kept on ice, and it was typically diluted 1/10 to 1/200 in the same cold buffer. The assays were carried out after 2- to 20-fold dilution of the homogenate in cold water, and immediately followed by the addition of cold assay buffer to a final concentration of 50 μM Syntide 2, 25 mM Hepes (pH 7.4), 10 mM MgCl, 0.5 mM DTT, 15 μM ATP and [γ-^{32}P]ATP at 50 μCi/ml. For the Ca^{2+}-CaM induced assays, we also added to this buffer 1.5 μM calmodulin and 2.0 mM $CaCl_2$. For the Ca^{2+}-CaM independent reactions, we added 0.5 mM EGTA to the assay buffer. After adding the assay buffer, reactions were briefly mixed and quickly placed at 30°C for 45 s. The reaction was terminated by spotting half of the reaction volume (25 μl) in perforated disks of phosphocellulose. These disks were then washed of nonincorporated [γ-^{32}P]ATP with 1 percent phosphoric acid and water. The radioactivity bound to the disks was counted and the values plotted. The phosphorylation results shown were derived from four independent experiments, each with at least three different concentrations of homogenates to check that substrate was not limiting, and with duplicates at each concentration point.

15. K. E. Burgin *et al.*, *J. Neurosci.* **10**, 1788 (1990).
16. We used 50 μM of peptide CaMKII272-302 described in reference 9.
17. R. J. Douglas, *Psychol. Bull.* **67**, 416 (1967).
18. All animal handling and tissue preparation were in accordance with a protocol approved by the Salk Institute and MIT Animal Use and Care Committee. Transverse hippocampal slices (~350 Im) were prepared from normal (wild) or mutant mice (male or female, 1 to 4.5 months old, mostly 1.5 to 3 months old). Slices were then maintained in an incubation chamber for at least 1 hour at room temperature (24° ± 1°C). An individual slice was transferred to a submerge–recording chamber where it was held by a net made with flattened platinum wire and nylon threads and continuously perfused with artificial cerebrospinal fluid (ACSF) at a rate of ~2 ml/min. The temperature in the recording chamber was 30.5° ± 0.5°C. The ACSF, equilibrated with 95 percent O_2 and 5 percent CO_2, is composed of (mM): NaCl (120), KCl (3.5), NaH_2PO_4 (1.25), $NaHCO_3$ (26), $MgCl_2$ (1.3), $CaCl_2$ (2.5), PCTX (0.05). The solution for dissection has the same composition as regular ACSF except there is no PCTX and NaCl is replaced with equimolar amounts of sucrose [G. K. Aghajanian and K. Rasmussen, *Synapse* **3**, 331 (1989)]. The CA3 region was usually removed to prevent epileptiform activity. The cell layer was visualized under an inverted microscope with phase contrast (Zeiss). Extracellular field excitatory postsynaptic potentials (f-EPSP's) were recorded in the stratum radiatum of CA1 with electrodes (1 to 2 Mohm) filled with ACSF. Excitatory post-synaptic currents (EPSC's) were recorded in CA1 pyramidal neurons with the whole-cell patch-clamp mode; electrodes (3 to 4 Mohm); no fire polishing; soft glass (Drummond) filled with (mM) cesium gluconate (130), $CsCl_2$ (5), EGTA (0.5), $MgCl_2$ (1), Mg-ATP (2), GTP (0.2), NaCl (5), Hepes (10); pH 7.25. The seal formed on cell bodies was typically 2 to 3 Gohm and the input resistance of cells was typically around 100 Mohm. The Bipolar tungsten stimulating electrodes (Frederick Haer & Co.) were positioned in Schaffer collateral-commissural afferents to evoke f-EPSP's (150 to 200 μm away) or evoke EPSC's (50 to 100 μm away). The stimulus intensity was adjusted to evoke pretetanic responses of similar sizes for all the neurons or slices. The stimulus duration was 100 μs. Recordings were performed with an Axopatch-1A (Axon Instruments, Inc.), filtered at 1 to 2 kHz, and sampled at 5 to 10 kHz. Data were collected and analyzed with programs written by C. F. Stevens in AxoBASIC/QuickBASIC. The data collected from normal other strain of mice were combined with the data from normal littermates, since they were indistinguishable. CNQX and D-APV (D-2-amino-5-phosphonovaleric acid) were from Cambridge Research Biochemicals and PCTX was from Sigma.
19. L. Chen and L. Y. M. Huang, *Nature* **356**, 521 (1992).
20. C. E. Jahr and C. F. Stevens, *J. Neurosci.* **10**, 1830 (1990); *ibid.*, p. 3178.
21. J. W. Lin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8257 (1990).
22. J. M. Bekkers and C. F. Stevens, *Nature* **346**, 724 (1990); R. Malinow and R. W. Tsien, *ibid.*, p. 177.
23. T. McGuiness, Y. Lai, P. Greengard, *J. Biol. Chem.* **260**, 1969 (1985).
24. W. Muller, J. A. Connor, *Nature* **354**, 74 (1991); P. B. Guthrie, M. Segal, S. B. Kater, *ibid.*, p. 76.
25. A. J. Silva, unpublished data.
26. W. A. Falls, M. J. D. Miserendino, M. Davis, *J. Neurosci.* **12**, 854 (1992); M. Davis, *Tips* **13**, 35 (1992).
27. The 6.1-kb genomic fragment present in p23 was obtained after Pvu II digestion of cosmid 14.4. This cosmid was cloned from a C57B1/6J library with a Sph I–Pvu II fragment from an α-CaMKII full-length cDNA that only detects α-CaMKII. The *neo* gene was inserted within the 6.1-kb genomic-fragment at the deleted 130 bp Sph I fragment, and its transcriptional orientation, is the same as the endogenous α-CaMKII.
28. The DNA samples (5 μg) shown in the autoradiograph were digested with Pvu II restriction enzyme, blotted to nylon membranes, and probed with radioactively labeled p23 (1 × 10^9 cpm/μg). We used only mutation homozygotes that were F1 progeny from crosses between these mutant heterozygous.
29. We have also analyzed the Western blots shown with a polyclonal antibody that recognizes α- and β-CaMKII. This antibody has a wider specificity, and it might have recognized any other proteins resulting from the fusion between α-CaMKII and the inserted *neo*.

