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PKCγ Mutant Mice Exhibit Mild Deficits in Spatial and Contextual Learning

Asa Abeliovich,* Richard Paylor,† Chong Chen,*‡ Jeansok J. Kim,§ Jeanne M. Wehner,† and Susumu Tonegawa*‡
*Center for Cancer Research and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
‡Howard Hughes Medical Institute
at the Center for Cancer Research
and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
†Institute for Behavioral Genetics and School of Pharmacy
University of Colorado
Boulder, Colorado 80309
§Neuroscience Program
University of Southern California
Los Angeles, California 90089–2520

Summary

We are undertaking a genetic approach to investigate the role that synaptic modulation in the mammalian central nervous system plays in learning and memory and to identify relevant molecular components. We have generated mice deficient in the γ isoform of protein kinase C (PKCγ), an enzyme that has previously been implicated in both long-term potentiation (LTP) and learning and memory. These mice have a modified LTP of synaptic transmission in the hippocampus. We demonstrate that PKCγ-mutant mice can learn to carry out hippocampus-dependent tasks, although mild deficits are evident. Thus, hippocampal CA1 LTP induced by the conventional tetanic stimulation is not essential for the mice to exhibit spatial and contextual learning. Furthermore, the modification of hippocampal synaptic plasticity correlates with the learning deficits we observe.

Introduction

Donald Hebb theorized that the physiological basis of learning and memory involves the long-term strengthening of synapses among neurons that are coincidentally active (Hebb, 1949). Subsequently, long-term potentiation (LTP), a form of synaptic strengthening that exhibits the properties Hebb initially postulated (Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973) was identified: coincident presynaptic and postsynaptic neuronal activity is required for its induction; the modulation is specific for the activated synapse; and it is long lasting. Long-term depression (LTD), a second form of synaptic change or plasticity that also conforms to these rules, except it involves the weakening of synaptic connections, has recently been defined in the hippocampus (Dudek and Bear, 1992; Mulkey and Malenka, 1992). Both forms of synaptic plasticity are present in the hippocampus, a structure implicated in learning and memory by lesion experiments (Squire, 1987).

Hippocampus lesions have served to define two classes of associative learning: a complex class of learning, termed declarative or configural, that is sensitive to hippocampal lesions, and a simple class of learning, termed procedural, that is insensitive to hippocampal lesions (Squire, 1987, 1992; Eichenbaum et al., 1991, 1992; Sutherland and Rudy, 1989; Jarrard, 1993; Hirsh, 1974; O'Keefe and Nadel, 1978). Hippocampus-dependent learning tasks commonly involve associations among multiple sets of cues, whereas hippocampus-independent tasks commonly involve a simple association. In rodents, configural learning is often evaluated in the hidden-platform Morris water maze navigation task. This task involves the association of multiple spatial cues, a process termed spatial learning, that is a subclass of configural learning. More recently, other configural tasks, such as context-dependent fear conditioning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992), have been identified in rodents.

It has been proposed that LTP might serve as a mechanism by which synapses are strengthened in the course of hippocampal-dependent learning and memory (Bliss and Lømo, 1973). To test this hypothesis, Morris and others examined the spatial learning capability of rats whose hippocampal LTP had been blocked by AP5, an antagonist for glutamate receptors of the N-methyl-D-aspartate (NMDA) class (Morris et al., 1986, 1991). Since these rats were defective in both LTP and spatial learning, at least under certain conditions, these data suggest that LTP is the cellular mechanism for this type of learning. More recently, a genetic approach was applied to examine further the relationship between hippocampal LTP and spatial learning (Silva et al., 1992a, 1992b). Mice genetically deficient in the α isoform of calcium, calmodulin dependent protein kinase II (αCaMKII), were generated using the gene targeting technique. These mutant mice, although normal in ordinary synaptic transmission, are markedly deficient in hippocampal LTP and are severely impaired in spatial learning. Thus, the αCaMKII mutant study supports the hypothesis that LTP is the cellular mechanism for spatial learning. A similar conclusion (Grant et al., 1992) was drawn from the analysis of mice deficient in *fyn*, the gene encoding the nonreceptor tyrosine kinase Fyn.

While the cumulative evidence cited suggests a critical role for LTP in certain types of learning and memory, several issues remain unresolved. For instance, blocking NMDA receptors disrupts not only LTP, but also disrupts synaptic function and thus potentially interferes with the in vivo computational ability of hippocampal circuits (see Bekkers and Stevens, 1990). Therefore, perhaps the failure of learning results not from the deficit in LTP but simply from incorrect operation of hippocampal circuits. In the *fyn* mutants, a clear defect was found in the arrangement of the granule cells and the pyramidal cells of the CA3

region. Thus, a developmental abnormality, rather than the LTP blunting, could be the cause of the observed impairment in spatial learning. Furthermore, in the three studies cited, LTD was neither examined nor considered as a candidate cellular mechanism for learning and memory. It is now known that the NMDA receptor antagonist AP5 blocks the induction of hippocampal LTD (Dudek and Bear, 1992; Mulkey and Malenka, 1992) as well as LTP, and, furthermore, hippocampal LTD is impaired in αCaMKII-mutant mice (C. F. Stevens, S. T., and Y. Wang, unpublished data).

