Spatial Memory and Learning in Transgenic Mice: Fact or Artifact?

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Spatial learning of transgenic mice is often assessed in the Morris watermaze, where mice must use distant cues to locate a submerged platform. Such learning is confounded by species-specific noncognitive swimming strategies. Factor analysis permits cognitive and noncognitive strategies to be disentangled and their association with electrophysiological phenomena to be investigated.

Gene targeting has become a popular technique to study the actions of proteins in the otherwise intact central nervous system. Selective inactivation of genes (knockout) allows one to study the functional chain from gene to behavior by investigating the effects of the genetic lesion at the cellular, electrophysiological, and behavioral level, including memory and learning. This approach is being met with enthusiasm. As with any new technique, however, methodological issues have been raised that have triggered debates about the validity of the conclusions drawn from such experiments (1). One important problem is the genetic background of knockout mice, because the most popular stem cell donor strains (129/Sv, 129/J, 129/Ola) are characterized by electrophysiological and behavioral peculiarities that call to question the validity of a generalized interpretation. Another problem is that most gene knockouts cause pleiotropic effects and trigger a variety of compensatory mechanisms that can mask the expected deficits.

A further problem is well known to behavioral biologists but has often escaped the attention of other fields: how can learning and memory be reliably quantified by behavioral measures? This would seem a prerequisite for establishing causal relations between cellular functions, electrophysiology, and higher brain functions. As we show here, precise assessment of memory is not a trivial task.

The Morris watermaze test

One of the most popular and widely used tests to measure spatial memory and learning is a swimming navigation test originally developed for rats by Richard G. M. Morris (4). Because of its elegance and technical simplicity, it has found widespread application in lesion and neuropharmacological studies. More recently, it has become a standard test for assessing changes in memory and learning of transgenic mice and for identifying neural correlates of behavioral changes.

In the specific protocol we use, the mouse is released into a circular water tank (diameter between 1 and 1.5 m) filled with water made opaque by milk or white paint (Fig. 1A). The task is to find an escape platform (~12 \( \times \) 12 cm) that is hidden below the water surface. Initially, mice swim along the wall of the tank. This instinctive behavior is called “thigmotaxis” after the Greek words \( \theta \gamma \mu \alpha \) (thigma), meaning “touch,” and \( \tau \alpha \xi \zeta \) (taxis), meaning “order” or “destination.” Another spontaneous reaction is motionless floating. This is typical for some inbred strains but rarely observed in animals with a hybrid genetic background. Because the escape platform is usually located at least 30 cm from the wall, neither of these behaviors permits active escape. After 120 s, the mice are removed from the water. After about two trials, most mice begin to adopt other swimming strategies. They eventually hit the platform by chance. If they climb on it and remain there for a short waiting period, they are returned to their home cage and are allowed to dry under an infrared lamp. The mice undergo six trials per day with an intertrial interval of 60–90 min. The platform remains in the same position for 3 days, but, on each trial, the mice are released from different starting points (Fig. 1B). Most mice learn the task surprisingly quickly, often after three to four trials (Figs. 1B and 2). This is only possible if the mice use the distant visual cues (Fig. 1A) to calculate the position of the invisible target and to guide their navigation. Learning curves are usually obtained by plotting successive escape latencies or swim path lengths (Fig. 2). The retention of...
spatial memory is assessed on the fourth day by a probe trial, during which the platform is either absent or is moved to a new position. Normally, the mice search at the previous platform position (Fig. 1B). A standard measure of spatial memory is the fraction of swim time spent in the former target quadrant in comparison to other quadrants. To test the flexibility of spatial orientation, the testing continues for 2 days, with the platform remaining constantly in the new position (Fig. 1B).

