hidden-platform task, thus showing that their deficit in spatial learning is specific. However, in the hidden-platform task, the control group showed no significant learning for 20 trials and the mutants showed none for 28 trials, at which point the training experiment was terminated. In many tasks, mice learn after a much larger number of trials. The questionable rationale for this early termination at 28 trials appears in note 29 of the research article by Grant et al.: “We used a training procedure that avoided overtraining the mice, because, in pilot experiments, overtraining masked the fyn- learning defect.” Thus, it seems that with a larger number of trials, the mutant mice do learn the hidden-platform task, albeit more slowly than the wild-type mice. This resembles the pattern that emerges in the visible-platform task, which was run for 48 trials.

In summary, we find no evidence that the mutant mice in either set of studies (1–3) suffered from a specific impairment in spatial memory. The interpretable evidence shows instead that nonmemory deficits played an important role in the performance of the mutant mice. Nevertheless, these are important experiments. The mutant mice, in spite of gross derangement of long-term potentiation, were clearly capable of learning. These are pioneering studies in disrupting targeted genes in order to elucidate the physiological bases of learning and behavior.

J. Anthony Deutsch
Department of Psychology,
University of California, San Diego,
La Jolla, CA 92039–0109

REFERENCES AND NOTES
4. Silva et al. (1) used mutant mice defective in the α isoform of calcium-calmodulin-dependent kinase II.

27 July 1992 and 19 January 1993; accepted 15 June 1993

Responses: The criticisms by Deutsch focus on the evaluation of performance variables that may be important in determining whether mutant mice are impaired (as compared with wild-type litter mates) in spatial learning performance. The criticisms relate to two issues; first, the use of latencies (the time taken to escape to a platform) to evaluate performance, and second, the statistical analysis and interpretation of the plus maze experiments.

The Morris water task is a learning task that is frequently used to assess spatial learning performance in rodents. However, to interpret performance in this task, several measures must be used. The major thrust of Deutsch’s criticism with regard to the use of the Morris water task is based on the presumption that success in learning was evaluated by measuring the time it took for mice to reach the platform. On the contrary, we conducted several tests—which provided multiple measures of spatial learning performance—to compare wild-type and mutant mice. Escape times, or latencies, during acquisition phases of the hidden platform task were not the only measures we relied on because they do not address the issue of special selectivity in the task.

Our experience with the hidden platform task (gained during the testing of at least 30 different strains of mice in the last 6 years) has indicated that latencies are not a good measure of the spatial learning strategies of mice in the Morris water task. Similarly, others have shown that latencies decrease as a function of training in rats with hippocampal lesions during acquisition training despite the fact that the rats showed impairment on other, better measures of spatial selectivity that have been derived from probe trial, or transfer tests (1). Stated simply, animals incapable of using a spatial strategy will revert to some other type of strategy to escape to the platform.

Deutsch discusses the latency curves in our report and argues that we cannot conclude that the mutant mice are impaired in spatial learning because no reliable difference was detected in the rate of learning between the mutant and control mice. However, conclusions with regard to spatial learning were not based only on the rate of learning in the hidden platform task, but on the results of probe trials and data acquired by assessing the behavior of mice when the platform was moved to new sites. In the trial the differential latency (the difference between the time taken to reach the original site and a new, randomly located, site) provides important information. First, each animal serves as an internal control for swim speed. Second, the trial measures further the selectivity of the animal’s search with procedures identical to those used during task acquisition (except for the location of the platform).