Thus, it is necessary to examine further the relationship between LTP (or LTD) and learning and memory. To this end, we have generated a strain of mouse mutant with a deletion in the gene encoding the γ isoform of protein kinase C (PKC). We chose to mutate PKC because PKC inhibitors have been shown to block LTP (Malinow et àl., 1988, 1989), indicating that PKC activity is required for LTP. Furthermore, several experiments have correlated hippocampal PKC with performance in learning tasks (Bank et al., 1989; Olds and Alkon, 1991), although no causal role for PKC has been established in learning and memory. For example, classical conditioning of the nictitating membrane response in rabbits has been correlated with increased phorbol ester binding in the hippocampus (Bank et al., 1988), and spatial learning performance of rats (Olds et al., 1990; Paylor et al., 1992) and mice (Wehner et al., 1990; Fordyce and Wehner, 1993) in the hidden-platform Morris water maze task have been correlated with hippocampal PKC activity.

PKC is composed of a family of at least ten isoforms encoded by at least nine genes (Nishizuka, 1988). In none of the LTP or learning experiments cited above has the specific functional role of the various isoforms been determined. In the present study, we chose to focus on the γ isoform because it is specific for neurons in the central nervous system (CNS) and is expressed postnatally (Hashimoto et al., 1988), in contrast with several other isoforms. Furthermore, an increase in the measured γ isoform in the hippocampus has been associated with spatial learning (Van der Zee et al., 1992), although the involvement of other isoforms cannot be excluded. An accompanying paper (Abeliovich et al., 1993 [this issue of *Cell*]) shows that LTP in the CA1 hippocampal region of PKCγ-mutant mice is abnormal: it can rarely be induced after conventional high frequency stimulation (tetanus), although apparently normal LTP can be observed if the tetanus is preceded by a low frequency (1 Hz) stimulation. Abeliovich et al. (1993) also show that the CA1 hippocampal region of mutant mice is normal in the induction of LTD as well as in ordinary synaptic transmission. In this paper, we analyze PKCγ-mutant mice in two different learning tasks, both of which require an intact hippocampus in rodents, but differ in their performance requirements and allow for the assessment of nonspecific behavioral impairments.

Results

PKCγ-mutant mice are viable and display normal grooming, feeding, circadian activity, and mating behaviors. However, PKCγ-mutant mice have an abnormal gait. For example, when walking on a floor made of round steel rods 1 cm apart, PKCγ-mutant mice often misplace their limbs between rods. In addition, mutant mice are more prone to falling off an inclined pole (3 cm diameter) than wild-type mice are. The observed coordination deficits appear to ameliorate with practice. This phenotype is typically observed in cerebellum-lesioned animals (Flourens, 1824) and, although mild, suggests a role for PKCγ in cerebellar physiology consistent with its high level of expression in cerebellar Purkinje cells (Nishizuka, 1988).

LTP is modified in PKCγ-mutant mice in that it can rarely be induced by conventional stimulation (Abeliovich et al., 1993). To test the correlation between hippocampal LTP and learning and memory, we subjected PKCγ-mutant mice to two learning and memory tasks, the performance of which is reported to require the hippocampus in rodents: a hidden-platform Morris water maze task that tests spatial learning and context-dependent fear conditioning, a task that tests contextual learning. Additionally, we investigated the performance of mutant mice in two tasks that, in rodents, do not require the hippocampus: a visible-platform water maze task (Morris et al., 1982; Sutherland et al., 1982) and tone-dependent fear conditioning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992).

Morris Water Maze

The Morris water maze (Morris, 1981) consists of a circular pool filled with opaque water that contains an escape platform submerged approximately 1 cm below the surface of the water. In the hidden-platform version of the Morris water maze task, mice are placed in the pool at 1 of 4 start sites and the platform location is kept constant throughout training. To escape the water, mice must learn to navigate to the hidden platform by mapping its position relative to visual cues outside of the pool, a process defined as spatial learning. In the visible-platform Morris water maze task, a cylindrical landmark is placed on the escape platform, indicating its position. Mice are placed in the pool at 1 of 4 start sites but, unlike the hidden platform, the visible platform is relocated to new quadrants of the pool between

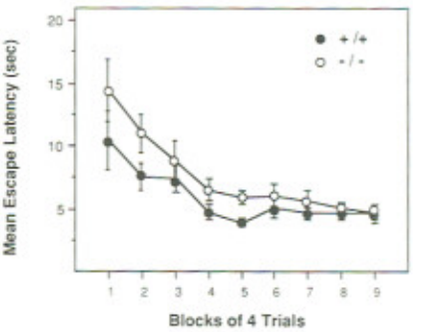

Figure 1. Average Time Taken to Locate the Visible Platform (Mean Escape Latency) for Wild-Type and PKCγ Mutant Mice

Mice were trained using a massed trial (12 trials per day) procedure. These data are from mice previously trained on the hidden-platform task. Performance improved for each genotype ($F[8, 104] = 15.547$, $p < .0001$) and there was no significant difference between wild-type and mutant mice ($F[1, 13] = 2.737$, $p = .122$).

