the vector MT-REVAB (Clegg et al., 1987) into the EcoRV site downstream of a 5′ untranslated leader containing a hybrid intron (Choi et al., 1991) in the plasmid pNN265 to create the plasmid pTA8011. The plasmid pNN265 (kindly provided by N. Nakanishi, Harvard Medical School) is a modified form of pcDNAI/Amp that contains the untranslated leader and SV40 sequences flanked by NotI sites. The expression plasmid CaMKII-R(AB) (see Figure 1D; pTA8136) was created by subcloning a 3.0 kb NotI fragment from pTA8011 downstream of 8.5 kb mouse *CaMKIIα* promoter (Mayford et al., 1996). All cloning junctions were verified by DNA sequencing.

Transgenic Mice

After purification by CsC1 gradient centrifugation, the *CaMKII-R(AB)* transgene was purified away from vector sequences by digesting with BssHII. The 11.5 kb transgene was isolated by agarose gel electrophoresis and purified after electroelution using Elutip. Transgenic mice were generated by injecting the purified *CaMKII-R(AB)* transgene into pronuclei of BL6CBAF2/J zygotes (Hogan et al., 1994). Founders were backcrossed to C57BL/6J mice. In some cases, because of breeding difficulties, mice from the *R(AB)-2* transgenic line were crossed to B6CBAF1/J animals. Mice were maintained and bred under standard conditions, consistent with NIH guidelines and approved by the IACUC. For genotyping, tail DNA was prepared (Laird et al., 1991) and analyzed by Southern blotting (Sambrook et al., 1989) using a transgene-specific probe.

In Situ Hybridization

Mouse brains were dissected and rapidly frozen in Tissue-Tek embedding medium. Sections (20 μm) were fixed and hybridized as described (Mayford et al., 1995) to an [α-^{35}S]dATP-labeled, transgene-specific oligonucleotide (5′-GCAGGATCCGCTTGGGCTGCAG TTGGACCT-3′), which hybridizes to sequences present in the 5′ untranslated leader within the transgenic transcript. Slides were exposed for 3 weeks to Kodak Biomax MR autoradiographic film.

Protein Kinase A Assays

Hippocampi were dissected into ice-cold artificial cerebrospinal fluid and homogenized in 200 μl of extraction buffer (Brandon et al., 1995). The extract was diluted to a protein concentration of 2 mg/ml (Bradford, 1976) and kept at 4°C until use. PKA activity was assayed in duplicate as described (Clegg et al., 1987). To some reactions, cAMP (5 μM) and/or PKI (40 μg/ml; Peninsula Labs) were added. PKA activities (pmol [γ-^{32}P]ATP/min/mg protein) were calculated relative to the background activity without added substrate.

Electrophysiology

Transverse hippocampal slices (400 μm thick) from 8- to 12-week-old transgenics and wild-type controls were maintained in an interface chamber at 28°C perfused (1 mL/min) with ACSF (Nguyen et al., 1994). After a 90 min recovery period, extracellular field EPSPs (fEPSPs) were recorded in the stratum radiatum layer of area CA1. The stimulation intensity (0.05 ms pulse width) was adjusted to give fEPSP slopes approximately 40% of maximum, and baseline responses were elicited once per minute at this intensity. Slices that showed maximal fEPSP sizes <3 mV were rejected. In some experiments, paired-pulse facilitation was tested at various interpulse intervals using baseline stimulus intensity. Synaptic "input–output" curves were generated by plotting fEPSP slopes and their corresponding presynaptic fiber volley sizes, elicited at different stimulus strengths. L-LTP was induced by applying four 1-second trains (100 Hz, test strength), spaced 5 min apart, to the stratum radiatum. In some experiments, the number of 100 Hz trains was reduced to either one or two (20 s apart) in order to elicit E-LTP (Huang and Kandel, 1994).

