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Research report

# Effects of environmental enrichment on exploration, anxiety, and memory in female TgCRND8 Alzheimer mice

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ABSTRACT

After we could recently demonstrate a beneficial effect of environmental enrichment on AD-like brain pathology in female TgCRND8 mice [Ambrée O, Leimer U, Herring A, Görtz N, Sachser N, Heneka MT, et al. Reduction of amyloid angiopathy and Abeta plaque burden after enriched housing in TgCRND8 mice: involvement of multiple pathways. Am J Pathol 2006;169:544–52] the present study focuses on the behavioural effects of environmental enrichment with special emphasis on learning and memory performance in this AD model.

In the first experiment spontaneous exploration, locomotor activity and anxiety-related behaviour were assessed as the performance in learning tasks can be biased substantially by exploratory behavioural traits. In the second experiment spatial memory in the Barnes maze test and object recognition memory were examined.

Regarding exploratory behaviour transgenic mice from standard housing condition were statistically indistinguishable from wild-type controls. Enrichment had comparable effects in both genotypes indicated by higher levels of exploration and locomotor activity. In transgenic mice the elevated plus-maze revealed less anxiety-related behaviour due to enrichment in contrast to wild-type mice that statistically did not differ in anxiety-related behaviour.

Concerning learning and memory performance, cognitive deficits of standard housed transgenic mice could be demonstrated in both learning tasks. Surprisingly, in both housing conditions a significantly higher number of transgenic mice refused to explore any objects compared to wild-type mice. Furthermore, the Barnes maze test revealed deficits of the transgenic mice in spatial memory compared to wild-type mice whereas no effect of environmental enrichment was detectable. Thus environmental enrichment increased exploratory behaviour and decreased anxiety-related behaviour but could not clearly ameliorate deficits in learning and memory performance of TgCRND8 mice.



## 1. Introduction

Epidemiological studies suggest that Alzheimer's disease (AD) can be modulated by environmental factors. Frequent participation in cognitively stimulating intellectual and physical activities is linked to a reduced risk of AD [11,40]. In laboratory rodents it is well known that environmental stimulation has a great impact on various behavioural parameters (for review, see [29]) and can also improve learning and memory [10,32,33,35,38]. Interestingly, there is no clear picture regarding the effects of environmental enrichment on neuropathology in various mouse models for AD. On one hand some groups found a remarkable reduction of Aβ plaque burden after exposure to an enriched housing [2,21] and after voluntary exercise [1]. On the other hand an increased plaque formation after exposure to an enriched housing was demonstrated [15,16]. Others again did not find any significant effect of environmental enrichment on β-amyloid deposition [3,41]. Regarding the effect of voluntary exercise or environmental enrichment on learning and memory skills in mouse models for AD, until now positive effects were reported exclusively on water maze performance [1,6,15,41]. After our group recently demonstrated a beneficial effect of environmental enrichment on AD-like pathology in female TgCRND8 mice [2], the present study focuses on the effects of environmental enrichment on learning and memory performance of female mice of this AD model.

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As performance in learning tasks can be considerably influenced by differences in exploratory and locomotor behaviour, we focused on these behavioural characteristics in the first experiment. In the second experiment of the present study learning and memory performance was examined in the Barnes maze test and in the object recognition task.

So, the aim of this study was to elucidate if environmental enrichment that was shown to reduce amyloid burden to a remarkable degree in female TgCRND8 mice [2] is furthermore able to compensate for learning and memory deficits in these mice.

## 2. Materials and methods

### 2.1. Animals and general housing conditions

#### 2.1.1. Animals

In this study female transgenic and wild-type mice of the TgCRND8 line, a transgenic animal model of AD [5,18] were investigated. (Because of escalated aggressive behaviour in group housed TgCRND8 males it was necessary to house males individually at an age of about 90 days. As group housing is a major component of the environmental enrichment used in this study we decided to investigate only female mice of the TgCRND8 line.) These transgenic mice express a double mutant form of the human amyloid precursor protein (APP) 695 transgene (K670/M671L and V717F: 'Swedish' and 'Indiana' mutations) under regulation of the Syrian hamster prion promotor (PrP) on a hybrid C3H/HeJ–C57BL/6 strain background. Animals derived from our local stock of breeding pairs consisting of wild-type females and transgenic males. Genotypes were identified by PCR amplification of a DNA fragment within the PrP promoter [5]. Tissue samples were taken from the tail tip at 21 ± 1 days of life. At 30 days of age, animals were transferred to the experimental housing conditions.

We conducted two independent experiments: from a total of 41 female mice, we used in the tests for exploratory behaviour (Experiment I), 21 mice (9 transgenic and 12 wild-type) were housed in the standard housing condition (SH) and 20 mice (8 transgenic and 12 wild-type) in the enriched housing condition (EH).