30. We thank A. Smith, Y. Ichikawa, M. B. Kennedy, M. Hooper, N. Waxman, and P. Kelly for advice or various invaluable reagents (or both); J. R. Pauly, G. Schneider, and R. Erzurumlu for help with the neuroanatomy. Supported by Howard Hughes Medical Institute (S.T. and C.F.S.), Human Frontier Science Program grant #76834 (S.T.), and NIH grant 5 R01 NS 12961-17 (C.F.S.).

10 June 1992; accepted 19 June 1992

Impaired Spatial Learning in α-Calcium-Calmodulin Kinase II Mutant Mice

Alcino J. Silva, Richard Paylor, Jeanne M. Wehner, Susumu Tonegawa

Although long-term potentiation (LTP) has been studied as the mechanism for hippocampus-dependent learning and memory, evidence for this hypothesis is still incomplete. The mice with a mutation in the α-calcium-calmodulin–dependent kinase II (α-CaMKII), a synaptic protein enriched in the hippocampus, are appropriate for addressing this issue because the hippocampus of these mice is deficient in LTP but maintains intact postsynaptic mechanisms. These mutant mice exhibit specific learning impairments, an indication that α-CaMKII has a prominent role in spatial learning, but that it is not essential for some types of non-spatial learning. The data considerably strengthen the contention that the synaptic changes exhibited in LTP are the basis for spatial memory.

Changes in synaptic strength may be critical for learning either as a mechanism for the direct storage of memories, or as a process that transforms information making it suitable for long-term storage (*1*). Long-term potential (LTP) is a stable and long-lasting potentiation of synaptic activity which follows Hebbian rules (*2*). Hence, it is widely thought that LTP is a physiological mechanism underlying learning and memory processes. The *N*-methyl-D-aspartate receptor (NMDAR) is a voltage-sensitive and glutamate-gated channel, and it regulates a calcium current essential for the induction of LTP (*3*). The evidence supporting the linkage between LTP and mammalian learning and memory primarily comes from the analysis of rats in which the NMDAR was blocked by the antagonist, aminophosphonovaleric acid (APV) (*4*). The interpretation of those results is difficult because inhibiting NMDAR function also disrupts synaptic function (*5*) and therefore might alter the character of information processing in the hippocampus. Thus, the deficits in learning could be due to this alteration in hippocampal synaptic function, and not to the deficits in LTP. A second line of evidence linking LTP to learning and memory comes from electrophysiological experiments that show that induction of saturating levels of LTP in the hippocampus impairs the ability of rats to acquire new spatial information (*6*). However, these findings have also been alternatively interpreted (*7*).

It is thus important to develop addition-

A. J. Silva and S. Tonegawa are at the Howard Hughes Medical Institute, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
R. Paylor and J. M. Wehner are at the Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

al experimental strategies for studying the relation between LTP and learning. One way of doing this would be to use embryonic stem (ES) cell-gene targeting (8) and produce mice with a mutation in individual enzymes likely to participate in the regulation of LTP. For our study, we chose the α isoform of calcium-calmodulin–dependent kinase II (α-CaMKII) (9). The α-CaMKII is postnatal (*10*) and neural-specific, and is present both pre- and postsynaptically (*11*). The postsynaptic densities of the hippocampus and cerebral corex are particularly rich in this enzyme (9). These two brains structures are essential for complex learning, such as spatial learning in rodents (*12*). Furthermore, pharmacological studies have implicated the CaMKII holoenzyme in the induction of LTP in the hippocampus (*13*).

As was described (*14*), we produced mutant mice defective in α-CaMKII and have shown that the CA1 hippocampal region of these mice exhibit little or no LTP although postsynaptic transmission appears normal. Therefore these mice are ideal for examining the association of hippocampal α-CaMKII activity, LTP, and learning and memory processes. In our present study, we examined whether α-CaMKII mutant mice can learn to perform a complex spatial learning task, such as the Morris water tasks (*15*). We now report that α-CaMKII mutant mice show a pronounced deficit in spatial learning performance compared to normal wild-type littermates. Our data demonstrate that α-CaMKII is important for spatial learning and support the hypothesis that LTP is the electrophysiological basis for certain types of learning processes.