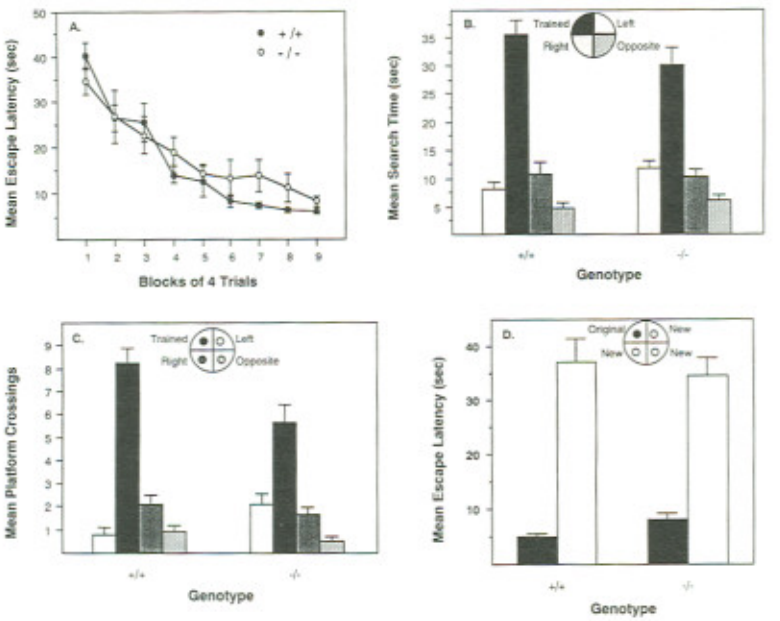

Figure 2. Performance of Wild-Type and PKCγ Mutant Mice Trained on the Hidden-Platform Version of the Morris Water Task

Mice were trained using a distributed trial (four trials per day) procedure.

(A) Average escape latency during training. Performance of wild-type and mutant mice improved during training ($F[8, 152] = 34.695$, $p < .0001$) and there was no difference between the two genotypes ($F[1, 19] = 0.286$, $p = .5987$).

(B) Average time subjects spent in each quadrant of the pool during the probe test.

(C) Mean platform crossings during the probe trial. Wild-type mice spent more time in the training quadrant than in the other quadrants ($F[3, 24] = 44.777$, $p < .0001$; Newman–Keuls post hoc comparison: trained > all other quadrants, $p < .01$) and crossed the site in which the platform was located more often than the alternate sites ($F[3, 24] = 57.517$, $p < .0001$; Newman–Keuls post hoc comparison: trained > all other quadrants, $p < .01$). Similarly, mutant mice spent more time in the training quadrant than in the other quadrants ($F[3,33] = 20.988$, $p < .0001$; Newman–Keuls post hoc comparison: trained > all other quadrants, $p < .01$) and crossed the site in which the platform was located more often than the alternate sites ($F[3, 33] = 13.251$, $p < .0001$; Newman–Keuls post hoc comparison: trained > all other quadrants, $p < .01$). Wild-type mice did not spend any more time in the training quadrant than mutant mice did ($t[19] = 1.145$, $p > 0.26$), but did cross the correct site more often than the mutants did ($t[19] = 2.337$, $p < 0.031$).

(D) Performance on the random platform test. Both wild-type and mutant mice located the platform more rapidly when it was in its original training site compared with when it was located in a new site ($F[1, 19] = 114.045$, $p < .0001$), and there was no difference between the two genotypes ($F[1, 19] = 0.001$, $p = 0.9846$).

trials. Therefore, mice must learn to associate the landmark with the location of the platform, and spatial information is irrelevant. In rodents, disruption of NMDA receptor function appears to impair LTP induction as well as performance on the hidden-platform Morris water maze task (Morris et al., 1986), whereas performance on the visible-platform task is unimpaired (Morris et al., 1991). Because the two tasks are similar in terms of motivation and the requirement for swimming ability, the visible-platform task serves as an important control for these factors.

Visible-Platform Morris Water Maze Task

PKCγ-mutant mice were tested in the visible-platform version of the Morris water maze, a nonspatial learning task, following training on the hidden-platform task. The performance of PKCγ-mutant mice ($n = 8$) was not significantly different ($p = .122$) from that of wild-type mice ($n = 7$), although mutant mice initially tended to perform somewhat more poorly than wild-type mice (Figure 1). In the course of training, mutant mice did reach the wild-type level of performance, demonstrating that mutant mice can learn this task and suggesting that the initial impairment displayed by mutant mice does not prevent them from learning. Similar results were observed when the mice were not trained on the hidden-platform task prior to visible-platform training (data not shown).

