Finding the right motivation: genotype-dependent differences in effective reinforcements for spatial learning

Jiun Youn1,2, Bart A. Ellenbroek4, Inti van Eck1, Sandra Roubos1, Matthijs Verhage2 and Oliver Stiedl1,3*

1Behavioral Neuroscience Group, 2Department of Functional Genomics and 3Department of Molecular and Cellular Neuroscience, Center for Neurogenomics and Cognitive Research (CNCR) and Neuroscience Campus Amsterdam (NCA), VU University Amsterdam, The Netherlands; 4School of Psychology, Victoria University of Wellington, New Zealand

*Corresponding author: Oliver Stiedl, Ph.D., Associate Professor

Behavioral Neuroscience Group, CNCR & NCA, VU University Amsterdam, De Boelelaan 1085, Room A-062, 1081 HV Amsterdam, The Netherlands

E-mail: oliver.stiedl@cnrc.vu.nl, Phone: +31 20 5987100, Fax: +31 20 5986968

Keywords: C57BL/6J, DBA/2J, modified Barnes maze, spatial learning, memory, aversive, appetitive, motivation
Abstract

Memory impairments of DBA/2J mice have been frequently reported in spatial and emotional behavior tests. However, in some memory tests involving food reward, DBA/2J mice perform equally well to C57BL/6J mice or even outperform them. Thus, it is conceivable that motivational factors differentially affect cognitive performance of different mouse strains. Therefore, spatial memory of DBA/2J and C57BL/6J mice was investigated in a modified version of the Barnes maze (mBM) test with increased complexity. The modified Barnes maze test allowed using either aversive or appetitive reinforcement, but with identical spatial cues and motor requirements. Both mouse strains acquired spatial learning in mBM tests with either reinforcement. However, DBA/2J mice learned slower than C57BL/6J mice when aversive reinforcement was used. In contrast, the two strains performed equally well when appetitive reinforcement was used. The superior performance in C57BL/6J mice in the aversive version of the mBM test was accompanied by a more frequent use of the spatial strategy. In the appetitive version of the mBM test, both strains used the spatial strategy to a similar extent. The present results demonstrate that the cognitive performance of mice depends heavily on motivational factors. Our findings underscore the importance of an effective experimental design when assessing spatial memory and challenges interpretations of impaired hippocampal function in DBA/2J mice drawn on the basis of behavior tests depending on aversive reinforcement.

1. Introduction

C57BL/6J (C57) and DBA/2J (DBA) mice are probably the most often compared mouse strains in behavioral research. They are non-albino strains with no known retinal degeneration or other gross abnormality that would confound behavioral performance based on visual information processing. Both strains have been used as founders for generating recombinant inbred strains[1], which are of a great importance to identify gene loci related to specific traits. Despite many attempts for replication, comparison studies between the two inbred strains do not yield a very coherent picture. Even the results from the seemingly simple open field test vary from C57 mice being more active [2] to DBA mice being more active [3]. Findings from anxiety tests such as the elevated plus maze are also inconsistent [3,4]. Since learning and memory tests are unavoidably influenced by locomotion and anxiety [5], several attempts were made to control for the confounding effect of non-cognitive factors. Interestingly, one study concluded that the difference in activity and emotionality does not account for the superior spatial memory performance of C57 mice [3], whereas another study indicated a significant correlation between anxiety and cognitive performance [6]. It should be noted that C57 mice showed reduced explorative behavior, higher levels of anxiety and superior spatial memory performance compared to DBA [3]. In contrast, it was suggested that DBA mice are a 'highly anxious strain' with superior performance in the modified holeboard test [6]. This superior performance in DBA mice was especially interesting since a large number of studies using the water maze reported that DBA mice performed worse than C57 mice [7,8]. Such conflicting results might be partly due to the difference in experimental conditions and procedures, but it is also possible that the aversive component (swimming) in the water maze and the appetitive reinforcer (almond chips) in the modified holeboard test influenced the strains in a different manner. In addition to this, DBA mice learned an olfactory discrimination task more readily than C57 mice when food reward was used as reinforcer [9]. The impact of motivational factor has been already shown in the studies with mice lacking the non-receptor-type tyrosine kinase Fyn. While knockout mice had been initially reported to have severe spatial learning deficit in the water maze test[10], these mice showed intact performance upon stimulation to swim and not float in the water maze task[11] and were unaffected in a radial maze task [12]. A similar task-dependent group difference was observed in a strain comparison study [13] with BALB/c mice being impaired in the water maze,
while performing comparably to C57BL/6N in the dry maze task. It was suggested that the impairment observed in the water maze was most likely due to poor motivation or motor skills rather than memory deficit.