The tests for learning and memory (Experiment II) were conducted with a total of 44 female mice, with 24 mice in the standard housing condition (10 transgenic and 14 wild-type) and 20 mice in the enriched housing condition (11 transgenic and 9 wild-type).

#### 2.1.2. Housing conditions

Female mice were housed in mixed genotype groups of 3–4 animals each in a standard laboratory cage (37 cm × 21 cm × 15 cm). All animals lived in a light/dark cycle of 12 h:12 h with lights on at 8 a.m. The cages of both housing conditions contained a thin layer of sawdust (Allspan, Karlsruhe, Germany). Commercial mouse diet (Altromin 1324, Lage, Germany) and bottled tap water were available *ad libitum*. The room temperature was maintained at 22 °C (±2), and humidity was 50 ± 10%. Cages were inspected daily but mice were handled only once a week while transferring them to clean cages.

The homecages of the environmentally enriched groups additionally contained a plastic inset, a wooden climbing frame and nesting material. For a detailed description, see [25].

In the dark phase the animals of the environmentally enriched group had the opportunity to explore an adjacent cage ('stimulus cage') that was connected by a Plexiglas tunnel. This cage contained a daily changing composition of different stimulus objects. For a detailed description, see [2,13].

The presented work complies with current regulations covering animal experimentation in Germany and the EU (European Communities Council Directive 86/609/EEC). All experiments were announced to the local authority and were approved by the 'Animal Welfare Officer' of the University of Muenster.

### 2.2. Behavioural investigations

#### 2.2.1. Exploratory behavioural parameters (Experiment I)

*Barrier test*: Spontaneous exploratory behaviour was measured at 140 ± 1 days of age by means of the barrier test. A standard cage (37 cm × 21 cm × 15 cm) was divided by a Plexiglas barrier (3 cm high and 0.5 cm wide) into two equally sized compartments. At the beginning of each test, the mouse was placed in one of the compartments according to a pseudo-random schedule, and the latency was measured until either the mouse climbed over the barrier (all four paws in the other compartment) or a maximum time of 5 min elapsed with the mouse staying in her half.

*Open-field test*: At day 141 ± 1 of age an open-field test was conducted. In this test, mice were placed into the centre of a square-shaped arena of 80 cm × 80 cm for 10 min. The arena was dimly lit (60 lx) by a bulb suspended above the centre of the maze to avoid any shadows. The animals' locomotor activity was measured using an automated tracking system [23]. After each trial the arena was cleaned with 70% ethanol.

*Elevated plus-maze test*: At day 142 ± 1 of age the elevated plus-maze has been carried out. In this test mice had the choice to explore two pairs of opposing arms, which were either shielded or open. The maze was elevated 50 cm above the floor and the arms were 30 cm long and 5 cm wide. The maze was dimly lit (60 lx) by a bulb suspended above the centre of the maze to avoid any shadows. At the beginning of each trial, mice were placed into the centre of the maze facing one of the open arms. Each entry into an open or closed arm was counted for 10 min by the automated tracking system [23]. Mice that refused to visit more than one arm were excluded from the analysis. After each trial the maze was cleaned with 70% ethanol.

#### 2.2.2. Learning and memory performance (Experiment II)

*Object recognition task*: The object recognition task is based on the spontaneous tendency of rodents to explore a novel object more often than a familiar one [7,8].

*Pre-training*: To avoid neophobic interference a habituation phase preceded the testing. Habituation comprised 5 consecutive days where mice were placed for 5 min into a circular open-field arena (50 cm in diameter) beginning on day 114 ± 1 of age. On the first 2 days of the handling phase the mice could explore freely the empty arena for five minutes followed by 3 days where the mice could explore the arena with one object inside. Finally, mice were subjected to a pretest following the protocol given below to habituate them to the testing procedure. The objects used for habituation were different from the test objects.

*Testing*: The object recognition task was conducted 1 day after the habituation phase and comprised two trials. During the first trial the animals could explore two identical objects while in the second trial the objects were replaced by a novel one and an identical copy of the two objects used in the first trial. Each trial lasted 5 min, with an intertrial interval of about 90 min.

Frequency and duration of object exploration was recorded using a palm-handheld computer (palmOne) with software for behavioural data recording (http://www.phenotyping.com/not.html). Exploration of an object was defined as directing the nose towards an object at a distance of less than a half head length and/or touching the object with the paws. Sitting on an object was not considered as exploratory behaviour [8]. Mice without any exploration behaviour towards the objects were excluded from the analysis of learning behaviour. Furthermore, a recognition index was calculated by dividing the amount of time spent exploring the novel object by the total time of object exploration during the second trial.

All objects were made of a biologically neutral material such as plastic or metal, and animals could not move them around in the arena. Objects were not known to have any ethological significance for the mice and they never had been associated with a reinforcer as suggested by [8]. To avoid object or place preferences, place and novelty-status for each object changed regularly. The test arena was cleaned with ethanol (70%) after each tested animal.