Morris Maze Experiments

Water maze experiments were done as described previously (Bourtchouladze et al., 1994) except mice were trained with two trials per day (30 s intertrial interval) for 14 days. In the probe test on day 15, the platform was removed. The amount of time that the mice spent in each quadrant was measured, and the number of times the mice crossed the platform site (or similar locations in other quadrants) during searching was determined. In the "one distal cue" experiment, we removed all but one distal cue and placed the submerged platform such that mice always had to swim toward that one cue to find the hidden platform. We trained mice with 2 trials a day for 14 days, and a probe test was given on day 15.

Fear Conditioning

Fear conditioning experiments were done as described (Bourtchouladze et al., 1994). On the training day, the mouse was placed in the conditioning chamber (Med Associates) for 2 min before the onset of the CS, which lasted for 30 s at 2800 Hz and 85 dB. The last 2 s of the CS were paired with the US, 0.7 mA of continuous footshock. After an additional 30 s in the chamber, the mouse was returned to a home cage. Conditioning was assessed by scoring freezing behavior, which was defined as complete lack of movement in intervals of 5 s. Contextual conditioning was assessed for 5 consecutive minutes in the chamber in which the mice were trained. In cued conditioning experiments, the mice were placed in a novel context (another conditioning chamber with a smooth flat floor, posters on the wall, and a novel odorant) for 2 min (pre-CS test), after which they were exposed to the CS for 3 min (CS test). Mice were tested 1 hr or 24 hr after training. For both contextual and cued conditioning experiments, mice were trained and tested at each of the different time points in three separate experiments. Since the experiments were done in a balanced manner and since the training procedure was identical, we pooled all of the training data.

For ANI studies, we used C57BL/6J mice of both sexes. ANI (Sigma) was dissolved in 0.9% saline and pH was adjusted with 1N HCl to 7.0–7.4. Mice were subcutaneously injected with 150 mg of ANI/kg of body weight, or an equivalent volume of saline, 30 min before or immediately (30 s) after training. At the dose used, anisomycin inhibits cerebral protein synthesis in mice by about 96% (Davis and Squire, 1984).

Conditioned Taste Aversion Experiments

Mice were shaped for 5 days before the start of the experiment. During shaping, water bottles were removed and a 23 hr, 40 min water deprivation schedule was introduced. The mice had access to water once a day, during a 20 min session in the test chamber. On the day of conditioning, the mice received 0.1% saccharin as a novel taste, and 45 min later they were injected with LiCl (i.p., 0.15 ml, 2% body weight).

Aversion was assessed 24 hr after conditioning by testing mice for 20 min in a multiple choice test situation using four specially designed cups, two with 2 ml of 0.1% saccharin each and two with 2 ml of water each. The exact amount of water and saccharin consumed was determined.

Data Analysis

For the analysis of behavioral experiments, results from male and female mice were collapsed, since analysis of variance (ANOVA; gender × genotype, or gender × drug) did not reveal any significant differences. Experiments were analyzed with one-way ANOVA and two-way ANOVA with one repeated measure. Planned comparison tests were used for posthoc analysis. Student's t test was used for electrophysiological data analysis. All values are expressed as mean ± SEM. The experimenter was blind to the genotype of the mice in all behavioral and physiological studies.

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References

Abel, T., Alberini, C., Ghirardi, M., Huang, Y.-Y., Nguyen, P., and Kandel, E.R. (1995). Steps toward a molecular definition of memory consolidation. In Memory Distortion, D.L. Schacter, ed. (Harvard Univ. Press: Cambridge, MA), pp. 298–325.

Abraham, W.C., Mason, S.E., Demmer, J., Williams, J.M., Richardson, C.L., Tate, W.P., Lawlor, P.A., and Dragunow, M. (1993). Correlations between immediate early gene induction and the persistence of long-term potentiation. Neuroscience *56*, 717–727.

Bliss, T.V., and Collingridge, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature *361*, 31–39.