In order to investigate whether motivational cues indeed influence the performance of C57 and DBA mice, we compared the spatial memory performance of these two mouse strains under two different conditions. During the first experiment, the animals were required to find a shelter to avoid an aversive component (strong wind from fans). In the second experiment, the mice were trained to find a palatable food reward (almond chips). The spatial configurations and the motor requirements to complete the task were identical in the two conditions. We hypothesized that the impairment in DBA mice previously shown in the Morris water maze and classical Barnes maze would be replicated in the aversive version of our test. However, when food reward was given in the appetitive version, it was expected that the impairment in DBA mice would be ameliorated. Since C57 mice show superior spatial learning in nearly all studies including both appetitive and aversive tests, we predicted that the performance level in C57 mice would not fluctuate to a great extent across the different conditions. Nonetheless, since the animals were not food-deprived, and C57 mice are known to be less willing to consume food in a novel environment than DBA mice [14], it may not be surprising if C57 mice would perform worse in the appetitive than in aversive test.

For these experiments, we modified the Barnes maze that is commonly used for mice to reduce the stress related to water exposure. However, it has been shown that even visually impaired mice can learn the Barnes maze task by using non-spatial strategy [15]. The modification of the Barnes maze was made to present a seemingly irregular pattern of holes and thereby imitating the water maze more closely and prevent mice from solving the task on the basis of a non-spatial, e.g. serial exploration strategy [16].

2. Methods

2.1. Animals

In total, 46 male mice were used for the experiment from which 24 mice (12 C57BL/6JCrI and 12 DBA/2JCrI) were tested in the aversive experiment and 22 mice (11 C57BL/6JCrI and 11 DBA/2JCrI) were tested in the appetitive test. All mice were provided by Charles River (Maastricht, The Netherlands) and were 6 weeks old at the arrival. The mice were subsequently housed individually in standard Macrolon cages (type 2: 22 cm x 13 cm x 16 cm). Food and water was given ad libitum with the exception of the shaping phase in the appetitive test (see below for the detail). All animals were kept at constant room temperature (21±1°C) and (60 ± 10%) humidity on a reversed 12-h dark-light cycle with the lights switched on at 19:00. The experiments were conducted in an illuminated room at a light intensity of 180 lx with visual cues on the walls. The animal experiments complied with EEC recommendations for the care and use of laboratory animals (86/609/EEC) and were approved by the local animal research committee. All the efforts were made to minimize the suffering and stress of the animals.

2.2. Equipment

The modified Barnes maze (mBM; Fig. 1) was designed to measure spatial learning in mice under different motivational conditions. It contained more holes (44 in total) than most of the ‘classical’ Barnes maze (cBM) designs (10-20). The holes are arranged in such way, that no pattern is obvious that could be exploited by a serial exploration strategy, i.e. running from one hole to the next to find the target hole as observed in the cBM. Mice were trained to locate a target hole using visual extra-maze cues on the distant walls as a guide. The visual extra-maze cues were placed on the walls surrounding the maze. The
cues were black and white patterns (cross, lines, square) of sizes varying between 2000 cm² and 4200 cm². The distance between the maze and the walls ranged from 117 cm and 189 cm.