Hidden-Platform Morris Water Maze Task

Spatial learning was tested in the hidden-platform version of the Morris water maze task. Both mutant ($n = 12$) and wild-type ($n = 9$) mice displayed significant improvement over the nine blocks of training ($p < .0001$), and the two groups did not differ significantly ($p = .598$) (Figure 2A). Mice can improve their performance in the hidden-platform task by adopting a learning strategy other than spatial learning. For instance, the mice may learn that the platform is located a certain distance away from the edge of the pool. This strategy is not as precise as the spatial learning strategy, but, nevertheless, it enables mice to find the platform more quickly than a random search does.

To confirm that the PKCγ-mutant mice used a spatial learning strategy, we subjected the trained mice to a probe test in which the platform was removed and the mice were allowed to search the pool for 60 s. Both mutant ($p < .0001$) and wild-type ($p < .0001$) mice selectively searched the quadrant in which the platform had been located during the training versus all other quadrants (Figure 2B). Furthermore, both mutant ($p < .0001$) and wild-type ($p < .0001$) mice crossed the exact location at which the platform had been located during training more frequently than any of the corresponding locations in the other quadrants (Figure 2C). However, in this platform site-crossing test, mutant mice did cross the correct site less often than wild-type mice ($p < .05$), indicating that mutants harbor a moderate deficit in spatial learning.

We also subjected the trained mice to another test, the random platform test, to evaluate again the search strategy of PKCγ-mutant mice. In this test, the platform was placed either at its original training location or at a corresponding location in any one of the other three quadrants, and the time required to locate the platform was compared for the training versus the new locations. Both mutant and

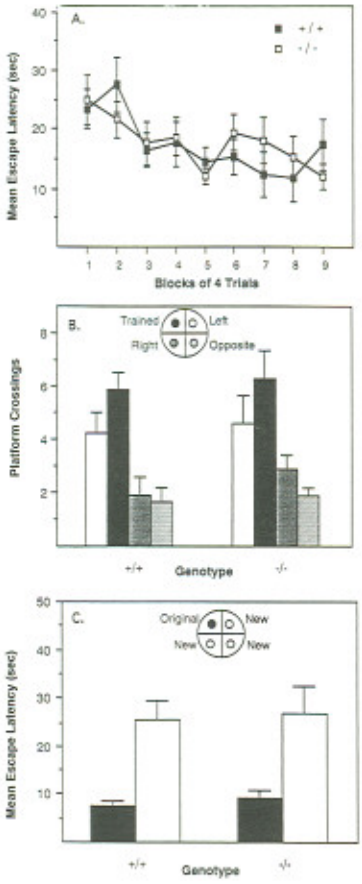

Figure 3. Performance of Wild-Type and PKCγ Mutant Mice Trained on the Hidden-Platform Version of the Morris Water Task

Mice of the C genetic background were trained using a massed trial (12 trials per day) procedure. These data are from animals previously trained on the visible platform task.

(A) Average escape latency during training. Performance of wild-type and PKCγ-mutant mice improved during training ($F[8, 104] = 3.90$, $p = .0005$) and there was no difference between the two genotypes ($F[1, 13] = 0.012$, $p = .9132$).

(B) Mean platform crossings during the probe trial. Wild-type mice crossed the site in which the platform was located more often than the alternate sites ($F[3, 21] = 15.63$, $p < .0001$; Newman–Keuls post hoc comparison: trained > left > right and opposite quadrants, $p < .05$). Similarly, mutant mice crossed the site in which the platform was located more often than the sites in the opposite and right quadrants ($F[3, 28] = 5.099$, $p = .0099$; Newman–Keuls post hoc comparison: trained > right and opposite quadrants, $p < .05$). Wild-type mice did not cross the correct site more often than the mutants did ($t[13] = -0.341$, $p < .65$).

(C) Performance on the random platform test. Both wild-type and PKCγ-mutant mice located the platform more rapidly when it was in its original training site as compared with when it was located in a new site ($F[1, 13] = 24.167$, $p = .0003$), and there was no difference between the two genotypes ($F[1, 13] = 0.165$, $p = .6914$).

wild-type mice were able to locate the hidden platform at the original location significantly more rapidly than at new locations ($p < .0001$) and the two groups of mice did not differ significantly in the time required to locate either platform ($p = .985$) (Figure 2D). Thus, the results of the so-called random platform test corroborated those of the probe test and demonstrated that the PKCγ-mutant mice can acquire spatial learning, although a mild deficit is detectable by the platform crossing test. Furthermore, when mutant mice were given more intensive training (massed training; see Experimental Procedures; data not shown), no significant genotype differences were observed on any hidden-platform task measures assessed.

Most of the behavioral analyses were performed in the 129/Ola × C57BL/6 (B6) genetic background, because preliminary experiments indicated that this genetic background performed best in the hidden-platform Morris water maze task. We also carried out an analysis of spatial learning with the PKCγ mutation in another genetic background, 129/Ola × BALB/c (C), to investigate the role of genetic background with respect to the PKCγ mutation. This second genetic background was chosen to allow for a comparison with the performance of αCaMKII-mutant mice. Learning was somewhat blunted in the C genetic background relative to the B6 genetic background for both wild-type ($n = 8$) and PKCγ-mutant mice ($n = 7$) (Figure 3). However, both wild-type and mutant mice of the C genetic background did show learning during acquisition (Figure 3A; $p < .001$), in the probe test (Figure 3B; $p < .001$), and in the random platform test (Figure 3C; $p < .001$). Furthermore, mutant and wild-type mice of the C genetic background did not differ significantly (all $p > .5$). This result confirms that PKCγ-mutant mice display spatial learning in the hidden-platform Morris water maze task and indicates that the difference in spatial learning performance between PKCγ-mutant mice and αCaMKII-mutant mice is not a consequence of the genetic background.