Blitzer, R.D., Wong, T., Nouranifar, R., Iyengar, R., and Landau, E.M. (1995). Postsynaptic cAMP pathway gates early LTP in hippocampal CA1 region. Neuron *15*, 1403–1414.

Bourtchouladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G., and Silva, A.J. (1994). Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell *79*, 59–68.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. *72*, 248–254.

Brandon, E.P., Zhuo, M., Huang, Y.-Y., Ming, Q., Gerhold, K.A., Burton, K.A., Kandel, E.R., McKnight, G.S., and Idzerda, R.L. (1995). Hippocampal long-term depression and depotentiation are defective in mice carrying a targeted disruption of the gene encoding the RIβ subunit of cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA *92*, 8852–8855.

Chetkovich, D.M., Gray, R., Johnston, D., and Sweatt, J.D. (1991). N-methyl-D-aspartate receptor activation increased cAMP levels and voltage-gated Ca^{2+} channel activity in area CA1 of hippocampus. Proc. Natl. Acad. Sci. USA *88*, 6467–6471.

Choi, T., Huang, M., Gorman, C., and Jaenisch, R. (1991). A generic intron increases gene expression in transgenic mice. Mol. Cell. Biol. *11*, 3070–3074.

Clegg, C., Correll, L.A., Cadd, G.G., and McKnight, G.S. (1987). Inhibition of intracellular cAMP-dependent protein kinase using mutant genes of the regulatory type I subunit. J. Biol. Chem. *262*, 13111–13119.

Davis, R.L. (1996). Physiology and biochemistry of Drosophila learning mutants. Physiological Rev. *76*, 299–317.

Davis, H.P., and Squire, L.R. (1984). Protein synthesis and memory: a review. Psychol. Bull. *96*, 518–559.

Deisseroth, K., Bito, H., and Tsien, R.W. (1996). Signaling from synapse to nucleus: postsynaptic CREB phosphorylation during multiple forms of hippocampal synaptic plasticity. Neuron *16*, 89–101.

Frey, U., Krug, M., Reymann, K.G., and Matthies, H. (1988). Anisomycin, an inhibitor of protein synthesis, blocks late phase of LTP phenomena in the hippocampal CA1 region *in vitro*. Brain Res. *452*, 57–65.

Frey, U., Huang, Y.-Y., and Kandel, E.R. (1993). Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science *260*, 1661–1664.

Ginty, D.D., Glowacka, D., DeFranco, C., and Wagner, J.A. (1991). Nerve growth factor–induced neuronal differentiation after dominant repression of both type I and type II cAMP-dependent protein kinase activities. J. Biol. Chem. *266*, 15325–15333.

Graf, P., and Schacter, D.L. (1985). Implicit and explicit memory for new associations in normal subjects and amnesic patients. J. Exp. Psych. *11*, 501–518.

Greengard, P., Valtorta, F., Czernik, A.J., and Benfenati, F. (1993). Synpatic vesicle phosphoproteins and regulation of synaptic function. Science *259*, 780–785.

Hogan, B., Beddington, R., Costantini, F., and Lacy, E. (1994). Manipulating the Mouse Embryo, 2nd edition (Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York).

Huang, Y.-Y., and Kandel, E.R. (1994). Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. Learning Mem. *1*, 74–82.

Huang, Y.-Y., Kandel, E.R., Varshavsky, L., Brandon, E.P., Qi, M., Idzerda, R.L., McKnight, G.S., and Bourtchouladze, R. (1995). A genetic test of the effect of mutations in PKA on mossy fiber LTP and its relation to spatial and contextual learning. Cell *83*, 1211–1222.

Huang, Y.-Y., Bach, M.E., Lipp, H.-P., Zhuo, M., Wolfer, D.P., Hawkins, R.D., Schoonjans, L., Kandel, E.R., Godfraind, J.-M., Mulligan, A.R., et al. (1996). Mice lacking the gene encoding tissue-type plasminogen activator show a selective interference with late phase LTP in both Schaffer collateral and mossy fiber pathways. Proc. Natl. Acad. Sci. USA *93*, 8699–8704.