All holes contained white double-floored cups underneath, which could easily be replaced (5 cm diameter). The upper layer of the cup was made of wire gauze on top of which food could be placed (gauze 1.6 mm deep, total of 3.2 mm deep). Mice were unable to access food below the gauze (holes of 1 mm diameter).

The mBM consisted of a circular light grey platform (diameter 122 cm) with 4 symmetrical quadrants. Each quadrant contained 2 holes, 3 holes and 6 holes in the inner, middle and outer ring respectively. The target was always located in the middle ring of holes. To prevent odor cues, the target location between each animal was varied and the mBM was thoroughly cleaned with 80% ethanol solution and rotated between trials. Once per day, all the cups were removed, washed under running water and dried. A dark cylinder (6.8 cm x 12 cm, diameter x length) served as transport container from the home cage to the mBM, to minimize the handling stress.

A video camera placed above the center of the mBM, monitored the performance of mice during mBM trials. Images were recorded and analyzed by a computer located in an adjacent room by using Viewer software (Biobserve GmbH, Bonn, Germany). The experimenter was not present in the experimental room during trials but observed the experiments on the computer screen. The distance and latency to reach the target location were recorded and tracked by the software. Additionally, errors (visits of incorrect holes) were counted manually by the experimenter. A hole visit was defined as a head dip into a hole, and multiple consecutive head dips were counted as single hole visit.
2.3. Aversively motivated Barnes maze test

2.3.1. Habituation

The mice were habituated to the maze and to entering an escape cylinder, made of dark plastic (6.4 cm x 6.1 cm x 15.3 cm), for 2 times. Animals that failed to locate the escape cylinder within 10 min were gently guided to it by the experimenter and were left there for 1 min. During the first habituation trial, the escape cylinder was filled with nesting material from the home cage to make it easy for the mice to venture into the novel space. During the 2nd habituation trial and all the training sessions, fans surrounding the maze were turned on to provide an aversive reinforcement. In total, three fans (Duracraft, Southborough, MA, USA) were placed at angles of ~120° apart from each other around the

![Figure 1: Schematic presentation of two Barnes mazes. The ‘classical’ Barnes maze (A) has 24 escape holes in the perimeter of the maze, whereas the ‘modified’ Barnes maze has 44 escape holes arranged symmetrically within each quadrant (B).](image)
maze. The rotor heads of the fans rotated at 90° angle in the direction of the mBM. The distance between the maze and fan was 60 cm. The wind velocity was 3 m/s in the center of the maze and 4.5 m/s at the edge of the maze.

2.3.2. Acquisition

Twice per day the mice were released from the center of the maze to find the target location. The transport cylinder was made of dark plastic, so the direction a mouse was heading to at the point of release varied randomly. The inter-trial period was approximately 3 hr. When the animal could not locate the target within 3 min, the experimenter gently guided it to the target and left it there for 1 min.

2.3.3. Probe trial

After the 13th trial, mice that made less than 5 errors for 2 consecutive times were tested on a probe trial to investigate if any non-spatial cues enabled the mice to locate the target (e.g. the odor from the escape cylinder). The probe trial took place ~4 hr after the last trial. Before the probe trial, the escape cylinder was replaced with a white double-floored cup identical to the rest of the holes. Animals were released from the center of the maze again as in the acquisition phase. To overcome the confounding effect of locomotion, the duration was set as 10-hole visits instead of limited time. Mice that did not meet the criteria even after 19 trials were subjected to the probe trial after the 19th trial.

2.4. Appetitively motivated Barnes maze test

2.4.1. Shaping

In a pilot study performed before the current experiment, it took considerably longer time for C57 to consume a palatable reward in a novel environment than DBA mice even when they were food deprived. Since severe food restriction is not only against the animal welfare policy but also can confound the performance level and locomotion, we tried to overcome the problem by making the habituation procedure more extensive than used in the aversive test. After 5 days of acclimation period after the arrival, a mild food deprivation regime took place. The weight of the animals was lowered to 90% of their original body weight. In addition, almond chips were given inside their home cage during the whole period of the experiment to familiarize the mice with the taste. In the next step, each mouse was placed on the maze 2 times per day for the maximum duration of 3 min. All holes on the maze contained almond chips, but only a few of them had accessible (on top of the gauze) chips placed inside. After 10 days of shaping sessions, all animals consumed the food reward given on the maze readily. After the last shaping trial, the mice were given free access to food and water again. During the shaping session, no preference for a specific quadrant was observed (data not shown).