*Barnes maze test*: Spatial memory was measured at 128 ± 1 days of age by means of the Barnes maze test. This test takes advantages of the natural preference of rodents to avoid brightly lit, unenclosed surfaces and no strong aversive stimuli are needed [4]. The apparatus consisted of a brightly lit (180 lx) circular platform (100 cm diameter), elevated 120 cm above the floor, from which the mouse could escape into 1 of 12 holes (3 cm diameter), evenly spaced around the perimeter. The escape hole was connected via a wire-mesh tunnel to the homecage that was placed directly beneath the centre of the platform, not visible for the mouse on the platform. The other 11 holes on the platform lead to short wire tunnels that ended blind after 4 cm. By learning the spatial relationship between the escape hole and visual cues in the experimental room, the task can be performed successfully [30].

The mice performed two trials per day with a maximum time of 5 min over a period of 5 days. The escape hole remained constant for any given animal over the first 4 days of testing. The total number of errors and the path length was recorded by an automated tracking system [23]. An error was defined as searching a hole that did not lead to the escape tunnel. At day 5, the escape tunnel was switched to a different, randomly chosen hole as probe trials (probes 1 and 2) ensuring the acquisition of spatial navigation indicated by a higher percentage of time spent in the target area (1/6 of the platform) as it would have been expected by chance (about 16.67%). A trial started by placing the mouse in a grey cylinder (11 cm diameter; 20 cm high), which was positioned in the centre of the platform. After about 30 s the cylinder was lifted and the trial started. If the mouse did not enter the escape hole within 300 s, it was gently guided there by the experimenter. After each trial the platform was cleaned with 70% ethanol.

*Statistics*: Graphics presented and statistics carried out were done using the statistical software "R" Version 2.2.0 (R Development Core Team, 2005). Deviation from normal distribution was analyzed by one-sample Kolmogorov–Smirnov tests. Additionally, Levene's test for homogeneity of variance was calculated. Data of the barrier test and the elevated plus-maze test were analyzed using non-parametric statistics [34] since the data sets showed non-Gaussian distributions that could not be transformed. Non-parametric comparison of two samples was done using the two unpaired sample Mann–Whitney *U*-test. A Bonferroni correction was applied to cope for multiple comparisons of the same sample. Paired data from the object recognition task was analyzed using the paired Wilcoxon rank sum test.

Data of the open-field test, recognition indices in the object recognition task, and area under the curve in the Barnes maze test (trial 2 to day 4), was analyzed by ANOVA in a two by two factorial design with genotype and treatment as between subject factors. Subsequent post hoc analysis was conducted by Bonferroni-corrected *t*-tests. The Binominal test was used to analyze if mice spent significantly more time in the former right sixth during the probe trial. To analyze whether

the number of animals that did not explore in the object recognition task differed between groups was tested using Fisher's Exact Test for Count Data. All tests were analyzed two-tailed except for the analysis of novel object recognition because only a preference for the novel object was considered as being meaningful. A significance level ($\alpha$) of 0.05 was selected. For consistency the presentation of all graphs of unpaired data are given as box plots representing the 25–75th percentile and location measures are given as medians (50th percentile). The graphs for the paired data of the object recognition task are given as medians (bars) and single values (dots) connected by lines for each set of paired data in order to illustrate a maximum of information.

## 3. Results

### 3.1. Barrier test

The latency to climb over the barrier in the barrier test, as a measure of spontaneous exploration did not differ between the two genotypes regardless of the housing condition (Fig. 1). However, we could demonstrate an effect of housing conditions for both genotypes in the barrier test. Transgenic mice (tg) as well as wild-type mice (w) from standard housing condition were less prone to show spontaneous exploration in the barrier test (*U*-test, tg: $p < 0.01$, w: $p < 0.001$) than both transgenic and wild-type mice from the enriched housing condition (Fig. 1).

### 3.2. Open-field test

ANOVA revealed a significant effect of housing condition in the open-field test concerning the parameters path length ($F_{1,37} = 8.87$, $p < 0.01$; Fig. 2) and percent centre time ($F_{1,37} = 5.37$, $p < 0.05$) with enriched housed mice of both genotypes covering a greater distance and spending more time in the centre. No significant effect of genotype and no significant housing × genotype interaction were detectable. Post hoc comparisons of individual groups did not reveal significant differences when Bonferroni correction was applied.

### 3.3. Elevated plus-maze test

In the Elevated plus-maze test the proportion of open arm vs. total arm entries did not reveal a significant difference between transgenic mice and wild-type controls of standard housing conditions. Furthermore, no housing effect was detectable.

However, a genotype effect was found for mice of the enriched housing condition with transgenic mice showing a significantly higher proportion of open arm entries than wild-type mice (*U*-test, $p < 0.01$; Fig. 3).