The Morris water task. In the Morris water task, mice are placed in a round pool filled with water that has been made opaque. To escape the water, the mice must swim to a submerged platform. In the "visible-platform" version of the Morris task, a visually conspicuous white flag is placed on top of the submerged platform which is positioned in random locations on each trial. To solve this task and swim directly to the platform, an animal needs only to learn that the flag indicates the location of the platform. Hence, distal extra-maze cues are irrelevant in this task. In the "hidden-platform" version of the Morris task, the escape platform is in a fixed location within the pool. Since there are no immediate proximal cues indicating where the platform is, and the platform cannot be seen through the water, the animal must learn the multiple spatial relation between distal objects in the room surrounding the pool and the platform in order to locate and swim directly to it. There are five phases to this test. All animals were subjected to each phase in the order presented below.

Solving the visible-platform task. In this first phase of the experiment, mice were tested on the visible-platform test. The platform location varied among four possible places within each block of trials (see legend to Fig. 1). Animals were tested for two consecutive days with three blocks of four trials per day, and the time required to reach the platform was recorded.

The α-CaMKII mutant mice initially took longer than the wild-type mice to reach the platform, but by the end of training they were locating it as rapidly as controls (Fig. 1A). Although α-CaMKII mutant mice appear to be initially impaired, they were able to overcome this deficit by training. Therefore, α-CaMKII mutant mice are (i) able to learn to associate the flag with the escape platform, (ii) motivated to escape the water, and (iii) have the coordinated motor skills needed to swim in water.

The exact nature of the initial impairment is not clear, but it is likely the impairment is due to the different response of the α-CaMKII mutant mice, compared to that of the wild-type animals, when placed on the platform. Unlike wild-type mice, the α-CaMKII mutant mice immediately jumped into the water. This "jumping response" occurred a number of times before the first trial, and thus by the time their jumping response habituated they appeared fatigued. Therefore, they may have taken longer on day 1 of visible-platform training because they were tired. On day 2, however, they did not show the jumping response and hence were not fatigued before the trials.

Training on the hidden-platform task. From 7 to 12 days after the visible-platform task, animals were trained on the Morris hidden-platform task. In the hidden-platform task, there are no direct proximal cues marking the location of the platform, and the platform remains submerged in a fixed place. Therefore, to locate the platform efficiently an animal needs to learn the multiple spatial relations between extra-maze cues and the escape platform.

Animals were subjected to the standard 3-day training used previously for inbred strains of mice (*16*). Wild-type controls quickly learned to locate the hidden platform, and by the end of day 3 of training they were navigating directly to it in less than 10 s (Fig. 1B). The α-CaMKII mutant mice took longer than the wild-type mice to locate the hidden platform, but the performance improved during training. However, after performance reached a plateau, the mutant mice still required approximately 20 s to locate the platform, approximately twice as long as the wild-type mice.

To ensure that mice were at asymptotic levels of performance, we provided some animals from each group (n = 5) with additional training for 2 days. Although wild-type animals were better than the mutant mice at locating the platform during extended training, neither group improved compared to day 3 of training (*17*). In all measures of performance during each phase, extended training did not significantly alter

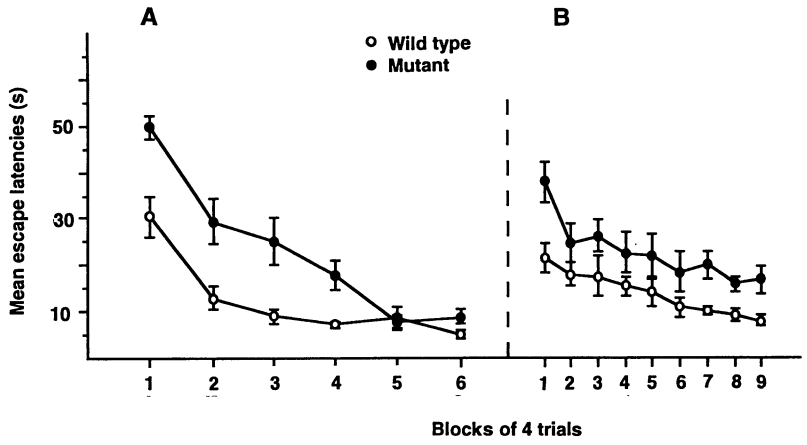

Fig. 1. Mean escape latencies for animals in the Morris water task. (**A**) In the first phase of the experiment, wild-type controls (n = 14) and α-CaMKII mutant mice (n = 11) were trained to navigate to a randomly located visible platform. The platform was rendered visible by attaching a small white flag to its top. Each animal was first trained to climb on the platform and given a 15-s practice swim to ensure that all animals could swim. On each trial, a subject was allowed to search the pool for 60 s. Once a subject found the platform, it was allowed to remain there for 45 s. Animals were given 12 trials a day, in blocks of 4 trials, on two consecutive days. The α-CaMKII mutant mice were initially impaired at locating the visible platform but overcame this deficit and learned to locate as rapidly as controls (*28*). (**B**) All animals were then trained to find a hidden platform located in a fixed location. The top of the platform was 1-cm below the surface of the water. Wild-type and α-CaMKII mutant mice were given either 3 or 5 days of training as described above (only 3 days are shown). The figure shows that wild-type controls had lower escape latencies than the mutants (*29*). The bars indicate the SEM.

the performance of wild-type or α-CaMKII mutant mice.