2.4.2. Habituation and acquisition

All mice were given time to rest and recover their original body weight for 2 weeks after the shaping period. During this period, they were handled regularly and given almond chips inside their cage. The habituation and acquisition procedure was identical to the aversive test with one exception. Instead of
fans generating a breeze as aversive reinforcement, there was an almond chip hidden in the escape cylinder for appetitive motivation. To prevent search bias by odor cues, all holes except the target location contained inaccessible almond chips below wire gauze.

2.4.3. Probe trial

The mice which met the criteria were tested in a probe trial ~4 hr after the 13th trial. Because of the slightly higher inter-trial variability in performance than in the aversive test, the criterion was set as less than 5 errors in 3 consecutive trials. During the probe trial, the target cylinder containing an almond chip was replaced with a double-floored cup containing an inaccessible almond chip. As in the aversive test, animals that did not meet the criteria by the 19th trial were subjected to the probe trial after their last training session.

2.5. Data analysis

The data was analyzed with SPSS Statistics version 15.0 (SPSS, Chicago, USA). Each acquisition data was analyzed using repeated ANOVA design with strain as an independent factor and trials as a within factor. The latency and distance to reach the target location were variables of interest. In addition, the strategy used in each trial was analyzed according to the protocol described by Bach et al. [16]. To avoid experimenter bias in the classification process, the strain information was blinded beforehand and the track record was used instead of actual video files. When a mouse visited more than 4 consecutive holes, the trial was classified as a serial strategy. When arbitrary direction changes occurred more than 2 times with center crossings, the behavior was classified as a random strategy. When a mouse explored holes in the vicinity of the target location and the distance covered in the target quadrant was more than 70% of the total distance, it was defined as spatial strategy. To quantify the strategy use preference, the 13 acquisition trials were grouped in 3 blocks (4-4-5 trials each). The percentage of each strategy use was calculated per block. When the sphericity assumption was violated, degrees of freedom were adjusted with the Huynh-Feldt correction. The differences were regarded as statistically significant when the p value was less than 0.05. When the p value ranged between 0.05 and 0.09, it was noted as trend. The probe data was analyzed by one-way ANOVA with strain as an independent factor. Target preference was defined as the percentage of time spent in the target quadrant divided by the total duration. When the Levene’s test of equality of error variances was significant, rank-transformed data was used for the analysis.