### 3.4. Object recognition task

Wild-type mice of both housing conditions demonstrated object recognition memory by exploring the novel object significantly more than the familiar one (Wilcoxon-test, SH: $p < 0.001$, Fig. 4A; EH: $p < 0.05$, Fig. 4B).

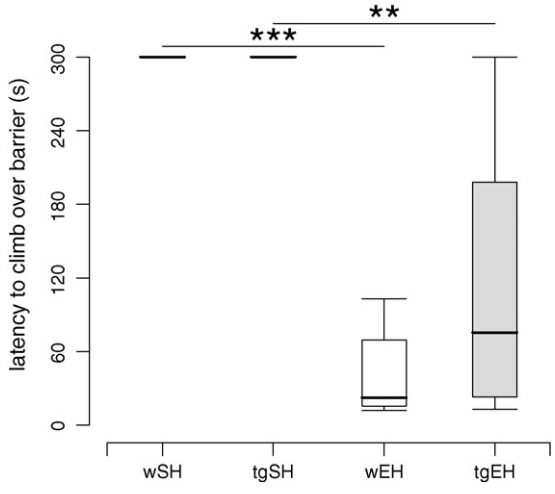


**Fig. 1.** Barrier test. Latencies to climb over a barrier are given as box plots. Each box represents the 25–75th percentile, and the horizontal line represents the median. Whisker lines extending below and above represent the extremes lying within 1.5 times the interquartile range (box height). Comparing effects of housing conditions with data separated according to genotypes (wSH: wild-type mice from standard housing condition, wEH: wild-type mice from enriched housing condition, tgSH: transgenic mice from standard housing condition, tgEH: transgenic mice from enriched housing condition). Statistics: *U*-test: $N_{wSH} = 12$, $N_{tgSH} = 9$, $N_{wEH} = 12$, $N_{tgEH} = 8$, $^{**}p < 0.01$, $^{***}p < 0.001$.

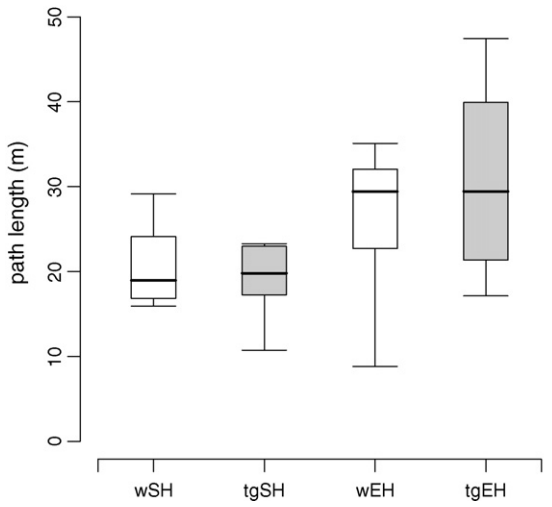


**Fig. 2.** Open-field test. Total path length is given as box plots (see Fig. 1). Comparing effects of housing conditions with data separated according to genotypes (wSH: wild-type mice from standard housing condition, wEH: wild-type mice from enriched housing condition, tgSH: transgenic mice from standard housing condition, tgEH: transgenic mice from enriched housing condition). Statistics: ANOVA: $N_{wSH} = 12$, $N_{tgSH} = 9$, $N_{wEH} = 12$, $N_{tgEH} = 8$, significant effect of housing condition ($p < 0.01$). Post hoc comparisons (Bonferroni-corrected *t*-tests): n.s.

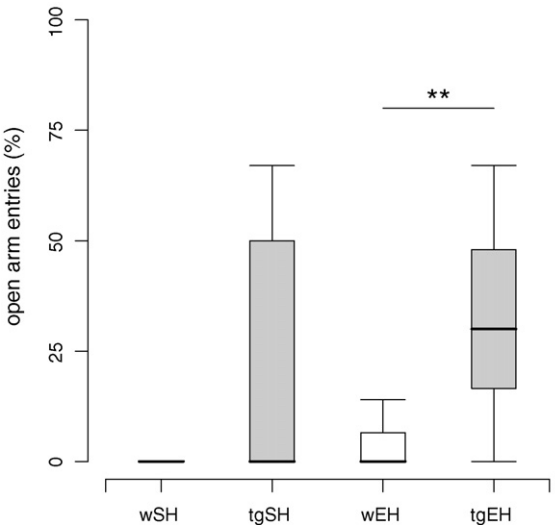


**Fig. 3.** Elevated plus-maze test. The proportion of open arm vs. total arm entries is given as box plots (see Fig. 1). Comparing effects of housing conditions with data separated according to genotypes (wSH: wild-type mice from standard housing condition, wEH: wild-type mice from enriched housing condition, tgSH: transgenic mice from standard housing condition, tgEH: transgenic mice from enriched housing condition). Statistics: *U*-test: $N_{wSH} = 9$, $N_{tgSH} = 9$, $N_{wEH} = 11$, $N_{tgEH} = 8$, $^{**}p < 0.01$.