Probe trial. In order to evaluate whether the mice indeed located the hidden platform by learning the multiple spatial relations between distal cues and the hidden platform, we subjected them to a probe trial. In this trial the platform was removed and the mice were allowed to search the pool for 1 minute. If an animal located the platform during training by using distal cues, it should selectively search the place where the platform was located during training more than other places in the pool. Animals were given their probe trial immediately after the last training trial (after 3 or 5 days of training) (Fig. 2).

The wild-type mice selectively searched the area where the platform had been located during training. They spent a larger percentage of their time in the quadrant where the platform had been during training (training quadrant) than in the other three quadrants. They also crossed the exact spot where the platform had been located during training (training site) more often than equivalent locations in other quadrants. In contrast, α-CaMKII mutant mice did not spend more time searching in the training quadrant than in the other quadrants; they spent an equal amount of time in all four quadrants. The mutant mice did not cross the training site more often compared to equivalent locations in the adjacent quadrants. They did cross the equivalent site in the opposite quadrant less frequently compared to the training site. This difference, however, may be an artifact of the testing protocol in which the animals always started the probe trial from positions in the opposite quadrant. The α-CaMKII mutant mice may have learned to swim directly away from the start location before implementing any search strategy. Since they were always started from places opposite of the training site, their starting strategy reduced the likelihood of crossing the opposite quadrant site early in the probe; the effect of random choice of the probe trial start location remains to be determined.

These data indicate that the wild-type mice learned the spatial relations between distal cues and the hidden platform (spatial strategy). In addition, these results suggest that the mutant mice improved their performance by developing an alternative strategy that does not depend on specific cues surrounding the pool. For example, mutant mice may have learned the distance between the wall of the pool and platform. Since the pool is circular and the wall is uniform, this strategy would not allow the

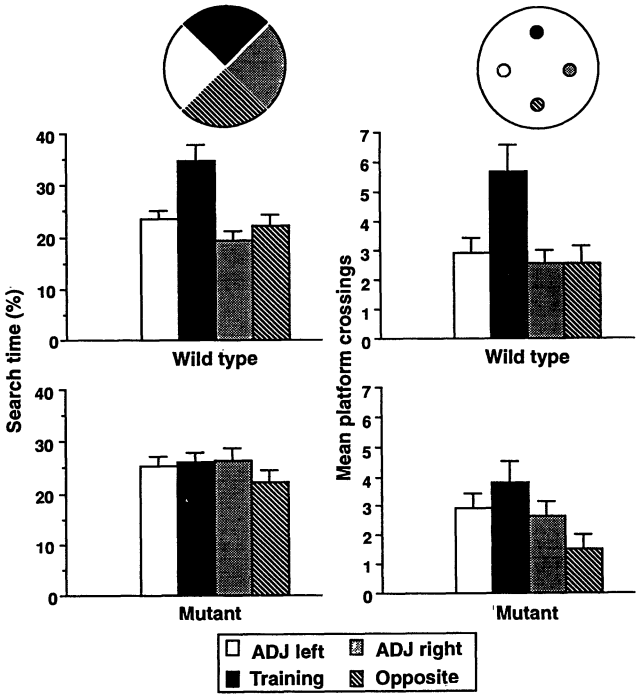

Fig. 2. Data from animals given a probe trial after 3 or 5 days of training. Since there were no differences in performance for wild-type or α-CaMKII mutant mice given 3 or 5 days of training prior to the probe trial (*17*), the data were analyzed together. These data show that wild-type animals selectively searched the place where the platform had been located during training, while in general the α-CaMKII mutants' search was not selective. (Upper) Data from the wild-type animals (*30*). (Lower) Data for the α-CaMKII mutant mice. In contrast to wild-type animals, α-CaMKII mutants did not selectively search any quadrant of the pool [ANOVA $F(3,30) = 0.689$, $P > .05$]. The α-CaMKII mutant mice also failed to cross the exact place where the platform had been located compared to both adjacent sites. However, they did cross the opposite site less often than the training site (*31*). Above the panels on the left, we show a schematic drawing of the pool with the four virtual quadrants used in the analysis, and on the right we show the four platform sites. The bars indicate the SEM.