3. Results

3.1. Aversive modified Barnes maze test

A clear spatial learning effect was observed in the aversive mBM test. The effect of trials was significant for latency and distance (F_{8,72, 191.82} = 8.39; p < 0.001; F_{8,95, 196.79} = 10.43; p < 0.001). Both, latency and distance to locate the target shelter decreased over time (Fig. 2). It took C57 mice less time and distance than DBA mice to complete the task (F_{1, 22} = 128.80; p < 0.001; F_{1, 22} = 61.19; p < 0.001). The interaction between trials and strain showed significant results (F_{8,72, 191.82} = 3.01; p < 0.001; F_{8,95, 196.79} = 3.22; p = 0.001). The strain difference was most noticeable between trials 5 and 11 and decreased thereafter. DBA mice were generally slower in finding the target especially during the initial trials. However, the distance graph (Fig. 2B) indicated that longer latencies were not accompanied by higher
levels of exploration. Many DBA mice were immobile or very slow before starting to explore the maze, which is reflected in the trend of strain effect in the velocity (F_{1,22} = 3.29; p = 0.08). There was a significant interaction between the strain and trial in the velocity (F_{9.59,191.77} = 5.13; p < 0.001). Although DBA mice were slower in the beginning, their speed was comparable to that of C57 mice in the later trials. During the probe trial, C57 mice spent significantly more time in the target quadrant than DBA mice (F_{1,22} = 11.69; p < 0.05). However, it should be noted that the time spent in the target quadrant was considerably higher than the chance level (25%) in DBA mice as well, reaching nearly 50%. In total, 11 out of 12 C57 and 6 out of 12 DBA mice reached the criteria before the end of acquisition training (\chi^2 = 5.05; p < 0.005). However, there was no difference in the target preference between mice that met the criteria and those that did not meet it (data not shown).
Figure 2: Spatial memory performance in the aversive version of the modified Barnes maze test. Overall, C57BL/6J performed better than DBA/2J mice (p < 0.001 in latency (A) and distance (B)). A significant strain x trial interaction was found for velocity with increased speed of C57BL/6J mice particularly at trials 1-4 and 13. There was a trend of strain effect as well (p = 0.08; C). In the probe trial (D), both strains performed significantly above chance level (25%) with C57BL/6J spending significantly more time in the target quadrant than DBA/2J mice (*p < 0.05).
3.2. Appetitive Barnes maze test

There was a clear learning effect in the appetitive version of the mBM test (Fig. 3). A significant effect of trials was determined for latency and distance ($F_{7,00,139.97} = 28.04; \ p < 0.001; F_{7.79,115.72} = 20.12; \ p < 0.001$). However, no significant difference existed between strains ($F_{1,20} = 0.02; \ p = 0.90; F_{1,20} = 0.32; \ p = 0.58$). DBA and C57 mice performed similarly during the training. There was also no interaction between the trials and strain ($F_{7.79,115.72} = 0.40; \ p = 0.92$). Interestingly, the velocity increased over time ($F_{9.59,191.77} = 14.09; \ p < 0.001$) in both strains in contrast to the results from the aversive test. However, the increase in velocity was much higher in DBA mice ($F_{9.59,191.77} = 5.13; \ p < 0.001$). In the probe test, both strains performed well above chance level (25%). There was no significant strain effect ($F_{1,20} = 0.32; \ p = 0.58$). In total, 9 out of 12 C57 and 8 out of 12 DBA mice reached the criteria before the end of acquisition training ($\chi^2 = 0.20; \ p = 0.88$). However, there was no difference in the target preference between mice that met the criteria and those that did not meet it (data not shown).
**Figure 3**: Spatial memory performance in the appetitive version of the modified Barnes maze test. There was no strain difference in the latency (A), distance (B) and probe trial performance (D). There was a significant velocity increase over the trials in both strains ($p < 0.001$; C), and the velocity increase along trials was more pronounced in DBA mice ($p < 0.001$). In the probe trial (D), both strains performed significantly above chance level (25%) without significant difference in the target quadrant time between DBA/2J and C57BL/6J mice.
3.3. Search strategies

During the mBM training with aversive reinforcement (Fig. 4A-C), the use of the random and serial strategy significantly decreased over time ($F_{2, 44} = 5.89; p < 0.01$ and $F_{2, 44} = 15.476; p < 0.001$ respectively), while the preference for the spatial strategy increased noticeably with increasing trials ($F_{2, 44} = 20.18; p < 0.001$). DBA used the random strategy more often than C57 mice ($F_{1, 22} = 10.52; p < 0.01$), and C57 used the spatial strategy more often than DBA mice ($F_{1, 22} = 15.71; p = 0.001$). There was a significant quadratic contrast between blocks and strain on the random strategy use ($F_{1, 22} = 5.22; p = 0.03$). The strain difference was most apparent on the second block (from trial 5 to trial 9) and decreased on the last block.

