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Genotype-Environment Interaction: Effect of Housing Conditions on Water Maze Performance in C57BL/6 and 129/SVEV Inbred Mouse Strains

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The Morris water maze (MWM) has been widely used as a diagnostic tool to detect alterations in hippocampal-dependent learning induced by pharmacological or genetic manipulations in rats and mice. However, with the frequent use of the paradigm some questions have arisen regarding the complex nature of the effects of environmental and biological factors that influence behavioral performance of rodents in this task. One of the most contentious issues is whether MWM can consistently detect genetic differences independent of environmental, i.e., laboratory and experimental conditions. In the present paper, we demonstrate that changes in environmental factors, due to holding mice in large vs. small home cages, can lead to significant and robust spatial learning alterations in one inbred strain (C57BL/6) while having minimal or no effects on another (129/SVEV). The detected genotype-environment interaction underscores the need for experimenters to diligently control and document all possible environmental factors in order to make their results comparable across multiple test environments and laboratories.

The Morris water maze (Morris et al., 1982) is perhaps one of the most popular behavioral tests with which the learning and memory performance of rodents is probed (Gerlai & Clayton, 1999; Grant & Silva, 1994). Its popularity lies in its methodological simplicity and the fact that it can provide information about hippocampal function, e.g., spatial learning in the hidden platform task, as well as nonhippocampal performance characteristics, e.g., motivation, perception, and motor function, tested in a control paradigm called nonspatial or visible platform test. Furthermore, the paradigm has significant construct validity. It is relevant for human clinical conditions because hippocampal behavioral function is crucial in human memory (declarative, relational, or episodic memory; Eichenbaum, 1996; O'Keefe & Nadel, 1978; Scoville & Milner, 1957; Squire et al., 1993) and is one of the traits that is most vulnerable to pathological changes associated with Alzheimer's disease and aging (Albert, 1996; Barnes et al., 1980; Chen et al., 2000).

Although the MWM paradigm appears simple from a technical viewpoint, it taps into complex cognitive processes that are mediated by numerous biochemical pathways and influenced by a potentially large number of environmental factors. For example, numerous molecules (receptors and their ligands, intracellular signaling molecules, retrograde messengers, etc.) have been shown to alter hippocampal learning processes or influence long-term potentiation (for review see Sanes & Lichtman, 1999), a synaptic mechanism that has been suggested to underlie hippocampal learning and memory (Bliss & Collingridge, 1993). Similarly,

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several environmental conditions, e.g., human handling, temperature of the water in the maze, habituation to testing procedures, level of illumination in the test room, etc., have been suggested to influence performance in the MWM (e.g., Gerlai & Clayton, 1999). Thus the question has arisen, can the water maze, or behavioral tests in general, consistently and correctly detect alterations in cognitive function in the face of such complexity (Crabbe et al., 1999)? The answer is heavily debated in the literature. Our paper attempts to contribute to this debate by providing an example of a genotype-environment interaction in water maze performance. Genotype-environment interaction means that changes in environmental conditions manifest differently at the phenotypical level depending on the genotype of the subjects, or alternatively, the effect of a genetic alteration leads to differential responses depending on environmental factors. In other words, genotype-environment interaction is observed when the effects of the environment and genetic factors are not additive.

In the present paper we show that a simple change in housing conditions (large vs. small holding cages) can make a dramatic difference in the spatial learning performance of one inbred strain (C57BL/6) but exerts no robust influence on the behavior of another inbred strain, 129/SVEV. The results demonstrate that seemingly irrelevant environmental factors can significantly affect behavioral performance, and the effect of the environment is genotype dependent. We conclude that rigorous control of the environment and perhaps a battery of behavioral tests are required to properly evaluate and interpret the behavioral effects of mutations in transgenic mice.

Method

Subjects

A total of 28 male C57BL/6 and 33 male 129/SVEV mice (Taconic, Germantown, NY) were used in the behavioral analyses. Mice were received at an age of 6-8 weeks and were allowed to acclimate to their new holding environment for 3 weeks before training and testing began. Mice from both strains were randomly assigned to two housing conditions. In condition one, they were housed in large plastic cages (45 cm x 25 cm x 15 cm, length x width x height) in groups of 10 (Big Cage), and in condition two, they were housed in small micro-isolator cages (30 cm x 15 cm x 15 cm, length x width x height) in groups of 4 (Small Cage). Both types of cages were placed in the same colony room (12:12 light-dark cycle) with identical maintenance and cleaning schedule for all cages. All mice were allowed access to food and water *ad libitum*. Although equal numbers of mice (16-17) were planned for each condition of the study, one group, the C57BL/6 mice housed in small cages, ended up having only 12 experimental animals as 4 males that suffered from fighting-related injuries were excluded from the study.