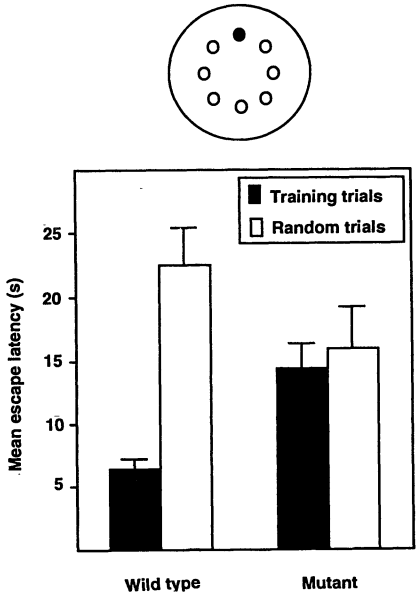

Fig. 3. Latency analysis of the random-platform task. Two days after the animals were given 3 or 5 days of training and subsequent probe trials, they were given a block of four trials with the platform in its original training location. On the next block of four trials, the platform was in the original location for the first two trials but was in two new (random) locations on the next two trials. On the final block of our trials the platform was placed in the original spot on the first trial and then in different random locations on the next three trials. (Top) Locations of the platform in the training trials (●) and in the "random" trials (○). In each random trial only one of the seven locations was used. Presented is the mean latency to find the platform on the seven trials when the platform was in its original location (black bars) compared to the five trials when it was in random locations (white bars). The wild-type controls took less time to find the platform when it was in its original location compared to when it was in random locations [correlated t test; $t(12) = -6.504$, $P < 0.001$]. The α-CAMKII mutant mice, however, were just as proficient at locating the platform in random locations as when it was in its original location (*32*). The bars indicate the SEM.

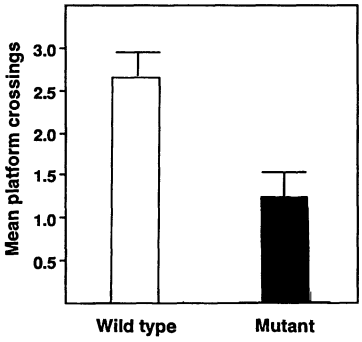

Fig. 4. Platform-crossing analysis in the random-platform task. Represented in this figure are the mean number of times mice crossed the original training site on the five trials with the platform in random locations. Wild-type controls crossed the training site significantly more often than α-CaMKII mutant mice on these five trials [$t(21) = 3.318$, $P < 0.01$]. The bars indicate the SEM.

mutant mice to distinguish among the four quadrants of the pool.

Random-platform task. The results of the probe trial suggest that mutant mice did not learn the spatial relations between distal cues and the hidden platform. However, these mice could improve their performance on the hidden platform task. In order to confirm that mutant mice are impaired at spatial learning, we subjected them to a random-platform task. The mice were given trials with the platform in its original location intermixed with trials where the platform was moved to any one of the seven other locations (Fig. 3). If the mutant mice were impaired in spatial learning, they should find the platform in the new locations as readily as when it was in the original training site.

The wild-type mice took significantly less time to locate the platform when it was in its original location (training trials) compared to new locations (random trials) (Fig. 3). In contrast, the α-CaMKII mice took as long to locate the platform when it was in new locations as when it was in its training site. These results support the hypothesis that the mutant mice developed a nonspatial strategy to find the platform. Another observation that confirms the validity of our hypothesis is that on the random trials wild-type mice crossed the training site more often than the α-CaMKII mutant mice (Fig. 4).

As would be expected from the data in Fig. 1B, mutant mice took approximately twice as long as the wild-type mice to locate the platform when it was in its training site. This performance is most probably a reflection of strategy used by the mutant mice, which does not result in as precise a localization of the platform as a spatial strategy does.

The plus (+) maze task. The results described thus far indicate that the mutant mice do not use a spatial strategy to locate the platform. This may mean that the mutant mice are impaired in learning the spatial relations between distal cues and the escape platform (true impaired spatial learning). However, an alternative explanation of the results is that the mice are impaired in another process (or processes), such as the ability to see and attend to distal cues, or to make an association between the distal environment and the escape platform. In order to exclude these latter possibilities, we tested the mice in a water-filled plus (+) maze (*18*).

The plus maze is a four-armed (+) clear Plexiglas maze filled with opaque water. An escape platform is placed in one arm of the maze with its top 1 cm below the surface of the water. The hidden escape position of the platform is kept constant throughout all trials. To find the platform, the mouse must learn to swim into the arm containing the platform and avoid the other possible arms. Because the maze is clear the animal can use prominent distal cues in the room to locate the platform. On each trial, a mouse was placed in one of the three arms that did not contain the platform and was allowed to swim toward the intersection. At this point, the mouse must choose one of the three remaining arms to enter. If the mouse chose the correct arm containing the platform it was allowed to climb onto the platform and then was removed from the maze and the choice was scored correct. When the animal swam into another arm of the maze that did not contain the platform, it was trapped in that arm and the trial was scored as a mistake. The arm used to start the animal on each trial was chosen more or less randomly with the restriction that each arm that did not contain the platform was used in a block of three trials. The plus (+) maze was placed directly on top of the Morris pool to ensure that the same distal cues were utilized.

Since on each trial there are three possible arms into which an animal can swim, random choices should result in the correct choice 33 percent of the time. Likewise, other predictable strategies such as always swimming left, right, or straight would also produce correct choices about 33 percent of the time because the start position was rotated such that in each ten trials each of the three start arms are used at least three times. The criterion used to assess whether mice had learned to locate the platform using a distal cue was 70 percent correct choices in ten trials.

The plus maze task has several features in common with the hidden-platform version of the Morris task. Both tasks require subjects to navigate through water and locate a hidden platform by using the environment outside the maze. Thus, in both tasks animals must be able to see and attend to distal cues. The differences between the tasks is that the "hidden-platform" version of the Morris task requires an animal to learn multiple spatial relations between distal cues and the escape platform. In the plus (+) maze, however, an animal always takes the same path to the platform; thus, it always has the same distal environment to swim toward for escape. Therefore, the (+) maze puzzle can be solved by learning the single relation between a particular distal environment and the escape platform.

