Spatial Learning in Mutant Mice

A. J. Silva et al. (1, 2) and S. G. N. Grant et al. (3) found that two types of mutant mice showed learning impairment, relative to wild-type mice (the control group), on an experimental task. These researchers argue that such mutant mice suffer from a memory deficit that is specific to spatial learning. Silva et al. state that their work "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" (1, p. 210). Grant et al. state that "mutations in the fyn gene in mice result in an impairment of both LTP (long-term potentiation) and spatial learning" (3, p. 1908). However, these interpretations in terms of a memory deficit are still open to question in view of the data presented in the two articles.

Two versions of the Morris water task were used by both groups of researchers (1–3) to measure behavioral differences between mutant and wild-type (control group) mice. In the "hidden-platform task," a fixed platform was submerged in a round tank of water that was rendered opaque; the task of the mice was to swim to the platform repeatedly and thus learn its location. In the "visible-platform task," the mice swam to a submerged platform, positioned in random locations, marked with a flag above water. Success in learning in both tasks was assessed by measuring the time it took for mice to reach the platform.

In the study by Silva et al. (1), mice were first tested on the visible-platform task. Mutant mice (4) "initially took longer than the wild-type mice to reach the platform," but "they were able to overcome this deficit by training" (1, p. 207). Silva et al. tentatively attribute this initial deficit to a "jumping response" by the mutant mice that led to fatigue on the first day of training (1, p. 207). On day 2 the mutant mice "did not show the jumping response and hence were not fatigued before the trials." This explanation is contradicted by figure 1B of the research article by Silva et al. (1, p. 207) and by their statistical analysis (1, p. 211). The mutant mice had large and significantly higher escape latencies (the time required to reach the platform) even on the first block of the second day, when they no longer showed a jumping response. The acquisition curve shows a sudden shift on day 2. As the poorer performance of the mutant mice occurred before learning had taken place, one can infer that the mutant mice suffered from deficits that were not related to memory. These deficits manifested themselves in the visible-platform task, which is held to be equivalent to the hidden-platform task except that it "does not require a spatial map"; (3, p. 1906). If so, then nonmemory factors could also be responsible for deficits in performance in the hidden-platform task.

In the hidden-platform task in the study by Silva et al., the mutant mice again showed a large deficit in performance at the outset of training that could not be a result of spatial or other memory. This deficit remained nearly constant throughout the experiment as shown by the statistical analysis performed by Silva et al. (1). This revealed a main effect, that of genotype, which was highly significant. However, "the interaction between genotype and trial block was not significant" (1, p. 211). This means that no reliable difference in the rate of learning between the mutant and control mice could be detected in the hidden-platform experiment. Only such a difference in rate could provide evidence for a possible difference in memory between the two genotypes in this experiment.

Further experiments by Silva et al. showed that the mutant and control mice relied on different strategies to find the hidden platform (1, p. 209)

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, [maybe] 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. ... The plus maze is a four-armed (+) 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. ... Because the maze is clear the animal can use prominent distal cues in the room to locate the platform.

Just because the animals can use prominent distal cues, they do not necessarily do so. The introduction of the Plexiglas maze could have provided proximal cues that were inconspicuous to human observers. Silva et al. present no control or transfer test data about what cues the mice might actually have used. Thus, the plus maze experiment may be irrelevant.

Silva et al. reject the hypothesis that the mutant and control mice differed in their performance in the plus maze on the grounds that it was not statistically significant (P = 0.326).

However, the plus maze experiment leaves unresolved the question of whether there is some intermediate impairment in a nonmemory process that is sufficient to produce the differences observed in the plus maze task. The performance of the mutant mice was 25% worse in this task, which, while not significant, is still the best estimate available. How the scores on the plus maze task translate into choice of the solution strategy used by the mice in the hidden platform task is unknown.

The results from the plus maze task do not exclude the possibility of nonmemory disabilities, nor do they indicate them; the plus maze task is too insensitive an instrument to do either. The scores of the mutant mice would have had to be at least twice as high as those of the control group to reach any level of statistical significance. Also, it is not clear why only five mice per group were used in this task as against 12 in the hidden-platform experiment.

In the visible-platform task, the mutant mice (1, p. 207) showed a clear nonmemory deficit, even though the visual cues toward which they had to learn to navigate were nearer than in the plus maze. Unless they were hyperopic, it would be unlikely that the mutant mice were unimpaired in the plus maze if or when such cues were more distant.

Grant et al. published a related study (3). In the main experiment, all the mice were trained for 7 days, with four trials per day (plus four more trials after the first transfer test) during which the animals would unlearn. Wild-type mice showed an eventual reduction in the time taken to find the hidden platform. This reduction did not occur with fyn mutant mice (3, p. 1905). To show that this difference was a result of a specific deficit in spatial learning in the mutants, Grant et al. performed a visible-platform (control) experiment. The results of this task were similar to those obtained in the same task in the study by Silva et al. (1). The wild-type mice at first performed with a latency of escape one-half that of the fyn mice; the latter improved so that they performed as well as the wild-type mice by day 6.

Grant et al. appear to regard the initial impairment of the mutant mice in this task as genuine and not an artifact of order: "In fact, both the fyn- and CamKII- mice show an initial impairment in the single-cue association task . . ." (3, p. 1908). If so, the control experiment reveals strong nonmemory factors. However, because the same mice were used in the main and control experiments without a counterbalanced design (3), one cannot exclude order effects as a possible cause of the initial difference in latencies in the performances on the visible-platform task or any other difference found in performances between the hidden and visible-platform tasks.

Grant et al. say (3, p. 1906) the visible-platform results demonstrate "that fyn- mice can learn some tasks" presumably in contrast to their inability to learn in the
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 fnm-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.

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REFERENCES AND NOTES

4. Silva et al. (1) used mutant mice defective in the \( \alpha \) isoform of calcium-calmodulin-dependent kinase II.

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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 \( \alpha \)-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
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