Apparatus

The water maze, a circular, white fiberglass tank (diameter = 180 cm, height of wall = 30 cm), was filled with water. Water temperature was maintained at 24-26° C by thermostat-controlled aquarium heaters. The maze was filled with water up to 5 cm from the top edge of the wall and the water was made opaque using nontoxic white tempera to block any potential visible cues underneath the water. Three round, clear plexiglass platforms (diameters of 20, 15, and 10 cm) were used in habituation, training, and testing. The surface of the platforms was treated with sandpaper and a grid of grooves were also cut to decrease slipping. The platform was placed approximately 1 cm below the surface of the water. Behavior was monitored and recorded by a digital camera interfaced to a computer running the Noldus EthoVision (Noldus Technologies, Wageningen, Netherlands) video imag-

ing software. The water maze was located in a room rich with extra maze cues. This sound proof room was isolated from all computer equipment. Furthermore, the background noise and illumination of the room as well as the video-tracking camera in the room could be controlled from a room adjacent to the water maze testing room. The cues in the testing room consisted of various shapes of styrofoam painted in different colors and fastened to the walls and ceiling. In order to reduce the stress of being placed in the water and to standardize the release of mice to the maze, a slowly sinking drop chamber was designed and used to start mice in the water maze. The drop chamber consisted of a plexiglass cylinder (diameter = 15 cm, depth = 18 cm) with extensions that fit over a set of posts located in the water maze. This allowed the chamber to slowly sink into the water at a constant speed exactly at the predetermined start locations.

Procedure

The study was conducted in three separate phases. In the first phase, ten 129/SvEv mice (129) and seven C57B/6 mice (B6) were housed in small cages and underwent training and testing in an order randomized for strain origin. Three weeks following the first phase, ten 129 and ten B6 mice were housed in large cages and underwent training and testing in an order randomized for strain origin. Three weeks following the second phase, 33 129 mice (16 mice housed in a big cage and 17 in small cages) and 28 B6 (16 housed in a large cage and 12 in small cages) underwent training and testing in an order randomized for strain and housing condition. All mice received an identical procedure detailed below. The effects of housing found during test phases 1 and 2 were replicated in phase 3; therefore, the data were pooled.

Prior to training, all mice received one 4-minute trial of water maze habituation. During habituation, a curtain was hung from the ceiling around the water maze to eliminate extra maze cues. Two large platforms (diameter = 20 cm) were placed in opposite ends of the water maze. Mice were started from the same location and the trial ended when either the mouse reached the platform and climbed on top of it, or when 4 minutes had elapsed. If the mouse had not reached the platform within the 4 minutes, it was gently guided onto the platform by the experimenter.

Two days after water maze habituation, all mice began hidden platform training. Mice received 3 days of training, followed by a 2 day break period, and then 2 more days of training. Each day the mice received 2 sessions of training conducted between 8:00 and 17:00 h. A session consisted of three 2-minute trials (inter-trial intervals of approximately 1 h), i.e., a total of 6 trials were administered each day. During training, the curtain was removed to allow the extra maze cues to be present. For the first 4 training sessions (days 1 and 2), a medium-sized goal platform (diameter = 15 cm) was placed in a fixed location in the water maze, with the edge of the platform approximately 20 cm from the wall of the tank. For the following training sessions a smaller goal platform (diameter = 10 cm) was placed in the same location. The platform "shrinking" procedure is used to facilitate the probability of the mice to accidentally bump into the platform (Eichenbaum et al., 1990; Gerlai et al., 1995). Mice were started from one of six predetermined locations along the wall of the water maze and allowed to search the water maze for a maximum of 120 s. The order of starting locations followed a semirandom sequence so that the sum of the distances of the first three starting positions from the goal platform (session one on each day) and the sum of the distances of the last three starting positions from the goal platform (session two on each day) were equated.

The mouse was released into the water maze using the sinking drop chamber as explained above. This method warranted that the release of the mice into the water maze was done in a standard manner and also allowed enough time (5 s) for the experimenter to leave the test room and start the video-tracking precisely when the mouse started swimming. The trial ended when the mouse either safely reached the goal platform or if the 2 min maximum trial length had elapsed. If the mouse did not reach the platform by the end of the trial, the experimenter gently guided the mouse onto the platform. Once on the platform, the mouse was allowed 20 s to rest before being gently returned to a holding cage.

On the day following the completion of the hidden platform training, all mice received two, 1-min probe trials. For the first probe trial, a curtain was hung from the ceiling around the water maze to eliminate extra maze cues. Extra maze cues were also removed from the ceiling and the water maze was illuminated by a 40-W red light bulb from the ceiling (which was covered during hidden platform training). The goal platform was removed from the water maze. Mice were randomly started from different quadrants during the probe trial, with an equal number of mice from each group started in each quadrant. When the trial was completed, the mouse was placed in the holding cage underneath the water maze. The second probe trial was conducted in a manner identical to the first probe

trial, except the curtain was removed, extra maze cues on the ceiling were returned, and the red light on the ceiling was covered. Thus, for the second probe trial mice were able to utilize all extra maze visual cues they may have previously observed during training.