Deutsch states that nonspecific behavioral impairments in α-CAMKII mutants could have lead to increased latencies on the visible platform version of the task. We attributed these longer latencies, which occurred on the first day of training, to fatigue caused by “jumpiness.” The fact that we said that the mutants were better habituated to the task by the second day is not in conflict with the data. It is true that their average latencies on the first block of trials were also longer on the second day. This might be expected if fatigue interfered with their using the information presented to them on the first day of training. The important point in this aspect of the study is that they caught up with the wild-type mice in their performance by the second block of trials on the second day. Thus, any performance factors that were problematic in the mutants were quickly overcome during the second day of visible platform training. The training in the hidden platform version of the task was accomplished in 3 days, and these same interfering factors should have been diminished by the second day. If such factors were a problem, a precipitous drop in latencies would again be expected in the mutants. This was not the case. In fact, to rule out this possibility, some animals were given an additional 2 days of training. Again, in the total 5 days of training, latencies on the hidden version remained different between the mutants and wild-types, which suggests that there was impaired spatial learning in the former. This impairment was then further verified by probe trial and random platform trial data.

Last, Deutsch suggests that the additional use of the plus maze to evaluate differences in performance between wild-type and mutants may be irrelevant. Deutsch is correct that a transfer test was not performed with the plus maze. However, the position of the maze was rotated between trials, and the start position varied such that it was unlikely that the animals could have used intramaze cues. Our data analysis, with the use of a two-tailed t-test, yielded a P value of 0.652, which suggests that a statistically reliable difference between the performances of the two genotypes on the plus maze was not observed. In support of this conclusion, we did a power analysis (2) of our data, based on an 80% chance of detecting a difference in the performance of wild-type and mutant mice in the plus maze. The analysis indicated that more than 170 animals would need to be tested for one to detect a difference. Regardless of how the two genotypes are solving the plus maze task, a heroic effort would be required before these slight differences would register as statistically significant.

In summary, we used the Morris water task with at least the same degree of stringency that has been applied in other studies of the effects of lesions and pharmacological agents on spatial learning performance, and we showed differences in performance on this task between the mutant and wild-type mice on the basis of various measures of spatial selectivity. As indicated in our research article, the mutant mice have other behavioral defects, and we evaluated the impact of such impairments on spatial learning performance. We stand by our
conclusion that the α-CaMKII mutant mice are impaired in spatial learning.

Alcino J. Silva  
Center for Learning and Memory,  
Cold Spring Harbor Laboratory,  
Cold Spring Harbor, NY 11724

Richard Paylor  
Jeanne M. Wehner  
Institute for Behavioral Genetics  
University of Colorado at Boulder,  
Boulder, CO 80309-0447

Sussumu Tonegawa  
Howard Hughes Medical Institute  
at Massachusetts Institute of Technology  
Center for Cancer Research  
Cambridge, MA 02139

REFERENCES


25 August 1992; accepted 15 June 1993

Response: Deutsch generously notes that the studies of mice with targeted disruptions of the α-CAMKII (1) and fyn (2) genes are pioneering in attempting to elucidate the physiological bases of learning and behavior. However, he seems to misinterpret some of the results of these studies and attributes to us conclusions to which we do not subscribe. Specifically, Deutsch addresses three issues in our paper (2) on fyn− mice. First, he states that “Success in learning in both [hidden- and visible-platform] tasks was assessed by measuring the time it took for mice to reach the platform.” This is not completely correct. Escape latencies in the hidden-platform version of the Morris maze (3) are by themselves poor indicators of spatial learning. A better measure comes from the additional use of the transfer test, a variation of the Morris maze specifically designed to measure spatial learning (3–6). We therefore also carried out this test. Here again, we found that wild-type mice had learned; they showed a significant spatial bias toward the quadrant of the pool where the platform was located during training. By contrast, the fyn− mice showed no such bias, thus indicating impaired spatial learning in this more specific task as well.

Second, Deutsch inaccurately attributes to us the conclusion that “mutant mice suffer from a memory deficit that is specific to spatial learning.” Although we found that fyn− mice had a spatial learning deficit, we did not conclude that this defect is specific to spatial learning. Rather, we point out that fyn− mice initially showed longer escape latencies than did wild-type mice in the visible-platform test. This led us to emphasize that other (nonspatial) forms of learning may also have been impaired (2, p. 1908). We wrote both the fyn− and CaMKII− mice show an initial impairment in the single-cue association task, a task that requires nonhippocampal regions. . . . This finding suggests either that the hippocampus can be involved in simple associative learning or that these kinases may be important for learning processes that require regions other than the hippocampus.