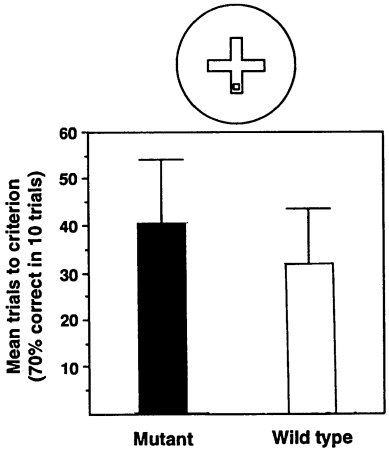

Fig. 5. Analysis of performance on the "+" maze showing the mean number of trials to reach the learning criterion of 70 percent correct in ten trials in a water-filled plus (+) maze. On each trial, an animal was placed facing the center of the maze in one of the three arms that did not contain the platform. An animal was allowed to swim into any of the arms, but if it chose an incorrect arm, it was trapped for 20 s. If it chose the correct arm, it was allowed to remain on the platform for 10 s. Each animal was given 15 trials on day 1, and 25 trials on each subsequent day. Each animal was given one trial at a time with an inter-trial of 1 to 3 minutes. These animals were given extended training in the Morris task. Both wild-type and α-CaMKII mutant mice were able to learn the location of a hidden escape platform in the plus (+) maze. The difference between the two groups' performance was not significant [$t(8) = .469$, $P > 0.05$]. (Top) The plus (+) maze placed on top of the Morris pool, and the location of the hidden platform. The bar indicates the SEM.

Both the wild-type mice and the α-CaMKII mutant mice learned to solve the task and there was no significant difference in the number of trials to reach criterion between the two genotypes [$t(8) = 0.469$, $P < 0.05$] (Fig. 5). These results demonstrate that the impairment of the α-CaMKII mutant mice in the Morris hidden-platform task was not due to an inability to see distal cues, attend to the environment outside the maze, or learn a simple association between escape and distal environment.

Other behavioral characteristics. Since α-CaMKII mutant mice have impaired spatial learning processes that appear similar to that of animals with hippocampal lesions, we were interested to see whether other behavioral responses displayed by the mutant mice were also similar to those observed in hippocampally lesioned rodents. For example, one prominent characteristic of rats with hippocampal lesions is their increased exploratory behavior and activity when placed in an open field (activity cage) or Y maze (*19*). To determine whether α-CaMKII II mutant mice also display this behavioral characteristic, we examined the behaviors of wild-type and α-CaMKII mutant mice in both an open-field arena and an enclosed Y maze.

The open field is a large white square Plexiglas arena (60 by 60 by 14 cm) that is divided by a series of photobeams into 16

squares and illuminated by white light (*20*). Each time the mouse moves and breaks a photobeam, a count of one is registered; total activity is recorded for 15 minutes. Thirteen wild-type and eight α-CaMKII mutant mice were used. The mutant mice displayed significantly more activity compared to that of wild-type (mean total activity; 498.4 ± 68.6 and 258.3 ± 44.9, respectively) (*21*).

The Y maze is an enclosed three-armed maze (each arm dimension is 26 by 6.3 by 9.8 cm) made of transparent red Plexiglas. The mouse is placed at the intersection of the three arms and then is scored on the number of times it enters the different arms. During the first minute the α-CaMKII mutant mice were no more active than the wild type, but their exploration during the next 2 minutes was significantly increased. The wild-type mice showed a constant amount of activity throughout the three-minute time period (*22*).

The results of these behavioral tests indicate that the α-CaMKII mutant mice have other behavioral modifications in addition to a spatial learning deficit. Both the spatial learning problem and increased activity seen in the mutant mice parallel responses seen in hippocampally lesioned animals (*19*).

Linkage of gene to behavioral deficit. The foregoing data demonstrate that the α-CaMKII mutant mice are impaired in performing a task that requires learning the multiple spatial relations among a hidden proximal object (a platform) and visible objects in the surrounding distal environment. In contrast, the mutant mice perform well in tasks that require a non-spatial association between a hidden platform and a visible object in either the proximal or distal environment. As previously demonstrated and as confirmed here, the wild-type mice can accomplish both types of learning equally well. We thus conclude that the α-CaMKII has a prominent role in spatial learning and memory processes, but that it is not essential for some other types of nonspatial learning.

In addition to the deficits observed in spatial learning, the mutant mice showed increased exploratory activity in an open field and in a Y maze. These behavioral characteristics are common among rodents with hippocampal lesions (*19*). Interestingly, the hippocampus is the brain site in which the expression of α-CaMKII is highest (*12*). Hence, our data are consistent with the hypothesis that the primary cause for the behavioral changes observed in the mutant mice is the lack of α-CaMKII in their hippocampi. However, as to the spatial learning deficit, a lack of α-CaMKII in neocortex may also be involved, since lesions in this brain structure have also been shown to result in this type of learning deficit (*23*).