Such swimming navigation is a complex process requiring intricate sensorimotor integration and computation. Thus it is very sensitive to lesions of various brain structures, in particular of the hippocampus.
Cognitive vs. noncognitive factors

Albeit elegant, the swimming navigation test has an intrinsic methodological problem. Reduced searching times during the probe trial are often taken as direct evidence for impaired spatial memory. This may, however, lead to false conclusions because the acquisition of spatial memory can be confounded by noncognitive strategies. For example, mutant mice often show excessive thigmotaxis, because of lack of orientation or merely because of increased anxiety. Whatever the reason, such mice tend to show imperfect acquisition even after prolonged training and will fail to show a preference for the old goal quadrant during the probe trial even if their capacity for memory is unaffected. This raises the question of whether some of the reported effects of gene targeting on spatial memory are misinterpretations.

To address this question, we have reanalyzed the swim paths of ~1,400 mice that had been recorded under constant experimental conditions. With the aid of Wintrack 2.0, a specifically developed software application, we have analyzed more than 50,000 swim paths and calculated a large number of behavioral variables. An earlier DOS-based version of this software has been described in detail elsewhere (12). Because many of the obtained variables were partially intercorrelated, factor analysis was used to reduce them to a small set of statistically independent behavioral factors.

The individual differences in swimming navigation behavior of the 1,400 mice could be largely described in terms of three statistical factors accounting for 81% of the observed variability in the behavior scores (Fig. 3A). Factor one ("thigmotaxis") explains 49% of the variability. It is associated with frequent swimming or floating near the wall, prolonged swimming times, and a low fraction of time spent in the actual target quadrant. Factor two ("passivity") explains 19% of the variability. It correlates with reduced swimming speed and frequent floating. Finally, factor three ("memory") accounts for 13% of the behavioral variability and reflects primarily the search time spent in the former target quadrant during the probe trial. The order of these factors does not reflect their functional importance but rather the amount of statistical variability they extract from the raw data. Nonetheless, more than two-thirds of the behavioral variability is accounted for by two factors that have no direct relation to spatial memory and learning and are, therefore, referred to as "noncognitive."

These findings prompted us to reanalyze part of our earlier data. Indeed, we found that some of the reported learning differences in strains and knockout mice appeared to be related to these noncognitive factors. We shall present an example in which a targeted mutation of the β-amyloid precursor protein (βAPP) entailed massive impairments of swimming navigation, mainly as a result of noncognitive alterations. On the other hand, by applying factor analysis, we were able to recognize a subtle spatial memory deficit in mice lacking tissue plasminogen activator (tPA) that had not been revealed by conventional statistics.

βAPP, long-term potentiation, and spatial memory

The β-amyloid peptide is an essential component of the plaques formed in the brain of patients suffering from Alzheimer's disease. It is formed by cleavage of the βAPP. βAPP is normally produced in the brain and most other tissues, but its in vivo function remains unclear. We have analyzed transgenic mice in which βAPP is expressed in truncated form and at low concentrations. These mice show prolonged swimming times, increased
One might conclude from these data that normal amounts of βAPP are required for intact spatial memory function. This is probably not the case, however. A factor analysis of these 115 mice extracted the same three factors as the analysis of all 1,400 animals, namely, thigmotaxis, passivity, and spatial memory. When we included genotype as an additional variable, we found that it was correlated with both noncognitive factors. The βAPP mutants were both more thigmotactic and more passive. But there was no correlation between genotype and the memory factor. This implied that the poor probe trial scores apparent in the raw data were due to noncognitive factors and thus provided no evidence for impaired memory.