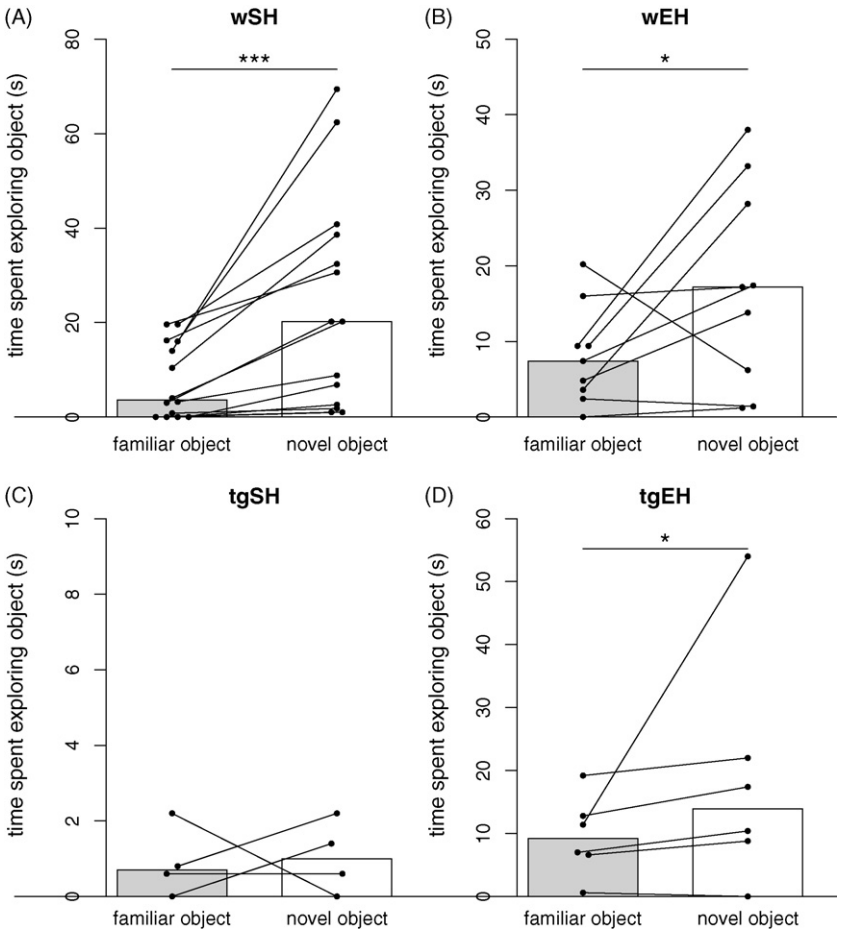


**Fig. 4.** (A–D) Object recognition task. Paired data of the exploration time of familiar and novel objects for wild-type (A) and transgenic mice (C) of standard housing conditions as well as for wild-type (B) and transgenic mice (D) of enriched housing conditions. Each bar represents the median and the dots represent the data of each animal with lines connecting paired values. Higher exploration of the novel object indicates the presence of an object recognition memory. Statistics: Wilcoxon-test: $N_{wSH} = 14$, $N_{tgSH} = 4$, $N_{wEH} = 9$, $N_{tgEH} = 6$, $^{***}p < 0.001$, $^{*}p < 0.05$.

In transgenic mice, 6 of 10 standard housed mice had to be excluded from the analysis because they did not even explore any of the two objects. Due to the reduced number of animals performing the task the Wilcoxon-test for paired data was not appropriate. In the group of enriched housed transgenic mice 5 of 11 mice had to be excluded from the analysis as well however the remaining animals explored the novel object significantly more than the familiar one (Wilcoxon-test: $p < 0.05$; Fig. 4D). In both housing conditions the number of transgenic mice that refused to explore objects was significantly higher compared to wild-types (Fisher's Exact Test for Count Data, SH: $p < 0.01$, EH: $p < 0.05$).

In order to compare the performance of different groups the recognition indices for each individual were calculated as time exploring the novel object divided by total exploration time. ANOVA revealed a significant genotype effect ($F_{1,29} = 4.38$, $p < 0.01$) with higher values in enriched housed mice but no effect of housing and no housing × genotype interaction. Post hoc comparisons of individual groups did not reveal significant differences when Bonferroni correction was applied.

### 3.5. Barnes maze test

In the Barnes maze test all four experimental groups showed a reduction in the median number of errors indicating acquisition of a spatial memory (Fig. 5). Spatial navigation and memory could be proofed for all experimental groups in the probe trial indicated by a higher percentage of time spent in the target area (1/6 of the platform) as it would have been expected by chance (about 16.67%, Binomial-test, wSH: $p < 0.05$; wEH: $p < 0.01$; tgSH: $p < 0.05$; tgEH: $p < 0.05$). Thus spatial learning was shown for transgenic and wild-type mice of both housing conditions in the Barnes maze.