The mechanism by which the loss of α-CaMKII impairs spatial learning is unknown. We demonstrated (*14*) that our α-CaMKII mutant mice are also deficient in LTP in the CA1 region of the hippocampus. Thus, it is likely that this deficit in LTP is responsible for the impairment in spatial memory. We also showed that the NMDAR function in the α-CaMKII mutant mice is normal, demonstrating that the role of α-CaMKII in LTP and spatial learning is not as a regulator of the activity of the NMDAR, the only other molecule known to be both required both for LTP and spatial learning (*4*). Thus, our work strengthens considerably the contention that the synaptic changes exhibited in LTP are the basis for spatial memory (*4*).

The work reported here demonstrates that a mutation in a known gene is linked to a specific mammalian learning deficit, and indicates that single genetic changes can have a selective but drastic impact on learning and memory. Previous studies have revealed that some inbred strains of mice, such as DBA/2, are also impaired in spatial learning (*24*), and that the impairments seem to be partly associated with a reduction in hippocampal protein kinase C (*25*). However, these previously reported differences among inbred mouse strains are clearly not the result of differences in a single gene, and the additional biochemical substrates that can account for strain differences in spatial learning remain to be described.

Despite the remarkable specificity of the learning impairments observed in the mutant mice, it is possible that they have other yet undetected learning deficits. Indeed, we suspect that the modulation of the acoustic startle response (*26*) is impaired in these mice, since they seem to have an abnormally enhanced acoustic startle response. Hence, it is interesting that the amygdala is another prominent site of α-CaMKII expression (*10*), and that LTP in this part of the brain might be involved in the modulation of the acoustic startle response (*26*). In contrast, our behavioral work did show that α-CaMKII is not essential for all learning. For instance, although it has previously been shown that α-CaMKII is expressed in the basal ganglia (*12, 13*) and that this structure is essential for learning the visible-platform version of the Morris task (*23*), our study indicated that the α-CaMKII mutant mice can learn this task.

Finally, we have demonstrated that α-CaMKII mutant mice are a useful model for studying the relations among a particular gene product (α-CaMKII), LTP, and behavior. We expect that other similarly constructed mice with mutations in judiciously chosen genes will be useful for studying mammalian behavior. In this regard, perhaps even more useful would be the mice with subtle rather than null mutations or mice with mutations directed to specific regions of the brain. Construction of such mutant mice may be feasible (*27*).

REFERENCES AND NOTES

1. D. O. Hebb, *The Organization of Behavior* (Wiley, New York, 1949); J. C. Eccles, *The Physiol. of Synapses* (Springer, Berlin, 1964).
2. T. V. P. Bliss and A. R. G. Medwin, *J. Physiol.* **232**, 357 (1973); T. V. P. Bliss and T. Lomo, *ibid.*, p. 331; P. A. Schwartzkroin and K. Wester, *Brain Res.* **89**, 107 (1975); B. L. McNaughton, R. M. Douglas, G. V. Goddard, *ibid.* **157**, 277 (1978).
3. G. L. Collingridge and W. Singer, *Trends Pharmacol. Sci.* **11**, 290 (1990).
4. R. G. M. Morris, E. Andersen, G. S. Lynch, M. Baudry, *Nature* **319**, 774 (1986); S. Davis, S. P. Butcher, R. G. M. Morris, *J. Neurosci.* **12**(1): 21 (1992); U. Staubli, O. Thibault, M. DiLorenzo, G. Lynch, *Behav. Neurosci.* **103**, 54 (1989).
5. J. H. Bekkers and C. F. Stevens, *Cold Spring Harbor Symp. Quant. Biol. Brain* **50** (1990).
6. C. A. Castro, L. H. Silbert, B. L. McNaughton, C. A. Barnes, *Nature* **342**, 545 (1989).
7. J. R. Keith and J. W. Rudy, *Psychobiology* **18**, 251 (1990); R. J. Sutherland, H. C. Dringenberg, J. M. Hoesing, R. W. Skelton, *Soc. Neurosci. Meet. Abstr.* **17**, 483 (1991).
8. M. R. Capecchi, *Science* **244**, 1288 (1989).
9. M. B. Kennedy, M. Bennett, N. Erondu, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7357 (1983); P. Kelly, T. McGuiness, P. Greengard, *ibid.* **81**, 945 (1984).
10. K. E. Burgin *et al.*, *J. Neurosci.* **10**, 1788 (1990); N. E. Erondu and M. B. Kennedy, *ibid.* **5**, 3270 (1985).
11. D. L. Benson, P. J. Isackson, S. H. C. Hendry, E. G. Jones, *ibid.* **11**, 1540 (1991).
12. R. G. M. Morris, P. Garrud, J. N. P. Rawlins, J. O'Keefe, *Nature* **297**, 681 (1982); R. J. Sutherland, B. Kolb, I. Q. Whishaw, *Neurosci. Lett.* **31**, 271 (1982).
13. R. C. Malenka *et al.*, *Nature* **340**, 554 (1989); R. Mallnow, H. Schulman, R. W. Tsien, *Science* **245**, 862 (1989).
14. A. J. Silva, C. F. Stevens, S. Tonegawa, Y. Wang, *Science* **257**, 204 (1992).
15. R. G. M. Morris, *Learn. Motiv.* **12**, 239 (1981).
16. M. Upchurch and J. M. Wehner, *Pharmacol. Biochem. Behav.* **29**, 325 (1988).
17. Extended training was analyzed by means of a one-way analysis of variance (ANOVA). Repeated measure on training data showed that the main effect of days [$F(2,20) = 2.155$] and genotype X days interaction [$F(2,20) = 3.162$, $P > 0.05$] were not significant. However, there was a significant main effect of mouse type [$F(1,8) = 9.832$, $P = 0.014$]. Performance on the probe trial was measured by quadrant search time or platform crossings, and did not significantly improve with extended training for either wild-type controls or α-CaMKII mutant mice. Wild-type training quadrant search time and platform-crossing values for animals trained for 3 days compared to those trained for 5 days, correlated $t(11) = 0.409$ ($P > 0.5$) and -1.868 ($P > 0.05$). The α-CaMKII mutant mice training quadrant search time and platform crossing values for animals trained for 3 days compared to those trained for 5 days, correlated $t(9) = 0.385$ and 0.426, respectively ($P > 0.05$).
18. R. Paylor, L. Baskall, J. M. Wehner, in preparation.
19. R J. Douglas and R. L. Isaacson, *Psychon. Sci.* **1**, 187 (1964); N. P. Foreman, *Physiol. Behav.* **30**, 711 (1983); D. P. Kimble, *J. Comp. Physiol. Psychol.* **56**, 273 (1963); R. N. Leaton, *ibid.* **95**, 813 (1981); W. W. Roberts, W. N. Dember, M. Brodwick, *ibid.* **55**, 695 (1962); H. Teitelbaum and P. Milner, *ibid.* **56**, 284 (1963).
20. Equipment to study mice in the open-field and Y