PKCγ-mutant mice display spatial learning in the hidden-platform Morris water maze task, although mutants are partially impaired relative to wild-type mice in the probe test. To investigate whether the PKCγ mutation is important in the retention of spatial memory, we tested wild-type and mutant mice in a probe 1 month subsequent to distributed training. Both mutant and wild-type mice remembered the location of the platform (Figure 4). Mutant mice did not differ significantly from wild-type mice in terms of time spent in the target quadrant (Figure 4A; $p > .2$) but crossed the target platform site significantly less often than wild-type mice did (Figure 4B; $p < .044$), reflecting their performance on the initial probe. Although both wild-type and mutant mice decreased their level of performance relative to the initial probe test, the decreases observed did not differ significantly. Thus, retention does not appear to be specifically affected by the PKCγ mutation.

Context-Dependent Fear Conditioning

We tested PKCγ-mutant mice in a second task, context-dependent fear conditioning (Kim and Fanselow, 1992), that has been shown to be dependent on the hippocampus and NMDA receptor function in rodents (Kim et al., 1992). In this task an initially neutral stimulus (an experimental chamber) is paired with an aversive, unconditioned stimulus (an electric shock). A conditioned freezing response, characterized by an immobile, crouching posture, is observed upon subsequent presentation of the experimental chamber. Hippocampus lesions in rodents serve to define two forms of fear conditioning: classical conditioning of

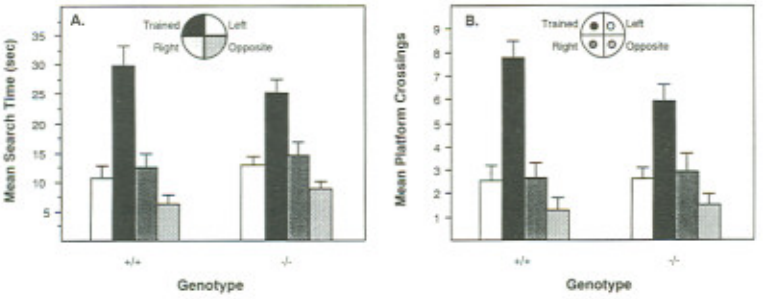

Figure 4. Performance of Wild-Type and PKCγ Mutant Mice on the 4 Week Retention Probe Test

These data are from mice trained with the distributed-trial procedure.

(A) Average time subjects spent in each quadrant of the pool during the retention probe test. (B) Mean platform crossings during the retention probe trial. Wild-type mice spent more time in the training quadrant than in the other quadrants ($F[3, 24] = 13.78$, $p < .0001$; Newman–Keuls post hoc comparison: trained > all other quadrants, $p < .01$) and crossed the site in which the platform was located more often than the alternate sites ($F[3, 24] = 17.629$, $p < .0001$; Newman–Keuls post hoc comparison: trained > all other quadrants, $p < .01$). Similarly, mutant mice spent more time in the training quadrant than in the other quadrants ($F[3, 33] = 7.743$, $p = .0005$; Newman–Keuls post hoc comparison: trained > all other quadrants, $p < .01$) and crossed the site in which the platform was located more often than the alternate sites ($F[3, 33] = 7.033$, $p < .0001$; Newman–Keuls post hoc comparison: trained > all other quadrants, $p < .05$). Wild-type mice did not spend any more time in the training quadrant than mutant mice did ($t[19] = 1.3.18$, $p < 0.2$), but did cross the correct site more often than the mutants did ($t[19] = 2.159$, $p < .044$).

fear to nonspecific cues, such as the context of an experimental chamber that is sensitive to hippocampal lesions; and conditioning to specific cues, such as a tone that is insensitive to hippocampal lesions (Kim and Fanselow, 1992; Phillips and LeDoux, 1992).

In the conditioning phase of the experiment, mice were placed in a shocking chamber and subsequently received three foot shocks. Mice were returned one day later to the shocking chamber and monitored for freezing behavior. Wild-type ($n = 14$) and PKCγ-mutant ($n = 13$) mice displayed comparable freezing during the conditioning phase of the experiment (Figure 5A), demonstrating that the PKCγ-mutant mice do not harbor a performance deficit in this task, such as an inability to freeze. The next day, both mutants and wild-type mice displayed the conditioned freezing response to the training context. However, mutants froze significantly less than wild-type mice (Figure 5B; $p < .05$), indicating a moderate deficit in context-dependent fear conditioning.