Concerning the acquisition of spatial memory (measured as the areas under the learning curves from trial 2 to day 4), however, a highly significant effect of genotype was detected by ANOVA ($F_{1,40} = 21.44$, $p < 0.001$). Post hoc analysis revealed that transgenic mice of standard housing conditions tended to perform worse (tgSH: mean = 54.68; wSH: mean = 26.02; Bonferroni-corrected $t$-test, $p < 0.066$) and enriched housed transgenic mice performed significantly worse than wild-types (tgEH: mean = 63.05, wEH: mean = 19.5; Bonferroni-corrected $t$-test, $p < 0.01$).

ANOVA confirmed this genotype effect in the parameter path length (ANOVA, $F_{1,40} = 36.3$, $p < 0.001$) with transgenic mice of both housing conditions performing significantly worse than wild-types (Bonferroni-corrected $t$-test, SH: $p < 0.05$, EH: $p < 0.001$). ANOVAs revealed no significant effect of housing conditions and there was no interaction between genotype and housing condition in both parameters, path length and errors.

## 4. Discussion

Transgenic TgCRND8 mice that were housed in standard housing conditions performed worse than wild-type controls in both learn-

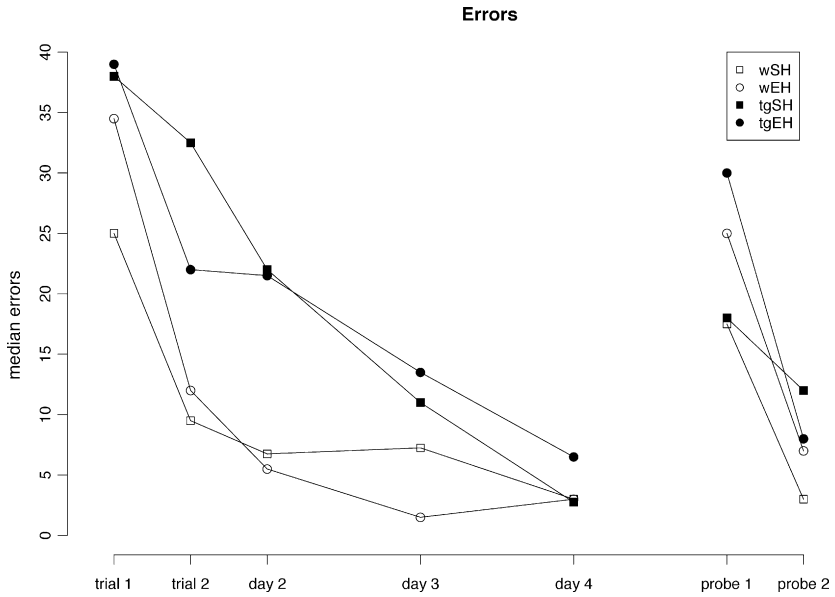


**Fig. 5.** Barnes maze. Learning curves for the parameter number of errors of the acquisition phase (trial 1 to day 4) and the probe trials 1 and 2 conducted on day 5. Data are given as medians.

ing tasks. Cognitive deficits were described earlier for this mouse model [5,14,17,19,20,24] and indicate the adequacy of TgCRND8 mice for studying AD. It could be shown that transgenic mice of the standard housing condition were statistically indistinguishable from wild-type controls concerning exploratory and locomotor behaviour. This is in line with other studies [14,36] and indicates that learning performance was not confounded by different levels of exploration in standard housed mice. The regime of enriched housing enhanced exploratory behaviour and locomotor activity in the barrier and open-field test. Additionally, in the elevated plus-maze enriched housed transgenic mice showed a higher proportion of open arm entries indicating reduced anxiety-like behaviour than wild-types of this housing condition. Environmental enrichment was not capable to convincingly compensate deficits of the transgenic mice in the Barnes maze tests as well as in the object recognition task as no significant housing effect was detectable. However, it is noteworthy that transgenic mice that were housed enriched showed sufficient exploratory behaviour to allow detection of object learning in contrast to transgenic mice that were housed in standard cages.

Studies regarding the effect of enriched housing on rodent models of brain disorders (for review, see [28]) have demonstrated that enriched housing delays behavioural symptoms and disease progression in mouse models of neurodegenerative diseases [9,22,37].

In a previous study of our group plaque burden was profoundly reduced due to environmental enrichment in female TgCRND8 mice [2]. Therefore it was hypothesized in the present study that environmental enrichment would also have a beneficial effect on learning and memory performance.