Impey, S., Mark, M., Villacres, E.C., Poser, S., Chavkin, C., and Storm, D.R. (1996). Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. Neuron *16*, 973–982.

Katz, B., and Miledi, R. (1968). The role of calcium in neuromuscular facilitation. J. Physiol. (Lond.) *195*, 481–492.

Kim, J.J., Rison, R.A., and Fanselow, M.S. (1993). Effects of amygdala, hippocampus and periaqueductal gray lesions on short- and long-term contextual fear. Behav. Neurosci. *107*, 1093–1098.

Laird, P.W., Zijderveld, A., Linders, K., Rudnicki, M.A., Jaenisch, R., and Berns, A. (1991). Simplified mammalian DNA isolation procedure. Nucleic Acids Res. *19*, 4293.

Mayford, M., Abel, T., and Kandel, E.R. (1995). Transgenic approaches to cognition. Curr. Opin. Neurobiol. *5*, 141–148.

Mayford, M., Baranes, D., Podsypanina, K., and Kandel, E.R. (1996). The 3′ untranslated region of CaMKIIα is a *cis*-acting signal for the localization and translation of mRNA in dendrites. Proc. Natl. Acad. Sci. USA *93*, 13250–13255.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P., and O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. Nature *297*, 681–683.

Müller, G.E., and Pilzecker, A. (1900). Experimentelle Beitrage zur Lehre vom Gedächtnis. Z. Psychol. *1*, 1–288.

Nguyen, P.V., Abel, T., and Kandel, E.R. (1994). Requirement of a critical period of transcription for induction of a late phase of LTP. Science *265*, 1104–1107.

Phillips, R.G., and LeDoux, J.E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav. Neurosci. *106*, 274–285.

Qi, M., Zhuo, M., Skålhegg, B.S., Brandon, E.P., Kandel, E.R., McKnight, G.S., and Idzerda, R.L. (1996). Impaired hippocampal plasticity in mice lacking the $C\beta_1$ catalytic subunit of cAMP-dependent protein kinase. Proc. Natl. Acad. Sci. USA *93*, 1571–1576.

Raymond, L.A., Blackstone, C.D., and Huganir, R.L. (1993). Phosphorylation of amino acid neurotransmitter receptors in synaptic plasticity. Trends Neurosci. *16*, 147–153.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd edition (Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York).

Sassone-Corsi, P. (1995). Transcription factors responsive to cAMP. Annu. Rev. Cell Dev. Biol. *11*, 355–377.

Schenk, F., and Morris, R.G.M. (1985). Dissociation between components of a spatial memory in rats after recovery from the effects of retrohippocampal lesion. Exp. Brain Res. *58*, 11–28.

Squire, L.R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys and humans. Psychol. Rev. *99*, 195–231.

Squire, L.R., and Alvarez, P. (1995). Retrograde amnesia and memory consolidation: a neurobiological perspective. Curr. Opin. Neurosci. *5*, 169–177.

Tsien, J.Z., Huerta, P.T., and Tonegawa, S. (1996). The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell *87*, 1327–1338.

Wu, Z.L., Thomas, S.A., Villacres, E.C., Xia, Z., Simmons, M.L., Chavkin, C., Palmiter, R.D., and Storm, D.R. (1995). Altered behavior and long-term potentiation in type 1 adenylyl cyclase mutant mice. Proc. Natl. Acad. Sci. USA *92*, 220–224.

Xing, J., Ginty, D.D., and Greenberg, M.E. (1996). Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science *273*, 959–963.

Yamamoto, T., Shimura, T., Sako, N., Yasoshima, Y., and Sakou, N. (1994). Neural substrates for conditioned taste aversion in the rat. Behav. Brain Res. *65*, 123–137.