maze was supplied by A. Collins.
21. Statistical analysis of open-field activity: $t(19) = 3.061$, $P = 0.0064$.
22. ANOVA with repeated measure for Y maze activity: main effects of genotype or time ($P > 0.05$), genotype X time interaction [$F(2,36) = 3.166$, $P < 0.05$]. Simple effects analysis of the interaction showed that α-CaMKII mutant mice significantly increased their activity over the 3 minutes, but wild-type animals did not show any change. Data from one wild-type mouse were lost (computer error).
23. I. Q. Whishaw and B. Kolb, *Behav. Brain Res.* **11**, 123 (1984).
24. M. Upchurch and J. M. Wehner, *Behav. Genet.* **18**, 55 (1988); *Pharmacol. Biochem. Behav.* **29**, 325 (1988).
25. J. M. Wehner, S. Sleight, M. Upchurch, *Brain Res.* **523**, 181 (1990).
26. M. Davis, *Trends Pharmacol. Sci.* **13**, 35 (1992).
27. P. Hasty, R. Ramirez-Solis, R. Krumlauf, A. Bradley, *Nature* **350**, 243 (1991); S. O'Gorman, D. T. Fox, G. M. Wahl, *Science* **251**, 135 (1991).
28. An ANOVA with repeated measures confirms this observation. There was main effect of strain [$F(1,23) = 22.778$, $P = 0.0001$], showing that the overall wild-type controls were better than the mutant mice. There was also a main effect of trial block [$F(5,115) = 43.371$, $P < 0.00001$], showing that overall, animals' latency to swim to the platform decreased during training. Finally, there was a significant strain X trial block interaction [$F(5,115) = 4.528$, $P = 0.008$]. Post hoc analysis of the interactions (Neuman-Keuls, $P < 0.05$) showed that the wild-type animals had significantly lower escape latencies compared to the mutant mice on trial block numbers 1, 2, 3, and 4, but were not different on trial blocks 5 and 6.
29. One wild-type animal floated on all trials, and therefore the data on this animal were excluded. Of the 13 wild-type and 11 mutants used, the main effect of genotype [$F(1,22) = 11.576$, $P = 0.0024$] showed that overall wild-type controls had significantly lower escape latencies than the mutants. The main effect of the trial block [$F(8,176) = 8.54$, $P < 0.00001$] showed that animals improved significantly during training. The interaction between genotype and trial block was not significant.
30. An ANOVA showed that wild-type mice spent more time in the quadrant where the platform had been located during training than in the other three quadrants [$F(3,36) = 7.29$, $P = 0.006$; Newman-Keuls post hoc analysis: training > opposite, adjacent left, adjacent right, $P < 0.01$]. Similarly, an ANOVA showed that wild-type mice crossed the exact site where the platform had been located more often than equivalent locations in the other three quadrants [$F(3,36) = 13.673$, $P < 0.0001$; Newman-Keuls analysis: training > opposite, adjacent left, adjacent right, $P < 0.01$].
31. ANOVA $F(3,30) = 3.85$, $P < 0.05$; Newman-Keuls analysis: training = adjacent left and adjacent right, but training > opposite, $P < 0.05$.
32. Correlated t test $t(10) = -0.336$, $P > 0.05$; one α-CaMKII mutant mouse appeared to have had a seizure, and it failed to swim normally on the day of random-platform training. Thus, its data were not analyzed.
33. Supported by Howard Hughes Medical Institute (S.T.), Human Frontier Science Program grant 76834, National Science Foundation BNS-882007 (J.M.W.), and an RSDA (AA-001410) (J.M.W.)

10 June 1992; accepted 18 June 1992