Additionally, we analyzed context-dependent fear conditioning of wild-type and mutant mice of the C genetic background. We found that freezing performance in both the conditioning and the testing phases was considerably blunted for all mice of the C genetic background (data not shown) relative to the B6 genetic background, similar to the performance of these strains in the hidden-platform Morris water maze task. We did not detect a deficit with PKCγ-mutant mice relative to wild-type mice of the C genetic background (data not shown), but this is likely to be due to the generally poor performance of mice of the C genetic background, which reduces the assay sensitivity.

Tone-Dependent Fear Conditioning

To evaluate the specificity of the fear-conditioning impairment observed, we tested mutant and wild-type mice in the tone-dependent fear conditioning task, which does not require hippocampal function in rodents. In this task, a 20 s long tone was presented immediately prior to each foot shock during the conditioning phase of the experiment (Figure 5C). In the testing phase of the experiment, animals were placed in a novel cage and subsequently the tone was presented (Figure 5D). Prior to the tone, mutant ($n = 8$) and wild-type ($n = 8$) animals did not freeze significantly in the novel cage. When the tone was presented, both wild-type and mutant animals displayed freezing behavior, and the two groups did not differ significantly ($p = .914$).

Discussion

We have shown that PKCγ-mutant mice can learn to carry out two learning tasks, the hidden-platform Morris water maze and context-dependent fear conditioning, although mild to moderate deficits are evident. The hidden-platform Morris water maze and context-dependent fear conditioning tasks both have been shown to require the integrity of the hippocampus in rats (Morris, 1981; Kim et al., 1992). In light of the known similarity of hippocampal functions across species (Eichenbaum et al., 1992), it is likely that the hippocampus also is needed for performance by mice in the hidden-platform Morris water maze and context-dependent fear conditioning tasks. Both of these tasks involve the configuration or integration of multiple cues or facts, a process broadly termed configural or declarative learning. However, these two tasks differ considerably otherwise, both in terms of the motivation involved and in the elicited response. Thus, it is striking that similar results were observed in both tasks. These findings suggest that PKCγ-mutant mice possess a mild deficit in configural or declarative learning per se rather than task-specific performance deficits.

Several additional results support the conclusion that the mild to moderate impairments we observed in these two tasks are due to learning impairments rather than task-specific performance deficits. Mutant mice reach the wild-type level of performance with training in the nonspatial visible-platform Morris water maze task. It is unlikely, therefore, that PKCγ-mutant mice harbor a significant performance deficit, such as a swimming impairment, a lack of motivation to escape the water, or poor vision. The deficit we observe in the hidden-platform Morris water maze task with PKCγ-mutant mice is dependent on the training regimen, as we found that with more intensive training, this deficit could be overcome (data not shown). Therefore,

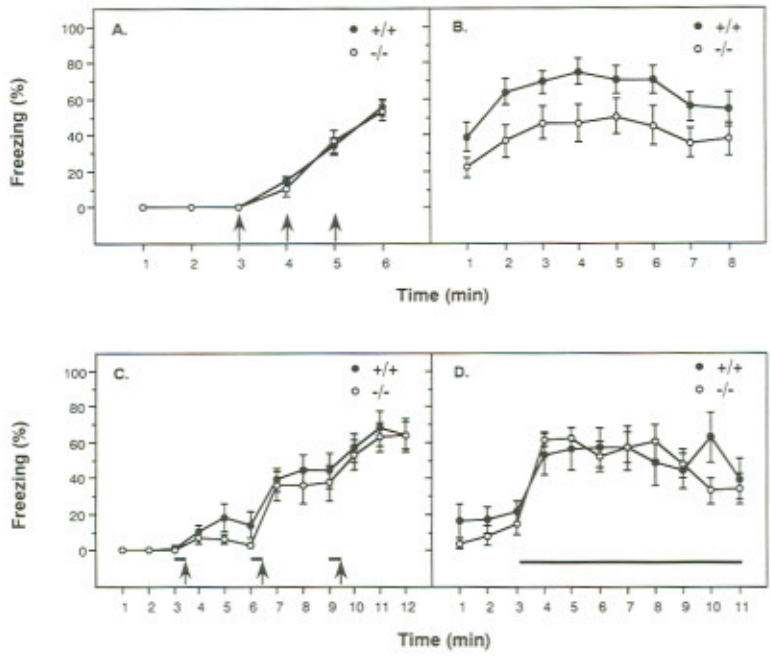

Figure 5. Mean Percent Freezing in the Context-Dependent and Tone-Dependent Conditioning Tasks

(A) Context-dependent fear conditioning, conditioning phase. Wild-type and PKCγ-mutant mice displayed a comparable degree of freezing ($F[1, 25] = 4.759$, $p = .864$) immediately after the foot shocks (arrows).

(B) Context-dependent fear conditioning, testing phase. Mutant mice displayed significantly less freezing than wild-type mice did when returned to the shocking chamber the next day ($F[1, 25] = 4.525$, $p = .043$).

(C) Tone-dependent fear conditioning, conditioning phase. Wild-type and PKCγ-mutant animals displayed a comparable degree of freezing ($F[1, 14] = .460$, $p = .5987$) immediately after the foot shocks (arrows) at the offset of the tone (solid line).