Thus, although fyn− mice eventually performed as well as wild-type controls in the visible-platform task, we considered this initial difference significant.

We agree with Deutsch that without a counterbalanced experimental design we cannot exclude the possibility that the initially longer escape latencies in fyn− mice represent an order effect and not a real behavioral phenotype produced by the fyn mutation. However, an order effect would not explain the results of the transfer test, which indicated significant differences in performance between mutant and wild-type mice, as the mice were experimentally naive before hidden-platform training. Moreover, Silva et al. (1, 2) observed a similar difference between mutant and wild-type mice in the visible-platform task despite their using the reverse order of experiments (visible-platform training first).

Third, Deutsch argues that, because fyn− mice learned the visible-platform task more slowly than wild-type mice, there is a defect in visual discrimination or motivation, which casts into doubt whether the results in the hidden-platform task really reflect a memory deficit rather than a nonmemory deficit. We disagree. While the fyn− mice showed a higher initial escape latency at the start of training in the visible-platform task than did wild-type mice, they showed a significant improvement on the very first day of training after as few as three trials (P < 0.05, Duncan’s multiple range test). By the sixth training trial, they performed as well as wild-type mice (Fig. 1). Thus, the same fyn− mice that made no progress over 7 days of training on the hidden-platform task showed immediate learning within the first few trials when the platform was visible. Because the fyn− mice learned to perform as well as wild-type mice in the visible-platform task, they did not appear to have gross sensory, motor, or motivational abnormalities that would preclude learning. This suggests that the differences we detected between the wild-type mice in the transfer tests (which followed training in the hidden-platform configuration) was specific to learning, although not necessarily to spatial learning.

Finally, Deutsch objects to the fact that we did not use the same number of training trials in the visible-platform task (48 trials; 8 days of six consecutive trials each at 30-second intertrial intervals) as in the hidden-platform task (28 trials; 7 days of four trials separated by 60-minute intertrial intervals). But why should one use the same training protocol (number of trials, intertrial interval) for tests that explore distinctly different types of learning? Although the transfer test is a sensitive assay of hippocampal-dependent spatial learning (3, 4), even animals with hippocampal lesions (that normally show large deficits) can compensate and exhibit spatial learning when they are overtrained (4, 5). Overtraining can mask a variety of learning deficits in other forms of learning as well.

To optimize our detecting potential differences, we therefore conducted pilot experiments to determine a training protocol that was most likely to avoid overtraining. This was particularly important in our case, as we had found that LTP was reduced but not completely absent in fyn− mice, and the degree of reduction was dependent on the stimulation protocol used to induce LTP (3). We were thus concerned that this defect in LTP might lead to a subtle learning deficit that would go undetected if animals were overtrained. Our pilot studies also indicated that the time interval between training trials, rather than number of trials, was an important variable. We found that the performance of fyn− mice improved when training trials were closely spaced together, perhaps because such spacing is less taxing on long-term memory. To optimize the detection of any learning defect that fyn− mice might have, we selected longer intertrial intervals and fewer training trials.

Deutsch concludes by saying that in both the fyn− and α-CaMKII mutant mice, despite “gross derangement of long-term potentiation, [the mice] were clearly capa-

Fig. 1. Training in the visible-platform task on the first day of the 8-day training protocol. Mice were placed into the pool at random locations and escaped by swimming to a flagged platform, also at a random location. Both fyn− (c) and wild-type (A) mice showed immediate improvement after the first trial and performed equally by the sixth trial. Error bars show mean and SEM.
Responses
Alino J. Silva, Richard Paylor, Jeanne M. Wehner and Susumu Tonegawa

Science 262 (5134), 761-762.
DOI: 10.1126/science.262.5134.761