Two days after the probe trials, all mice received 3 days of visible platform training (2 sessions of 3 trials per day for the first two days, and one session of three trials on the last day). The schedule and procedural aspects of training were identical to those of the hidden platform training and consisted of 2 sessions of 3 trials, i.e., a total of 6 trials per day for two days. However, on the last training day mice received only one session, i.e., a total of 3 trials. During visible platform training, a curtain was hung from the ceiling around the water maze to eliminate extra maze cues. Furthermore, extra maze cues were removed from the ceiling. For this training, a visible cue (a cylinder approximately 15 cm high, 4 cm in diameter wrapped in bright tape) was placed on a small (diameter = 10 cm) platform submerged approximately 1 cm below the surface of the water. Mice were started from a fixed location (the location where the goal platform was in hidden platform training), but the platform with the visible cue was moved to one of six predetermined locations for each trial. The sequence of platform locations corresponded to the sequence of starting locations previously applied in the hidden platform training. This arrangement made certain that the minimum total distance the experimental animals were required to swim to reach the goal platform, i.e., the motor requirement of the task, was equal for all sessions of the visible and the hidden platform training. All other parameters of the visible platform training were identical to those of the hidden platform task.

Data Analysis

Water maze performance was analyzed using numerous variables calculated on the basis of the swim path pattern of the experimental subject using the Noldus EthoVision software. Cumulative distance from target has been suggested to be one of the most precise measures of spatial navigation performance (Gallagher et al., 1993). Cumulative distance from target was calculated as the distance between the experimental subject and the target platform sampled once every 0.05 s throughout the trial and summed for the period of the trial. Relative duration of time spent in the target quadrant (dwell time) is calculated as the amount of time the subject spent in the quadrant of the maze that contained the platform compared to the total length of the trial. Swimming speed was measured as the total distance traveled divided by trial length for each trial. Relative duration of time spent motionless (compared to trial length), was also calculated. Similarly, duration of time spent in the perimeter of the maze (a 15 cm deep annulus) relative to trial length, was calculated. All measures were averaged for three trial sessions for each subject in order to equate differences associated with different starting locations, as explained above.

Data analyses were carried out using Systat 9 for Windows. Repeated measures analysis of variance (ANOVA), t-Tests, and post hoc multiple comparison Tukey's Honestly Significant Difference tests were conducted. Alpha was set at 0.05 in all statistical tests.

Results

Hidden Platform Training

A significant behavioral impairment was observed in B6 mice housed in small cages compared to B6 mice housed in large cages during hidden platform training. Interestingly, this behavioral impairment was not seen in the 129 mice. Figure 1 shows that B6 after having been housed in small cages remained further from the platform throughout training than did B6 mice housed in large home cages, but 129 strain mice housed in small cages and 129 strain mice housed in large cages exhibited similar levels of performance throughout training. Repeated measures ANOVA of the between-group factors Strain and Housing, and the within-subject factor of Session, found significant main effects of strain, $F(1, 57) = 7.67$, housing, $F(1, 57) = 5.47$, and session, $F(9, 513) = 24.22$, a significant interaction of the factors strain and session, $F(9, 513) = 3.37$, and a nearly significant interaction of the factors strain and housing, $F(1, 57) = 3.44$. Because ANOVA is

insensitive to detect interaction of main factors (Wahlsten, 1990) and because the interaction term was nearly significant, we decided to explore the effect of housing separately for the two mouse strains. The separate repeated measures ANOVA including only B6 mice revealed a significant main effect of housing, $F(1, 26) = 6.08$, and a significant main effect of session, $F(9, 234) = 5.06$, but did not find a significant interaction of these factors, confirming that throughout training, B6 mice housed in small cages before training searched further away from the platform than B6 mice housed in big cages. However, although the B6 mice housed in small cages were impaired throughout this task, they showed a similar rate of learning to B6 mice housed in large cages. A repeated measures ANOVA including only 129 mice did not find a significant main effect of housing, $F(1, 31) = 0.18$, but found a significant main effect of session, $F(9, 279) = 35.24$, and a significant interaction of session and housing, $F(9, 279) = 1.98$. However, post hoc Tukey's HSD tests showed no significant differences, between small and big cage housed 129 mice for any session, indicating a lack of effect of housing on the performance of 129 strain mice.

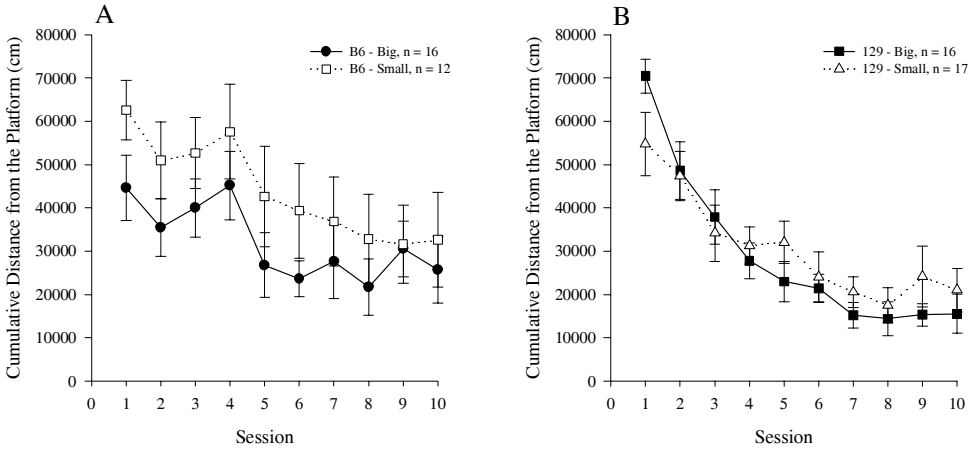

Figure 1. Cumulative distance from target is significantly affected by housing conditions (big vs. small holding cages) in C57BL/6 mice (B6) but not in 129/SVEV mice (129). A, B6 mice housed in small cages are significantly impaired compared to B6 mice housed in big cages. B, 129 mice are unaffected by housing conditions. Data are expressed as mean $\pm$ standard error.