(D) Tone-dependent fear conditioning, testing phase. Wild-type and mutant mice displayed a comparable degree of freezing ($F[1, 14] = 0.012$, $p = 0.9142$) when presented with the tone (solid line) in a novel context the next day.

PKCγ-mutant mice are capable of performing well in this task. In the context-dependent fear conditioning task, mutant mice exhibited normal freezing immediately following a shock (Figure 5A), indicating that the deficiency in the conditioned freezing response (Figure 5B) observed 1 day later is not likely to be a consequence of performance deficits associated with motor, sensory, or motivational factors. Furthermore, PKCγ-mutant mice displayed normal freezing in the tone-dependent fear conditioning task, again arguing against a performance deficit. Finally, the context-dependent fear conditioning appears to be specific to the conditioned context, as freezing in an unconditioned novel context was minimal (see Figure 5D; the first 3 min correspond to freezing in the novel context).

Abeliovich et al. (1993) show that hippocampal LTP is abnormal in PKCγ mutants: it is absent or greatly attenuated when induced in vitro by conventional tetanic stimulation, although apparently normal LTP can be enabled by prior low frequency stimulation. Thus, our overall data show that LTP, as assessed by conventional tetanic stimulation, is not essential for mice to exhibit hippocampus-dependent learning capabilities (Table 1). However, the modified properties of hippocampal LTP correlate with mild to moderate deficits in spatial and contextual learning, consistent with the notion that LTP is a synaptic mechanism for these forms of learning. The learning deficits observed in PKCγ-mutant mice may be causally related to the LTP modification.

Another interesting candidate synaptic mechanism for learning that has emerged from this and other recent studies is LTD. While there has been no direct evidence, LTD satisfies the same criteria for a synaptic learning mechanism as does LTP (Siegelbaum and Kandel, 1991). Furthermore, LTD appears to correlate with spatial and contextual learning capabilities. LTD is intact in PKCγ-mutant mice (Abeliovich et al., 1993), which display spatial and contextual learning, whereas it is impaired in αCaMKII-mutant mice (Table 1; C. F. Stevens, S. T., and Y. Wang, unpublished data), which are deficient in spatial learning (Silva et al., 1992b). It is also of note that AP5, which has been shown to impair spatial and contextual learning (Morris et al., 1986; Kim et al., 1992), is now known to block not only LTP but also LTD (Dudek and Bear, 1992; Mulkey and Malenka, 1992). Therefore, it is possible that the learning impairments observed in AP5-treated animals result from the disruption of both LTP and LTD.

Although PKC has been implicated in synaptic plasticity and in learning and memory, previous studies rarely addressed the functional significance of specific PKC isotypes. It had been hypothesized that PKCγ might serve a specific function in the CNS because of its unique expression pattern among the PKC isotypes (Nishizuka, 1988). Van der Zee et al. (1992) found that the level of PKCγ immunoreactivity in the CNS correlates with spatial learn-

Table 1. Summary of Data from Mutant and AP5-Treated Mice

Analysis	PKCγ (−/−)	αCaMKII (−/−)	AP5 Treated
LTP (conventionally induced)	−	−	−
LTP (primed)	+	−	ND
LTD	+	−	−
Learning (spatial/contextual)	+[a]	−	−

[a] Partial impairment.

ND, not done.

LTP and LTD in PKCγ-mutant mice are from Abeliovich et al. (1993). LTP in αCaMKII-mutant mice is from Silva et al. (1992a); LTD in αCaMKII mice is from C. Stevens, S. T., and Y. Wang (unpublished data). Learning in αCaMKII mice is from Silva et al. (1992b). AP5 LTP is from Collingridge and Singer (1990); AP5 LTD is from Dudek and Bear (1992); and AP5 learning is from Morris et al. (1986).

ing, consistent with a role for PKCγ in spatial learning. Our data indicate that PKCγ does play a role in both LTP and in learning and memory, but is not essential for either process. One possible interpretation of these findings is that other PKC isotypes, such as the more abundant PKCβ isotypes, can compensate for the PKCγ deficiency. However, this is clearly not always the case, as conventionally induced LTP is severely deficient in the PKCγ-mutant mice. Therefore, we find it likely that PKCγ possesses certain unique functions. Another (and perhaps a more interesting) interpretation of these findings is that second messenger systems (Abeliovich et al., 1993; Silva et al., 1992a, 1992b) play a regulatory role in LTP and in configural learning so that the deficits we have observed reflect a failure in the regulation of LTP and configural learning rather than a deficit in their mechanisms. Our data suggest a role for the γ isotype of PKC in the regulation of configural learning.

It is now of interest to characterize further the physiological differences between PKCγ- and αCaMKII-mutant mice to identify additional candidate mechanisms and anatomical regions that may be involved in the different behavioral phenotypes observed in these mutants. It is also of interest to determine whether these different mutations function within the same genetic pathway by generating mice that harbor both mutations.