In this light our results were surprising as several other studies revealed a compensation of spatial memory deficits attributed to enrichment procedures in AD models. These effects were even found independently from the development of amyloid plaques and Aβ levels. Voluntary exercise in a running wheel enhanced the rate of spatial water maze learning and reduced plaque load and Aβ levels in female TgCRND8 mice [1]. The same was true for enriched housed PS1/PDAPP mice [6]. Despite stable Aβ deposition enhancement in water maze learning was also demonstrated in APPsw and APP23 mice which had access to complex large cages containing various objects [3,41]. What is more, Jankowsky et al. [15] reported an increased plaque formation in enriched housed female APP/PS1 mice, which nevertheless showed improved water maze performance.

In contrast to the present study using the Barnes maze to measure spatial memory the studies reviewed above applied the water maze. The escape to a secure base through a small hole in the Barnes maze test is probably reflecting spatial memory demands in the natural habitat of mice [12,39] whereas the escape from water most likely reflects an emergency situation. In stressful situations the secretion of epinephrine is elevated which leads to an increase in blood glucose levels. Epinephrine itself as well as moderate levels of glucose can enhance learning and memory function [26,27]. Thus the water maze possibly tests a kind of emergency learning that is maybe differentially affected by enriched housing conditions than learning in less stressful situations. Nevertheless, the differences between genotypes that were demonstrated in this study lead to the assumption that the Barnes maze test is indeed a suitable task to measure spatial memory performance. Further investigations have to clarify why improvements of genetically predetermined deficits of spatial memory can be revealed in the water maze but not in the Barnes maze in TgCRND8 mice.

In the object recognition task considerable effort of pre-training with a handling and a habituation phase was conducted to minimize possible interference of neophobic effects due to the testing situation. Wild-type mice of both housing conditions discriminated between novel and familiar object in this non-spatial learning task indicating the suitability of the test procedure. Nevertheless, in transgenic mice, significantly higher numbers of mice did not even investigate any of the two objects compared to wild-types. This was striking and one might assume neophobic-like behaviour against the objects [31]. In contrast transgenic mice were rather less anxious in the elevated plus-maze test conducted in the first experiment. Thus, TgCRND8 transgenic mice might exhibit an unbalanced ratio of these different types of anxiety. With regard to learning and memory those standard housed mice that showed sufficient exploration did not consistently discriminate between the novel and the familiar object. Transgenic mice that were housed enriched and showed an adequate amount of exploration were able to differentiate between novel objects and the familiar objects. We can,

however, not discriminate whether the object learning was due to an increased exploration and reduced anxiety of enriched housed mice or due to an amelioration of memory deficits.