Unlike in the aversive test, there was no significant strain difference in the use of different search strategies in the appetitive mBM test (Fig. 4D-F). While the preference for the random strategy decreased over time ($F_{2, 44} = 27.25; p < 0.001$), there was no significant block effect on the serial strategy use, and mice began to use the spatial strategy more frequently in the later trials ($F_{1,587, 44} = 23.71; p < 0.001$).
Figure 4: The percentage of search strategies used across trial blocks in the aversive and appetitive mBM tests. There was a significant block effect on random ($p < 0.001$; A), serial ($p < 0.01$; B) and spatial strategy ($p < 0.001$; C). The strain effect was significant on the random strategy ($p < 0.01$) and serial strategy ($p = 0.001$). There was a significant quadratic contrast on the interaction between blocks and strain on the random strategy use ($p = 0.03$). In the appetitive mBM training there was a significant block effect on random ($p < 0.001$; D) and spatial strategy ($p < 0.001$; F), but there was no strain difference. Block 1 covered trials 1 to 4, block 2 covered trials 5 to 8 and block 3 covered trials 9 to 13.
3. Discussion

Our results illustrate the importance of the reinforcing properties exploited in spatial learning tasks. The significant strain difference observed in the aversively motivated Barnes maze task disappeared in the appetitive version of the test when a palatable food reward was provided. This finding demonstrates that spatial learning capacity in DBA mice can be facilitated or is unimpaired when the proper motivation is used. To the best of our knowledge, this is the first experiment to compare the spatial memory in DBA and C57 with identical spatial cue configurations and motor requirements exploiting different reinforcing properties.

Upon aversive reinforcement, a clear difference existed between the two strains in locomotion. DBA mice were much slower and sometimes even immobile for prolonged time. This immobility of DBA mice was also observed in the cBM test (Dr. S. Spijker, personal communication). As velocity increased after the 4th trial, latency and distance began to decrease concomitantly. Although the strain difference persisted throughout the most part of the experiment, on the very last (13th) trial, the distance to find the target did not differ between the two strains anymore, suggesting that DBA mice had acquired the task by the end of the training.

This was confirmed in the probe trial during which DBA mice spent nearly 50% in the target quadrant. In contrast, C57 mice acquired the task within 5 trials, and the latency and distance value stayed stable without much fluctuation till the end. This superior performance was also observed in the probe trial, where C57 mice spent nearly 80% of the time in the target quadrant. It should be also noted that nearly all C57 mice (11 out of 12) met the criterion for the probe trial of two consecutive trials of less than 5 errors, while half of the DBA mice did not reach this criterion, which requires a stable performance.

In addition, C57 mice employed the spatial strategy more often while their use of less effective strategies decreased rapidly. Despite that the use of the spatial strategy increased over time in DBA mice as well, the serial strategy use increased again in the last block, suggesting that the spatial memory performance in DBA was not as stable as in C57 mice. Hence, despite the profound difference in locomotion between the two strains, it is concluded that C57 mice demonstrated better spatial learning and memory when the reinforcer was of an aversive nature. This result is in line with previous studies using the classical Barnes maze [8,15].

In initial pilot tests (data not shown), DBA were faster than C57 mice to consume almond chips in a novel environment. This observation is in agreement with the data from a study where a similar novelty-induced hypophagia test was performed [14]. This data, in combination with the fact that one of the few instances in which DBA outperformed C57 mice in a spatial task was in the presence of palatable food reward, led us to hypothesize that the motivation to locate a food reward might ameliorate the spatial memory impairment in DBA mice to some extent. Nonetheless, the complete ‘rescue’ shown in Fig. 3 was still quite surprising considering the large number of publications, including our own data from the aversive mBM test, reporting inferior performance of DBA to C57 mice. There was also no difference in the preferences of strategy use, and the number of mice that reached the criterion for the probe trial. The higher motivation level in DBA mice was also noticeable in the gradual increase in velocity that nearly doubled in the last trial compared to the first trial.