Experimental Procedures

Animals

Animal care was in accordance with institutional guidelines. Animals were typed for the PKCγ genotype by tail biopsy (Abeliovich et al., 1993). Animals utilized were of the B6 or C genetic backgrounds, as stated. B6 albino animals were not utilized to avoid the possibility of visual impairments. Mice were housed in standard animal cages in an animal facility with a 12 hr light–dark cycle: lights on at 0700 hr. Standard laboratory chow and water was provided ad libitum.

Morris Water Task

Naive adult mice (6–10 weeks old) were utilized. The training apparatus was a circular polypropylene (Nalgene) or galvanized steel pool 120 cm in diameter. The water was maintained at 26°C. Nontoxic Crayola paint was added to make the water opaque and to blend with the color of the pool wall. The pool was located in a laboratory room that had a number of items that could be seen by an animal swimming in the pool (for details see Paylor et al., 1993).

Visible-Platform Training

The visible platform was 11.5 × 11.5 cm in diameter. A cylinder (13 cm tall and 6.5 cm in diameter) was attached to the top of the platform such that the bottom of the cylinder was 10 cm above the platform top. The top of the platform was 1 cm below the surface of the water. Prior to training, each mouse was acclimated to the water and escape platform by placing it on the platform for 15 s followed by a 15 s swim and three practice climbs onto the platform. The platform location varied among four possible places within each block of four trials. A trial was started by placing an animal along the edge of the pool facing the wall in 1 of 4 start locations. A subject was allowed 60 s to locate the platform. Animals not finding the platform in 60 s were guided there by the experimenter. Animals were allowed to remain on the platform for 20 s. Each mouse was given 12 trials a day, in blocks of 4 trials for three consecutive days (massed-trial procedure). The time taken to locate the escape platform (escape latency) was determined on each trial. Mice in these visible-platform experiments started training either on the visible-platform task first, followed by training on the hidden-platform task, or they were started on the hidden-platform task, followed by visible-platform training. Only the latter data are presented in the results.

Hidden-Platform Training

Data presented in this study from the hidden-platform experiments were obtained using the same procedures that were used for the visible-platform task, with the following exceptions. First, there was no visible cylinder attached to the platform. Second, the platform location varied among different animals, but always remained in the same place for any given subject. Finally, mice were given four trials a day for nine consecutive days. Each trial during a day was separated by 1 hr (distributed trial procedure). During training, escape latencies were determined.

After the last training trial (1 hr), each animal was given a probe trial. During the probe test, the platform was removed and each animal was allowed 60 s to search the pool. Two measures of search behavior were determined. A quadrant search time measure was obtained by dividing the pool into four equal quadrants and determining the amount of time spent in each quadrant. A platform crossing measure was obtained by counting the number of times a subject crossed the exact place in the training quadrant in which the platform had been located during training. For comparison, the number of times a subject crossed the equivalent location in each of the other quadrants was determined.

Following the probe trial mice were given a random platform test. On day one of the random platform, test animals were given four trials with the platform in its original location. On the next day, subjects were given one trial with the platform in its original location and three trials with the platform in one of the platform sites in the other three quadrants. The average time taken to locate the platform on the trials prior to the platform being relocated in a new location was used as the original platform escape latency of an animal, and the average time taken to locate the platform when it was in its three new locations was used as the new platform escape latency of an animal.

Fear Conditioning

Naive adult mice (6–10 weeks old) were housed individually for at least 1 week prior to behavioral testing. Mice were handled routinely for 1 week prior to behavioral experiments to reduce stress. Fear conditioning and testing were conducted in a small rodent chamber (Coulbourn) containing a stainless steel rod floor (5 mm diameter, spaced 1 cm apart) through which scrambled foot shocks could be administered. The chamber was placed inside a sound-attenuating chest (Coulbourn) with a ventilation fan providing background noise. The chamber was cleaned with 1% acetic acid and dried completely before each animal was placed inside. Freezing was assessed by a time sampling procedure in which an observer blind to mouse genotype scored each mouse every 2 s. Percent freezing was calculated per minute. Experiments were recorded on videotape.

Context-Dependent Fear Conditioning

In the conditioning phase, animals were placed in the shocking chamber for 3 min and subsequently subjected to three foot shocks (0.5 mA intensity, 1 s duration, 1 min apart). Mice were removed from the chamber 1 min after the last foot shock. In the testing phase (the next day), animals were returned to the shocking chamber and freezing was monitored for 8 min. Preliminary experiments indicated that these conditions (the interval between the conditioning phase and the testing phase, the chamber, and the shock intensity) are sufficient for observing a freezing impairment (data not shown).

Tone-Dependent Fear Conditioning

Animals were placed in the shocking chamber for 3 min and then presented with three 20 s loud tones (approximately 75 db, 1000 Hz, 3 min apart) through a speaker mounted on the chamber. A foot shock (0.5 mA intensity, 1 s duration) was presented at the offset of each tone. Mice were removed from the chamber 1 min after the last foot shock. In the testing phase (the next day), mice were placed in an empty plastic cage different from the shocking chamber (to minimize freezing due to context-dependent fear conditioning) and freezing was scored for 3 min prior to the tone and subsequently for 8 min in the presence of the tone.

Data Analysis

Both male and female mice were tested, but since there were no signifi-

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