Figure 2 shows the relative duration of time mice spent in the quadrant of the maze that contained the goal platform. The results suggest that all mice improved their performance with training, i.e., spent an increasing amount of time searching in the target quadrant. Furthermore, a slight impairment in both strains of mice housed in small cages was also apparent. A repeated measures ANOVA confirmed these observations. Significant main effects were found for the factors housing, $F(1, 57) = 9.41$, and session, $F(9, 513) = 14.65$, but significant main effects of strain, or significant interactions of the factors strain, session, or housing were not detected, Fs < 1. Nevertheless, a separate repeated-measure ANOVA including only B6 mice found a significant effect of housing, $F(1, 26) = 5.9$, but a similar analysis including only 129 mice did not, $F(1, 31) = 3.16$, indicating that

housing conditions significantly affected performance only in B6 but not in 129 mice.

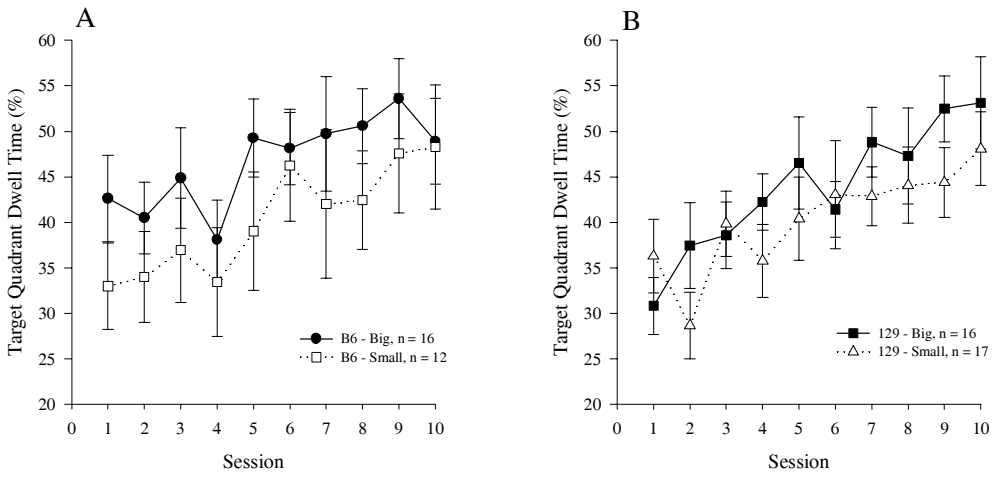

Figure 2. Target quadrant dwell time (duration relative to trial length) is significantly reduced in C57BL/6 mice (B6) but not in 129/SVEV (129). A, B6 mice. B, 129 mice. Data are expressed as mean ± standard error.

The deficits in spatial learning observed in this study were accompanied by other performance impairments, for example, alterations of swimming speed (Figure 3). ANOVA revealed a significant main effect of session, $F(9, 513) = 4.79$, and a nearly significant interaction of the factors housing and srain, $F(1, 57) = 3.49$. ANOVA conducted separately for B6 mice found significant main effects of housing, $F(1, 26) = 4.34$, and session, $F(9, 234) = 2.63$, but the interaction of these factors was nonsignificant, $F < 1$. A similar analysis including only 129 mice revealed a significant effect of session only, $F(9, 279) = 11.19$; all other Fs < 1. These results again suggest that B6 mice were more influenced by housing conditions than 129: B6 mice housed in small cages swam more slowly compared to B6 mice housed in large cages.

Another performance characteristic shown to influence spatial learning is the amount of time spent floating or not moving during training (Wolfer et al., 1997). Figure 4 shows that 129 mice spent more time floating than B6 mice, a finding that is in line with previous observations (Wolfer et al., 1997). ANOVA confirmed this observation and revealed a significant strain effect, $F(1, 38) = 6.33$, and also showed a significant interaction of housing and session, $F(9, 342) = 2.03$. However, the effects of housing, session, or any other interaction of these three factors were not found significant (all ps $> .05$). Separate ANOVAs for B6 or 129 mice failed to find any significant main effects of housing or session, or an interaction of these factors (all ps $> .05$).

Swimming in the perimeter of the water maze, or thigmotaxis, is another important trait that can significantly influence spatial learning performance in the water maze (Wolfer et al., 1997). Figure 5 suggests that unlike previously thought (Wolfer et al., 1997), 129 mice are not only not impaired in this measure but appeared to show superior performance, i.e., exhibited less thigmotaxis compared to B6 as the training progressed. ANOVA confirmed these observations and found significant main effects of strain, $F(1, 57) = 28.12$, and session, $F(9, 513) = 27.55$,

a significant interaction of the factors session and strain, $F(9, 513) = 5.71$. A nearly significant main effect of housing, $F(1, 57) = 2.44$, was also found. This latter finding was further investigated by ANOVAs conducted separately for B6 or 129 mice. ANOVA for B6 found a significant main effect of session, $F(9, 234) = 3.33$, and a nearly significant main effect of housing, $F(1, 26) = 3.33$. Whereas ANOVA for 129 mice failed to find a significant main effect of housing, $F < 1$, or an interaction of the factors housing and session, $F < 1$, but confirmed a significant main effect of session, $F(9, 279) = 61.7$, suggesting that while B6 mice housed in small cages spent a somewhat elevated amount of time in the perimeter of the water maze compared to those housed in large cages, 129 remained unaffected by housing conditions.

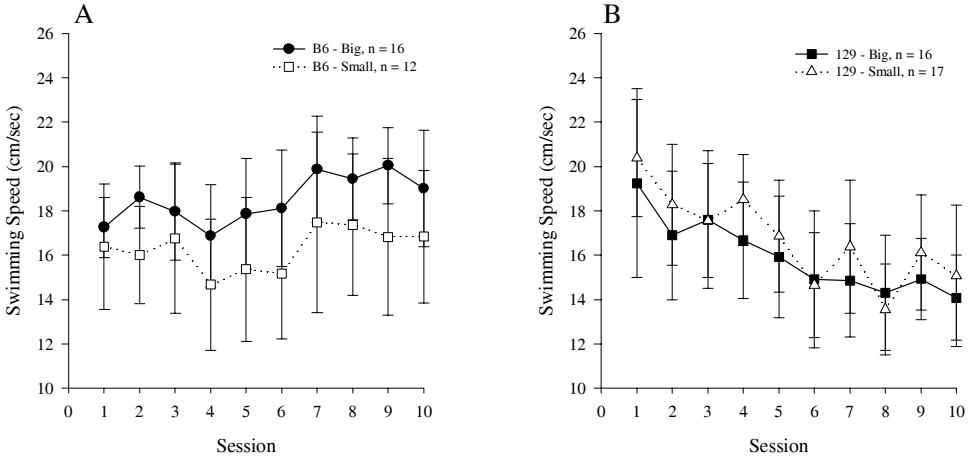

Figure 3. Swimming speed is significantly reduced by small cage housing in B6 mice while remaining unaffected in 129 mice. A, B6 mice. B, 129 mice. Data are expressed as mean ± standard error

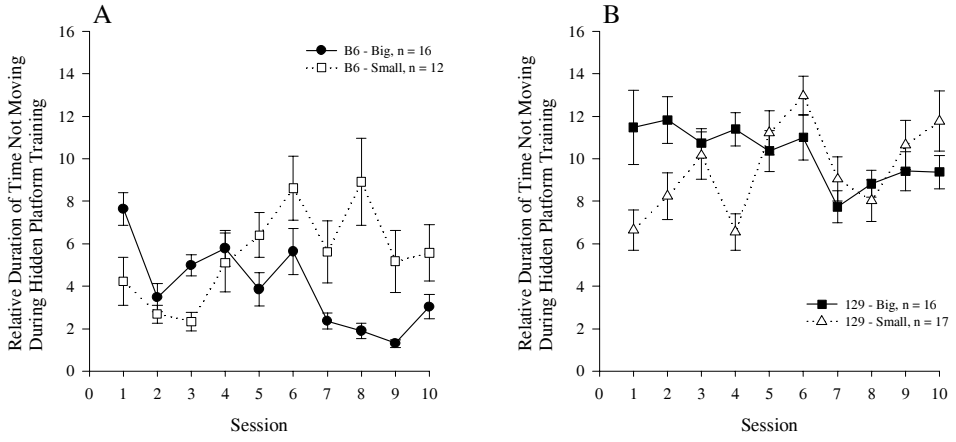

Figure 4. 129 mice spend significantly more time floating compared to B6. Floating, measured as duration of time not moving relative to trial length, is expressed as mean ± standard error.

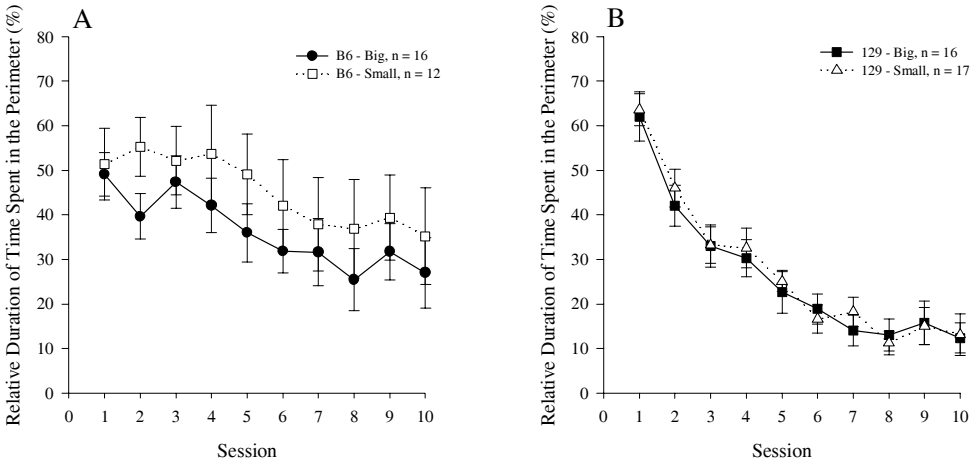

Figure 5. Thigmotaxis, measured as relative duration of time (mean ± standard error) spent in the perimeter (15 cm wide annulus) of the water maze is significantly increased in B6 mice housed in small cages vs. those housed in large ones, while such effects of housing conditions are not observable in 129 mice. A, B6 mice. B, 129 mice.

Probe Trials

The results obtained for probe trials 1 and 2 are shown on Figure 6. Variance analyses of the time spent by the mice searching in the target quadrant revealed no significant main effects or interactions of the factors housing and group, *F*s < 1. Furthermore, the analysis also revealed that the relative duration of time the mice spent in the target quadrant during Probe trial 1 (access to extra maze cues was blocked) was significantly better than 25% random chance level, $t(60)= 3.697$, which suggests that mice could navigate based either on intra-maze visual cues or some putative nonvisual cues that were not affected by the obstruction and removal of extra maze visual cues. Importantly, however, the relative duration of time spent in the target quadrant during the second probe trial (in which access to extra maze cues was restored), was significantly higher (ANOVA probe trial effect: $F(1, 57) = 27.17$; all other factors including housing, strain and all interaction terms: $F < 1.64$) suggesting that extra maze visual cues are important for the mice in their search of the target platform.

One potential concern regarding the probe trial is that it requires the mice to search for the target platform for 60 s, a period which is significantly longer than the escape latency (5-15 s) usually achieved by mice by the end of hidden-platform training. Thus, the probe trial may test not only spatial preference but also persistence or lack of extinction. In order to monitor the potential progression of changes of target quadrant dwell time we analyzed 10 s time bins of performance for the 60 s long probe trials.

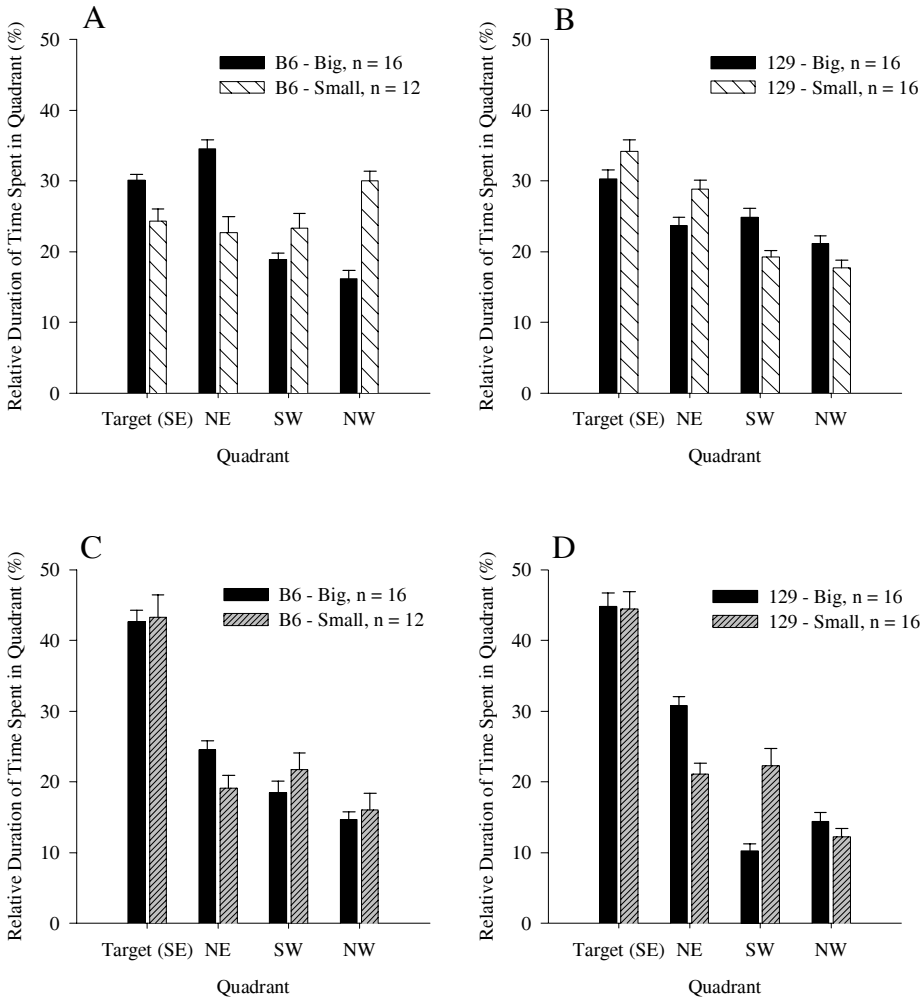

Figure 6. The behavior of B6 and 129 mice in probe trials. Panels A and B show relative duration of time spent by B6 and 129 mice, respectively, in four quadrants of the water maze in probe trial 1. Note that in this probe trial access to extra maze visual cues was blocked. Panels C and D show relative duration of time spent by B6 and 129 mice, respectively, in four quadrants of the water maze in Probe trial 2. Note that in this probe trial extra maze visual cues were present. Data are expressed as mean $\pm$ standard error.

Figure 7 shows that all groups exhibited a similar and stable level of target quadrant preference, i.e., no extinction of spatial preference was evident. These observations were confirmed by an ANOVA that demonstrated no significant main effects or interactions of the factors group, housing, or interval, $Fs \leq 1.10$.

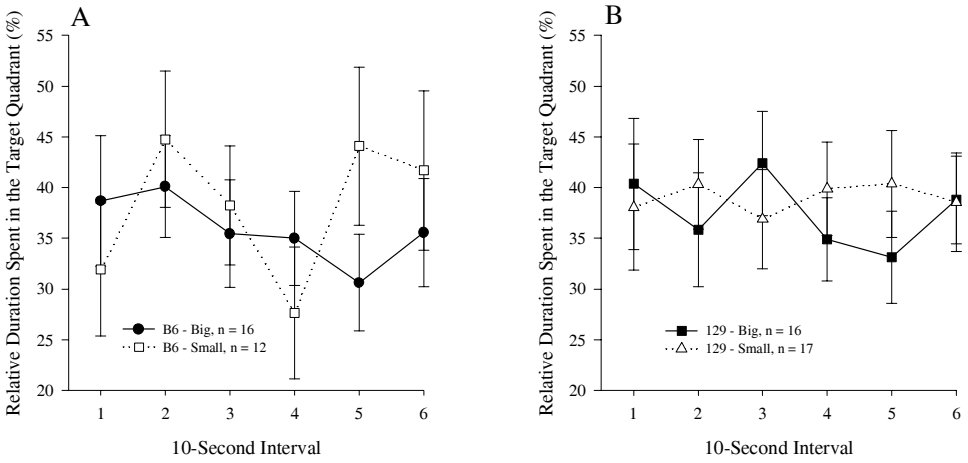

Figure 7. Target quadrant dwell time does not significantly fluctuate during the 60 second of Probe trial 2. Means ± standard error are shown.

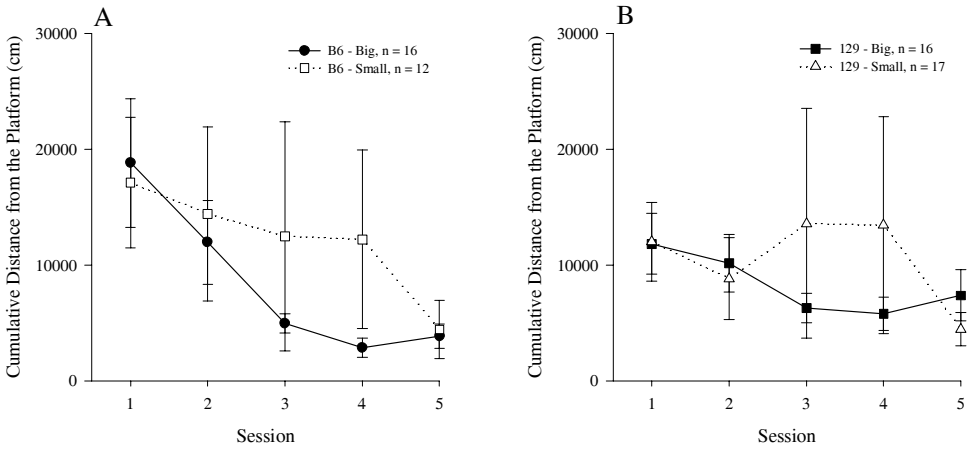

Figure 8. Nonspatial learning performance in the visible platform task is not affected by strain, housing condition and session. Means ± standard error are shown.

Visible Platform Training

During visible platform training, all groups showed a similar acquisition of the task. An analysis of training results revealed a significant main effect of session, $F(4, 156) = 12.41$, but did not find significant main effects of housing or block, or any interactions of these three factors, Fs < 1, suggesting that nonspatial learning remained unaffected by all these factors.

