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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