FIGURE 3. Correlation coefficients (factor loadings) between factors extracted by factor analysis and experimental variables. Strong correlations are shown against a dark background. A: in a data set of 1,400 mice, 49% of the behavioral variability is accounted for by factor 1 (thigmotaxis), which is associated with excessive wall contact, motionless floating near the wall, long escape latencies, and a small fraction of swim time spent in the actual goal quadrant; 19% of behavioral variability is explained by factor 2 (passivity), which reflects reduced swim speed and frequent floating. Finally, 13% of the variability is accounted for by factor 3 (memory), which correlates with a larger fraction of time spent in the actual goal quadrant and better scores in the probe trial. B: in an experiment involving mice expressing the β-amyloid precursor protein (βAPP) at low concentrations and in a shortened form, factor 3 correlates with the individual potentiation scores obtained when long-term potentiation was assessed in the dentate gyrus. C: in tissue plasminogen activator (tPA)-deficient mice, factor analysis reveals a strong negative relation between the mutation and factor 3. KO, knockout; EPSP, excitatory postsynaptic potential.
Long-term potentiation (LTP) was assessed a few weeks later in the dentate gyrus of a subsample of 45 mice. LTP is a long-lasting increase of synaptic efficacy induced by high-frequency stimulation and is thought to play a role in the formation of memory. Mutant and control mice did not differ with respect to LTP overall, although there were individual differences within the groups (unpublished data). These differences prompted us to extend the factor analysis by including the LTP scores as an additional variable, as well as the same variables used in the other samples. The analysis again showed the three behavioral factors: thigmotaxis, passivity, and spatial memory. However, the LTP scores showed a strong positive correlation with the memory factor (Fig. 3B): animals with high scores for the memory factor also had stronger LTP. These findings reveal that a positive statistical relation between LTP and spatial memory can be masked by confounding noncognitive factors. The importance of LTP for memory is still controversial, and dissociations between deficits in memory and LTP have been reported on several occasions (3, 6, 8, 10, 11). In view of these uncertainties, a careful data analysis that takes into account potential masking effects by noncognitive factors appears particularly important.

**tPA and spatial memory**

tPA plays an important role in fibrinolysis. In addition, it is induced in the brain by increased neuronal activity (7) and learning processes (9) and is believed to play a role in the formation or modification of synaptic connections. We have compared the swimming navigation learning of 34 mice carrying a targeted disruption of the tPA gene with matched control animals. A second group of mice was studied in another laboratory for hippocampal LTP. LTP could be induced in both mutants and controls but faded faster in the mutant mice (2). This finding is in accordance with the hypothesis that tPA supports certain aspects of synaptic plasticity. In the behavioral studies, the mutants needed slightly more time than controls to find the platform but spent an equal amount of time in the actual target quadrant during acquisition. According to conventional analysis of variance (ANOVA), their raw probe trial scores were statistically indistinguishable from those of wild-type mice (2). Was this now an example of intact spatial memory in mice with impaired LTP?

The behavioral data of tPA mutant mice were reanalyzed by factor analysis. As expected, the three factors (thigmotaxis, passivity, and memory) appeared again (Fig. 3C). After the genotype was included in the analysis, it was found that the mutation was negatively correlated with the memory factor. tPA-deficient mice earned lower scores for the memory factor than wild-type mice. Thus conventional ANOVA had led to the false conclusion of intact spatial memory in the mutants. Obviously, the probe trial scores had been biased by the individual variability in the two noncognitive factors, which had masked the subtle genotype difference in memory function. By analogy to electronics, noncognitive behavioral variability might be thought of as “noise” from which the signal memory must be extracted by filtering through factor analysis.

**Conclusions**

Our two examples show that effects of targeted mutations on behavior must be interpreted with caution, particularly in the context of memory and learning. On the other hand, they also demonstrate that an improved statistical analysis can discover new or masked relationships calling for experimental clarification. Clearly, the three factors emanating from the factor analysis presented could be specific for our protocol. Certain training protocols, such as extensive pretraining with a visible platform, tend to reduce floating and thigmotaxis, although we have found that they will not abolish these influences on performance.

Problems of interpretation are no reason to dismiss the swimming navigation test. First, confounding behavioral variables occur in every test. Second, successful swimming navigation is based on complex processes, and the test allows many different behavioral aspects to be extracted, provided that it is coupled with a suitable analysis, such as factor analysis or other statistical approaches that allow the amount of variability accounted for by different variables to be determined. Thus it is well suited for behavioral screening of mutant mice and the recognition of even subtle mutation-induced changes in the brain. Recognizing specific effects on spatial memory requires statistical separation from confounding behavioral variables. But these so-called noncognitive factors are themselves based on complex neuronal interactions that deserve physiological analysis as well. They remain facts, although they may appear as artifacts.

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Copies of the Wintrack 2.0 software can be requested at the authors’ address.
References