Discussion

The results demonstrate that seemingly modest environmental changes can induce significant alterations in learning performance in the water maze and this change is dependent upon the genetic makeup of the subjects studied. The performance of C57BL/6 mice (B6) was significantly affected by housing conditions. Mice from this strain exhibited good spatial learning performance when housed in groups of 10 in large cages; however, they showed robust impairment when housed in groups of 4 in small micro-isolator cages. The effects of small vs. large cage housing were almost undetectable in the 129/SVEV inbred strain (129). Over all, these mice performed well in the water maze irrespective of housing conditions. The impaired performance of B6 mice housed in small cages was characterized by increased cumulative distance from target and decreased target quadrant dwell time, measures that suggest impaired spatial preference and precision. Increased thigmotaxis was also evident in these mice, suggesting a potentially abnormal escape reaction induced by the water maze procedure. Lastly, decreased swim speed was also detected in these mice, which may be interpreted as reduced motivation to explore the maze.

At this point it is unclear what aspects of the different housing conditions elicited these changes. However, it is possible that aggression-induced stress is the culprit. Stress has been shown to lead to performance deficits in the water maze in a spatial-task-dependent manner in rodents (Hölscher, 1999; McEwen, 1999; McEwen & Sapolsky, 1995). Fighting or aggression has been demonstrated to be associated with stress (Benus et al., 1991). We observed elevated aggression in the small cages containing B6 mice possibly as a result of the smaller territory and the smaller number of opponents present. Although we did not quantify the amount of fighting, B6 mice housed in the small cages often exhibited visible bite marks whereas 129 mice did not. This finding is in line with the common observation in animal colonies on the usual docile nature of 129 mice as well as with the results of the analysis of the aggression levels of mice from these strains (Hughes, 1988). In the large cage, mice could hide better and also the attention of an aggressive dominant male could presumably be divided among multiple targets. Thus the effects of aggression and dominance hierarchy might have been diminished in the large home cage. Factors other than aggression might also have influenced learning performance. Housing in a large cage may be viewed as a form of environmental enrichment. It allows more space to be explored and more cage mates to interact with. Environmental enrichment has been shown to lead to improved spatial learning performance in the water maze (Pham et al., 1999; van Praag et al., 1999). Lastly, it is also possible that other parameters such as access to food and water, control of temperature and humidity, level of illumination, etc., might have been different between the small and large cage housing conditions. These aspects of housing conditions will need to be systematically explored in order to understand the relative importance of different environmental factors that influence water maze learning performance.

Another notable aspect of our results was the apparent lack of impairment of water maze learning performance in 129/SVEV mice compared to C57BL/6. 129 mice have been shown to possess numerous neuroanatomical (Wahlsten, 1982) and electrophysiological (Nguyen, Duffy & Young, 2000a) abnormalities.

Behaviorally, these mice have also been found to exhibit impairment in the water maze (Wolfer et al., 1997), which has been of concern as embryonic stem cells used for gene targeting are derived from this strain (Gerlai, 1996). However, the generality of the impairment of the 129 strain has been questioned (Montowski et al., 1997), and the fact that numerous and genetically distinct substrains of 129 exist has been pointed out (Simpson et al., 1997). Some of these substrains have been found to perform well in spatial learning paradigms such as the hidden platform task of the MWM (Montowski et al., 1997; Nguyen, Abel & Kandel, 2000b). Our results do not contradict the latter notion as they show that 129/SVEV mice are not impaired in spatial or nonspatial learning. Furthermore, in our present study, these mice were found to exhibit no thigmotaxis. Thigmotaxis was shown to be deleterious for spatial learning performance and was previously demonstrated to be prevalent in 129 (Wolfer et al., 1997). Although slightly increased immobility in 129/SVEV was detected, as also known in the literature (e.g., Paulus et al., 1999; Wolfer et al., 1997), apparently this behavioral trait did not impair the ability of the 129/SVEV mice to learn to locate the target platform efficiently. The lack of impairment in 129/SVEV, however, remains controversial because, although a similar finding has been shown before (Montkowski et al., 1997), mice from this substrain of 129 were found significantly impaired in water maze spatial learning in other studies (e.g., Balogh et al., 1999; Wolfer et al., 1997). The discrepancies may be due to genotype-environment interaction that altered the manifestation of genetic effects, and thus the relative difference among inbred strains, in an environmental-condition-dependent manner.

The more information is gathered on the effects of mutations on learning processes in mice, the clearer it becomes that the experimenter needs to be concerned about the complicated biology of such processes and also about the perhaps even more complex problem of how genes and the environment interact. Examples of genotype-environment interaction in the classical quantitative genetic literature (Ehrman & Parsons, 1976; Fulker et al., 1972; Gerlai & Csányi, 1990; McClearn, 1960; Tyron, 1940) are now again appreciated (Gerlai, 2000) as dramatic strain-dependent environmental effects are demonstrated (Cabib et al., 2000). Our present experimental example confirms the importance of genotype-environment interaction in mouse neurobehavioral genetic research. It demonstrates that one needs to rigorously control all possible environmental factors in order to gain understanding of the interaction of genes and the environment and to properly interpret the results of neurobehavioral genetic analyses, including transgenic and gene targeting experiments. Furthermore, environmental factors may exert their effects differentially in different behavioral paradigms. Therefore, our results underscore the importance of the recommendation made by numerous investigators (e.g., Crawley, 2000) regarding the need to apply a battery of behavioral tests with different idiosyncratic performance characteristics in the analysis of mutation effects in animal models of cognition.

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