It is possible that other factors than the difference in reinforcing properties may have played a role in the difference observed here. The mice in the appetitive test went through a more extensive handling procedure during the shaping phase with a mild food deprivation regime. This resulted in the age difference of ~4 weeks and prolonged the single housing period. Although ageing and single housing are both relevant factors in learning and emotion, it was shown that it is actually DBA mice which are more
susceptible than C57 mice to the detrimental effect of prolonged single housing \[^{14}\] and aging \[^{17}\]. For instance, DBA mice are prone to develop glaucoma with advanced age \[^{17}\], making them less suitable for tests depending on visual acuity such as spatial tasks. Consequently, if anything, the confounding factors from the shaping period should have been more disadvantageous for DBA than for C57 mice. In any case, the mice were still in their young adulthood at the age of 3 months during the appetitive test. Therefore, it is unlikely that age difference played a significant role here.

The shaping period was introduced to avoid food restriction during the acquisition training. Not only does food restriction interfere with learning-related neurotransmitter systems such as the dopaminergic system \[^{18,19}\], as during the pilot experiment it was observed that food restriction renders DBA mice hyperactive, which is in line with previous results \[^{20}\]. Interestingly, it was shown recently that the motivation to obtain food under different workloads was significantly different between DBA and C57 mice \[^{21}\]. DBA were more motivated to find food even when the workload increased and consumed significantly larger amounts of food than C57 mice. The hyperactivity might partly explain why DBA often performed worse than C57 mice in the radial maze task despite the use of palatable food rewards. Indeed, DBA mice exhibited a higher velocity and shorter latency to complete the task in a radial maze study with food deprivation of up to 20% body weight loss, while committing more errors than C57 mice \[^{22}\]. Taken together, it can be assumed that the combination of high motivation to find food with hyperactivity can render DBA mice more susceptible to errors when food-deprived than when satiated.

DBA mice have been regarded as genetic model of hippocampal dysfunction in many studies. Importantly, the spatial memory impairment in DBA mice was linked to the impairment in LTP maintenance and altered protein kinase C (PKC) level in the hippocampus \[^{8,23-25}\]. Exercise increased the PKC level thereby improving the spatial memory performance in DBA mice \[^{26}\], suggesting that the hippocampal ‘dysfunction’ in DBA mice is not of an irreversible nature. Our finding that DBA mice performed optimally under appetitive condition also implies that DBA mice are capable to recruit hippocampal function to perform the spatial memory task under conditions of high motivation and low levels of ‘stress’.

It remains to be investigated how motivational factor could have recruited hippocampal function in DBA mice to solve this task using a spatial, and thus, presumably hippocampus-dependent strategy. One might speculate that the anticipation before each trial in the appetitive test increased the dopamine level in a different manner in C57 and DBA mice, which in turn would have influenced attention and memory during the training sessions. It has been shown that C57 and DBA mice are different in gene expression levels of dopaminergic receptors \[^{27}\] and brain areas, such as the nucleus accumbens, which are rich in dopaminergic neurons \[^{28,29}\]. Dopamine is known to be important in the stabilization of place fields in the hippocampus \[^{30}\] thereby directly linking it to spatial learning. It is also possible that DBA mice do not rely on the hippocampus to the same extent as C57 mice do during the spatial memory task as has been shown for passive avoidance studies \[^{31}\]. Moreover, during the appetitive test, other brain areas such as the parietal cortex may become activated sufficiently enough to compensate the hippocampal dysfunction reported in DBA mice.