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## References

[1] Adlard PA, Perreau VM, Pop V, Cotman CW. Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. J Neurosci 2005;25:4217–21.
[2] Ambrée O, Leimer U, Herring A, Görtz N, Sachser N, Heneka MT, et al. Reduction of amyloid angiopathy and Abeta plaque burden after enriched housing in TgCRND8 mice: involvement of multiple pathways. Am J Pathol 2006;169:544–52.
[3] Arendash GW, Garcia MF, Costa DA, Cracchiolo JR, Wefes IM, Potter H. Environmental enrichment improves cognition in aged Alzheimer's transgenic mice despite stable beta-amyloid deposition. Neuroreport 2004;15:1751–4.
[4] Barnes CA. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J Comp Physiol Psychol 1979;93:74–104.
[5] Chishti MA, Yang D-S, Janus C, Phinney AL, Horne P, Pearson J, et al. Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. J Biol Chem 2001;276:21562–70.
[6] Costa DA, Cracchiolo JR, Bachstetter AD, Hughes TF, Bales KR, Paul SM, et al. Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms. Neurobiol Aging 2007;28:831–44.
[7] Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats: behavioural data. Behav Brain Res 1988;31:47–59.
[8] Ennaceur A, Michalikova S, Bradford A, Ahmed S. Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. Behav Brain Res 2005;159:247–66.
[9] Faherty CJ, Raviie Shepherd K, Herasimtschuk A, Smeyne RJ. Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism. Brain Res Mol Brain Res 2005;134:170–9.
[10] Frick KM, Fernandez SM. Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice. Neurobiol Aging 2003;24:615–26.
[11] Friedland RP, Fritsch T, Smyth KA, Koss E, Lerner AJ, Chen CH, et al. Patients with Alzheimer's disease have reduced activities in midlife compared with healthy control-group members. Proc Natl Acad Sci USA 2001;98:3440–5.
[12] Gerlai R, Clayton NS. Analysing hippocampal function in transgenic mice: an ethological perspective. Trends Neurosci 1999;22:47–51.
[13] Herring A, Yasin H, Ambrée O, Sachser N, Paulus W, Keyvani K. Environmental enrichment counteracts Alzheimer's neurovascular dysfunction in TgCRND8 mice. Brain Pathol 2008;18:32–9.
[14] Hyde LA, Kazdoba TM, Grilli M, Lozza G, Brusa R, Zhang Q, et al. Age-progressing cognitive impairments and neuropathology in transgenic CRND8 mice. Behav Brain Res 2005;160:344–55.
[15] Jankowsky JL, Melnikova T, Fadale DJ, Xu GM, Slunt HH, Gonzales V, et al. Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. J Neurosci 2005;25:5217–24.
[16] Jankowsky JL, Xu G, Fromholt D, Gonzales V, Borchelt DR. Environmental enrichment exacerbates amyloid plaque formation in a transgenic mouse model of Alzheimer disease. J Neuropathol Exp Neurol 2003;62:1220–7.
[17] Janus C. Search strategies used by APP transgenic mice during navigation in the Morris water maze. Learn Mem 2004;11:337–46.
[18] Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 2000;408:979–82.
[19] Janus C, Welzl H, Hanna A, Lovasic L, Lane N, St George-Hyslop P, et al. Impaired conditioned taste aversion learning in APP transgenic mice. Neurobiol Aging 2004;25:1213–9.
[20] Janus C, Westaway D. Transgenic mouse models of Alzheimer's disease. Physiol Behav 2001;73:873–86.
[21] Lazarov O, Robinson J, Tang Y-P, Hairston IS, Korade-Mirnics Z, Lee VM, et al. Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. Cell 2005;120:701–13.
[22] Lazic SE, Grote HE, Blakemore C, Hannan AJ, van Dellen A, Phillips W, et al. Neurogenesis in the R6/1 transgenic mouse model of Huntington's disease: effects of environmental enrichment. Eur J Neurosci 2006;23:1829–38.
[23] Lewejohann, L. Digital image processing in behavioral sciences; 2004. Available at: http://www.phenotyping.com/digital.html; accessed 11/26/2007.
[24] Lovasic L, Bauschke H, Janus C. Working memory impairment in a transgenic amyloid precursor protein TgCRND8 mouse model of Alzheimer's disease. Genes Brain Behav 2005;4:197–208.
[25] Marashi V, Barnekow A, Sachser N. Effects of environmental enrichment on males of a docile inbred strain of mice. Physiol Behav 2004;82:765–76.
[26] McGaugh JL. Involvement of hormonal and neuromodulatory systems in the regulation of memory storage. Annu Rev Neurosci 1989;12:255–87.
[27] Messier C. Glucose improvement of memory: a review. Eur J Pharmacol 2004;490:33–57.
[28] Nithianantharajah J, Hannan AJ. Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat Rev Neurosci 2006;7:697–709.
[29] Olsson IAS, Dahlborn K. Improving housing conditions for laboratory mice: a review of "environmental enrichment". Lab Anim 2002;36:243–70.
[30] Pompl PN, Mullan MJ, Bjugstad K, Arendash GW. Adaptation of the circular platform spatial memory task for mice: use in detecting cognitive impairment in the APP(SW) transgenic mouse model for Alzheimer's disease. J Neurosci Methods 1999;87:87–95.
[31] Powell SB, Geyer MA, Gallagher D, Paulus MP. The balance between approach and avoidance behaviors in a novel object exploration paradigm in mice. Behav Brain Res 2004;152:341–9.
[32] Rampon C, Jiang CH, Dong H, Tang YP, Lockhart DJ, Schultz PG, et al. Effects of environmental enrichment on gene expression in the brain. Proc Natl Acad Sci USA 2000;97:12880–4.
[33] Rampon C, Tsien JZ. Genetic analysis of learning behavior-induced structural plasticity. Hippocampus 2000;10:605–9.
[34] Siegel S. Nonparametric statistics for the behavioral sciences. New York: McGraw-Hill; 1956.
[35] Tang Y-P, Wang H, Feng R, Kyin M, Tsien JZ. Differential effects of enrichment on learning and memory function in NR2B transgenic mice. Neuropharmacology 2001;41:779–90.
[36] Touma C, Ambrée O, Görtz N, Keyvani K, Lewejohann L, Palme R, et al. Age- and sex-dependent development of adrenocortical hyperactivity in a transgenic mouse model of Alzheimer's disease. Neurobiol Aging 2004;25:893–904.
[37] van Dellen A, Blakemore C, Deacon R, York D, Hannan AJ. Delaying the onset of Huntington's in mice. Nature 2000;404:721–2.
[38] van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 1999;2:266–70.
[39] Whishaw IQ, Tomie JA. Of mice and mazes: similarities between mice and rats on dry land but not water mazes. Physiol Behav 1996;60:1191–7.
[40] Wilson RS, Mendes De Leon CF, Barnes LL, Schneider JA, Bienias JL, Evans DA, et al. Participation in cognitively stimulating activities and risk of incident Alzheimer disease. JAMA 2002;287:742–8.
[41] Wolf SA, Kronenberg G, Lehmann K, Blankenship A, Overall R, Staufenbiel M, et al. Cognitive and physical activity differently modulate disease progression in the amyloid precursor protein (APP)-23 model of Alzheimer's disease. Biol Psychiatry 2006;60:1314–23.