Alternatively, the high stress level in aversive tests may confound cognitive function in DBA mice as indicated by deficits in hippocampus-dependent tests including contextual fear conditioning of DBA vs. C57 mice \[^{32,33}\] and deficient latent inhibition in passive avoidance learning after pre-exposure \[^{31,34}\]. Consequently, DBA mice may be particularly sensitive to cognitive impairment under aversive test conditions as ‘stress’ particularly impairs spatial (hippocampus-dependent) learning performance \[^{35,36}\].
Compared to the drastic difference between the tests observed in DBA mice, the performance level in C57 mice remained fairly constant regardless of the reinforcement used. However, it still is unclear why C57 mice did not show such a high target preference in the appetitive test as displayed in the aversive test. The simplest explanation is that C57 mice are more motivated by the aversive component (fan breeze) than to find the food reward. Another possibility is that mice remembered from the shaping phase that the target location could be changed. Hence, once mice found out that the food was not in the target location, some of them could have switched to other locations. This raises another question why DBA mice persisted more in the target location compared to C57 mice despite the same shaping phase they went through. This type of behavior is reminiscent of the results from a Pavlovian conditioning test [37]. In the study, DBA mice acquired the conditioning task easily but when the conditioning stimulus was not associated with the reward anymore, they had more difficulty in discontinuing the instrumental response, while C57 showed a robust extinction pattern. Hence, it is possible that the shaping phase influenced the strains in a different manner and behavioral flexibility was more evident in C57 mice.

The current findings underscore the importance of using multiple behavioral tests especially when there is a distinct possibility that non-cognitive factors may affect cognitive function. Thus, a more careful approach is required to claim spatial learning deficits and attribute them to hippocampal dysfunction. Indeed, there are studies showing that the same group of mice performed differently under appetitive and aversive conditions [38-40]. However, the commonly used combination of Morris water maze and radial maze (aversive vs. appetitive) to assess spatial learning and memory may not be an ideal choice. The visuospatial information during swimming can drastically differ from that obtained while walking on the dry maze. In that respect, the mBM setup offers the opportunity to study the influence of different motivations with identical spatial configuration, thereby providing a more balanced approach to determine the impact of motivation on cognition in mice as demonstrated here for DBA mice. In addition, the sustained and even increased motivation to locate the target in the appetitive mBM test in both strains suggests that the appetitive model would be a suitable choice for longitudinal studies such as the effect of aging or reversal learning. It would also be worth to investigate if an appetitive reinforcer can motivate other strains such as 129S1/SvImJ and A/J mice which show a similar immobility as DBA mice on the Barnes maze [15].

In the current study, only male mice have been used to investigate the strain difference. It has been shown that there is a gender-related effect on spatial memory performance [41,42]. Considering that both genders responds differently to aversive stimuli [43], it would be interesting to investigate the interaction between gender and reinforcement properties (aversive vs. appetitive) in the mBM test.
5. Conclusion

The present findings underscore the importance of an effective experimental design when assessing spatial memory in different strains or genetically modified mice. Spatial performance strongly depends on motivational factors that differ in their saliency between mouse strains. It remains to be clarified whether appetitive reinforcement resulted in an improvement or whether aversive reinforcement caused an impairment of hippocampal function resulting in the dissociation of spatial learning and memory performance as a function of the reinforcement properties in DBA/2J mice. This results challenges interpretations of impaired hippocampal function in DBA/2J mice drawn on the basis of behavior tests with aversive reinforcement only, and this may extend to behavioral phenotyping results of genetic mouse models.

Acknowledgements

We thank Anton W. Pieneman for his valuable contribution in designing the modified Barnes maze and generating the figures, and Dr. René F. Jansen for statistical advice. This work was supported by the Dutch BSIK program (NeuroBsik Mouse Phenomics Consortium, BSIK03053), by the Netherlands Organization for Scientific Research (Pionier/VICI900-01-001 and ZonMW 903-42-095 to M.V.), and the European Union Seventh Framework Programs under grants agreements no. HEALTH-F2-2009-241498 (EUROSPIN project to M.V.) and PEOPLE-ITN-2008-238055 (BrainTrain).
References


[27] Loos M, Staal J, Schoffelmeer ANM, Smit AB, Spijker S, Pattij T. Inhibitory control and response latency differences between C57BL/6J and DBA/2J mice in a Go/No-Go and 5-choice serial reaction time task and strain-specific responsivity to amphetamine. Behav Brain Res 2010;214:216-24.


Disclosure Statement
All authors, Jiun Youn, Bart A. Ellenbroek, Inti van Eck, Sandra Roubos, Matthijs Verhage and Oliver Stiedl, disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted.