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ScienceDirect
Behavioural Brain Research 190 (2008) 74–84
BEHAVIOURAL BRAIN RESEARCH
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Research report

Wheel-running in a transgenic mouse model of Alzheimer's disease: Protection or symptom?

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Received 11 December 2007; received in revised form 31 January 2008; accepted 4 February 2008
Available online 8 February 2008

Abstract

Several studies on both humans and animals reveal benefits of physical exercise on brain function and health. A previous study on TgCRND8 mice, a transgenic model of Alzheimer's disease, reported beneficial effects of premorbid onset of long-term access to a running wheel on spatial learning and plaque deposition. Our study investigated the effects of access to a running wheel after the onset of Aβ pathology on behavioural, endocrinological, and neuropathological parameters. From day 80 of age, the time when Aβ deposition becomes apparent, TgCRND8 and wildtype mice were kept with or without running wheel. Home cage behaviour was analysed and cognitive abilities regarding object recognition memory and spatial learning in the Barnes maze were assessed. Our results show that, in comparison to Wt mice, Tg mice were characterised by impaired object recognition memory and spatial learning, increased glucocorticoid levels, hyperactivity in the home cage and high levels of stereotypic behaviour. Access to a running wheel had no effects on cognitive or neuropathological parameters, but reduced the amount of stereotypic behaviour in transgenics significantly. Furthermore, wheel-running was inversely correlated with stereotypic behaviour, suggesting that wheel-running may have stereotypic qualities. In addition, wheel-running positively correlated with plaque burden. Thus, in a phase when plaques are already present in the brain, it may be symptomatic of brain pathology, rather than protective. Whether or not access to a running wheel has beneficial effects on Alzheimer-like pathology and symptoms may therefore strongly depend on the exact time when the wheel is provided during development of the disease.

Keywords: Running wheel; Physical exercise; Stereotypic behaviour; Object recognition memory; Spatial learning; TgCRND8 mice; Alzheimer's disease; Adrenocortical activity

1. Introduction

Physical exercise has benefits for overall brain health, brain plasticity and cognitive function in both humans and animals [11,15,51]. It is known to prevent or ameliorate the effects of a variety of diseases, including cardiovascular disease, some forms of diabetes, cancer types and also Alzheimer's disease [11,19,26]. Clinical data are supported by animal research analysing the relationship between wheel-running and brain function in mice. Physical exercise was found to increase neurogenesis in the hippocampus, improve learning abilities and lead to a regional increase in the number of newly generated cortical microglia [15,51]. Moreover, synaptic plasticity, neurotransmission, and growth factor gene expression are increased in the hippocampus of wheel-running rodents [11]. Altogether, beneficial effects of voluntary physical exercise on general brain health and function are widely accepted.

The effect of wheel-running itself on cognitive function and pathology in transgenic mouse models of Alzheimer's disease (AD) remains controversial. While wheel-running reduced

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doi:10.1016/j.bbr.2008.02.005

amyloid load and improved water maze learning in TgCRND8 mice [1], APP23 mice with a running wheel did not differ from standard housed controls in spatial learning, plaque load, and hippocampal neurogenesis [55]. These conflicting findings might be caused by differences in the onset of access to a running wheel as well as the duration of access to the wheel. TgCRND8 mice had access to a running wheel for a period of 5 months, starting when the mice were 4 weeks of age [1], while APP23 mice started running at the age of 10 weeks and were tested when 11 months old [55]. The findings in TgCRND8 mice might thus provide a picture of young-adults with access to a wheel at a presymptomatic stage of development [1], while those in APP23 mice might reflect the situation after the onset of symptoms [55]. To further investigate this hypothesis, we decided to study the effects of three months of access to a running wheel starting at about the onset of pathology in TgCRND8 mice.

TgCRND8 mice carrying human $APP_{Swe+Ind}$ are known to develop several characteristics similar to symptoms observed in Alzheimer's patients. They are characterised by altered activity patterns and hyperactivity [2], cognitive deficits in spatial memory performance [9], age- and sex-dependent hyperactivity of the hypothalamic pituitary adrenocortical (HPA) axis [48], and amyloid deposition present in mice at about 80 days of age [9], while general health state, gross sensory functions, reflexes, and motor abilities were not found to differ between wildtype and transgenic mice of this model [48]. Additionally, a marked feature of TgCRND8 mice is spontaneous stereotypic behaviour [2], which is regarded to be an analogue to non-cognitive symptoms such as wandering, restlessness or disturbed sleep–wake patterns in Alzheimer's patients [2,18,29]. Stereotypies may be causally related to brain damage, which is discussed to be one reason for the occurrence of stereotypic behaviour [44].

Since access to a running wheel was shown not only to have beneficial effects on brain parameters but also to reduce stereotypic behaviour [36], the aim of this study was to examine the effects of access to a running wheel on behavioural, cognitive, endocrinological, and neuropathological parameters in TgCRND8 mice. Wheels were provided at about the time when plaque deposition starts to develop in order to elucidate whether or not a running wheel in this phase has beneficial effects on Alzheimer-related symptoms and pathology. Home cage behaviour with a focus on stereotypic behaviour and activity patterns, as well as faecal corticosterone metabolite concentrations were analysed. In addition, cognitive abilities regarding object recognition memory and spatial learning in the Barnes maze were assessed, and amyloid deposition was quantified.

2. Methods

2.1. Animals and housing conditions

This study was carried out with male mice of the TgCRND8 line, a transgenic murine model of Alzheimer's disease. These mice carry a double mutated form of the amyloid precursor protein 695 (APP), the 'Swedish' (K670N/M671L) and 'Indiana' (V717F) mutations, on a hybrid C57Bl/6-C3H/HeJ genetic background (for further details see [9]).

The animals derived from the internal breed of the Department of Behavioural Biology, University of Münster, Germany. Breeding was carried out with pairs of transgenic (Tg, APP +/−) males and wildtype (Wt, APP −/−) females. In total, 54 male mice, 31 Wt and 23 Tg animals, were investigated. After weaning at 21 ± 1 days of age, a tissue sample for genotyping was taken from the tail and mice were housed in unisex groups of 2–5 littermates per cage. At the age of 80 days, the mice were transferred to the experimental housing conditions, where they were housed individually in transparent standard polycarbonate cages (Macrolon, type III, 38 cm × 22 cm × 15 cm) with sawdust (Allspan, Höveler GmbH & Co. KG, Langenfeld, Germany) as bedding and tissue as nesting material (referred to as 'standard housing', SH). Single housing conditions were chosen to avoid aggression among group-housed male mice, which might influence both activity and stress hormone levels, and to determine locomotor activity in the running wheel individually. Out of the 54 mice, 27 subjects, 15 Wt and 12 Tg mice, had free access to a running wheel (RW). The control group (standard housing without running wheel: SH) consisted of 16 Wt and 11 Tg mice. The running wheel was 11.5 cm in diameter with a 5.0 cm-wide running surface. Each running wheel was interfaced with a magnetic sensor and a bicycle computer attached to the outside of the cage. The bicycle computer was activated by wheel rotation and converted the number of wheel rotations into running distance (360 mm/rotation). Wheels were checked on a daily basis to ensure that they turned freely, and running distance was read off twice a week. Seven Wt and four Tg mice did not run in the wheel (=run less than 4 m/day) and are referred to as non-runners in the following. Instead of running in the wheel, these animals were observed to use the wheel as climbing structure and/or nest site, which prevents the wheel from turning freely. To distinguish between the effects of wheel-running and the effects of access to a running wheel, data were analysed twice, including or not including non-runners, and presented separately in the results part.

All mice had free access to commercial mouse diet (Altromin no. 1324, Altromin GmbH, Lage, Germany) and bottled tap water. A photoperiod of 12 h light and 12 h dark, lights on at 1 a.m., temperature of 22 ± 2 °C, and relative humidity of 45 ± 10% were maintained in the animal housing room.

Experiments were approved by the local animal care and use committee as well as by the animal welfare officer of the University of Muenster. All efforts were made to minimise the number and suffering of animals in these experiments.

2.2. Procedures

2.2.1. Behavioural analysis

At 101 ± 3 days of age, a 3-week testing period started. In the first week, mice were handled daily to increase familiarity with the experimenter. Handling consisted of lifting the mouse by the tail and placing it in a separate "handling cage", similar to the home cage, for 5 min before placing it back in the home cage. The procedure was repeated twice daily on five consecutive days. Thereafter, mice were tested in the open field arena over a 3-day period, followed by 2 days of object recognition test. At 113 ± 3 days of age, spontaneous behaviour in the home cage was assessed from a 24 h video recording. The testing period was completed by 5 days of testing on the Barnes maze to assess spatial learning performance. Four weeks later, the mice were retested on the Barnes maze to assess long-term memory. All tests were carried out in a separate testing room.

Within the scope of another study all animals were intraperitoneally injected with physiological saline solution (5 μl/g) about 1 h before the learning tests started and with BrdU 4 h later to assess neurogenesis (for a later study, not part of this article).

2.2.1.1. Object recognition test. The one-trial object recognition paradigm utilizes the innate tendency of mice to prefer novel over familiar objects. The test measures spontaneous behaviour, and, thus, requires no lengthy training or preparation. It has now become a powerful tool in neurogenetical memory research, although the neural basis of this test is far from being understood [13].

The animals were exposed to the open field (30 cm × 30 cm plywood box, with walls 40 cm high) for 10 min daily on three consecutive days, followed by 2 days of object recognition test (day 1, D1; day 2, D2). On each of the 2 days, the same procedure was conducted, starting with a 10 min exploration phase followed by a 10 min choice phase, with 60 ± 5 min in-between [16]. During the exploration phase, there were two identical objects in the arena. The mouse was placed in the centre of the box between the two objects that were located in the middle of, and in direct contact with, two opposite side walls. For the

choice phase, one object was replaced by a novel object and the other by an identical copy of itself to avoid odour cues. The objects to be discriminated in the object recognition test were made of biologically neutral material such as plastic or metal, and animals could not move them around in the arena. None of them is known to have any ethological significance for the mice or has ever been associated with a reinforcer [17]. To avoid place preferences, the position of the novel object changed in regular intervals. Furthermore, each object was used as known and as novel object to avoid measuring an object preference instead of novelty-exploration. Faecal boli were removed, and the walls and the floor of the open field arena were cleaned with ethanol (70%) after each tested animal. Open field and object recognition tests were conducted during the activity peak of the mice at 2 p.m., in the dark phase.

For the object recognition test a palm-handheld computer (PalmOne, Zire^{TM} 31) with software for behavioural data recording (www.phenotyping.com/not.html) was used for data collection. The time spent exploring each object was determined, whereby exploration of an object was defined as directing the nose towards the object at a distance of less than one head length and/or touching the object with the nose and/or paws. Sitting on top of the object was not considered as exploratory behaviour [17]. Mice with a total exploration time of less than 5 s in either the exploration phase or the choice phase were excluded from the analysis of learning behaviour on that test day. This resulted in following group sizes: Wt SH: 14, Wt RW: 15, Tg SH: 10 (day 1), 11 (day 2), Tg RW: 12.

2.2.1.2. Spontaneous behaviour. The spontaneous behaviour was recorded in home cages at 113 ± 3 days of age, using a 24-h-time lapse video recorder (Panasonic AG6730). Four animals were recorded simultaneously by a light sensitive (0.5 lux) B/W CCD camera through the long sidewall of the cage. A 40 W red light provided illumination for video recording during the dark period. For behavioural analysis, individual data on activity patterns and stereotypies were collected by focal animal sampling and continuous recording [31]. The entire 24 h of each recording was analysed using 'The Observer Video Pro 4.0' (Noldus). If more than four animals had to be observed on the same day, the four test animals were randomly chosen. Frequency and duration of all bouts of the following behaviours were recorded for 41 males, 23 Wt (11 without and 12 with running wheel) and 18 Tg (9 without and 9 with running wheel) mice:

Activity patterns

Resting: Beginning with a bout of motionless lying for at least 10 s, ending when the animal moved at least one body length away. Changes of the resting position and intermediate bouts of grooming were considered as resting as long as the animal did not leave the resting place.

Activity: All behaviours other than *Resting*.

Stereotypies

Jumping: A bout of repetitive jumping up and down or scratching with the paws along the cage wall.

Climbing: A bout of repetitive movement in a straight or circular direction at the cage lid.

Digging: A bout of repetitive scratching the substrate with the forelegs in one corner of the cage and/or kicking out with the hind legs.

Bar-chewing: A bout of repetitive gnawing/chewing at any bars of the cage lid.

Circling: A bout of repetitive movement in a circular direction on the ground.

Looping: A bout of repetitive movement in a circular motion between the ground and the cage lid, i.e. jumping up to the cage lid, climbing at the cage lid in a straight direction, running back to the start place on the ground and repeating this movement in a continuous fashion.

Hanging: A bout of repetitive movement, starting with the mouse biting into the bars of the cage lid at a particular spot and holding on to it, thereby scratching with the paws along the wall.

Wheel-running activity

The mouse runs either inside the running wheel or outside, balanced on its tail and/or hindpaws.

Definitions were adopted from Ambrée et al. [2], Wiedenmayer [54] and Würbel et al. [56]. Resting behaviour was only recorded if bout length exceeded 60 s. Stereotypic behaviour is defined as repeated and invariant movements without any goal or function [32,33]. To ensure the repetitive character of the recorded stereotypies, stereotypic behaviours were only recorded if bout length exceeded 10 s. Intervals between two consecutive bouts of stereotypic behaviour were defined to last for a minimum of 5 s. For the statistical analysis, the six stereotypic behaviour patterns were summed to form the category *Stereotypies*. In order to normalise for general activity differences between Wt and Tg mice, the total amount of stereotypic behaviour was divided by the total activity per day.

Behavioural observations started at 11 a.m., 15 min after placing the animals in test cages identical to their home cages containing fresh bedding material and the home-cage running wheel for mice of the RW groups. Providing clean cages was necessary for the collection of faeces deposited during the 24 h video observations (see Section 2.2.2).

2.2.1.3. Barnes maze test. The Barnes maze [7] consisted of a white circular plywood disk of 100 cm in diameter. Twelve holes (3 cm diameter) were located at equal distance to each other, 13 mm from the edge. A wire-mesh tunnel connected one randomly chosen hole via a special cage lid with the home cage of the tested animal. The other holes were closed by blind wire-mesh tubes. The maze was elevated 125 cm above the floor to prevent the animals from jumping down. The platform was illuminated by a 100 W electric bulb located 110 cm above the centre of the maze providing an illumination of 180 lux to motivate the animals to escape into the hole.

Beginning at 115 ± 3 days of age, all animals performed two trials per day on four consecutive days (T1–T8). On day 5, a probe trial (T9) was completed that was repeated 4 weeks later to test long-term retrieval (T10). At the beginning of each trial the animals were placed inside a dark gray start cylinder that was lifted after about 30 s to start the trial. If the animals did not enter the correct hole within 300 s they were gently forced to enter it and go back to their home cage. During the probe trial all holes were closed. After 300 s the formerly correct hole was opened to let the animals go back to their home cage. All trials were tracked automatically by a digital tracking system (www.phenotyping.com/digital.html) assessing *path-length*, *latency to entering the correct hole* as well as *time spent in the correct sixth of the maze*. After each trial the platform was thoroughly cleaned with 70% ethanol. Barnes maze tests were conducted at 9 a.m., at the end of the light phase. Two animals of the Wt RW group died before the Barnes maze test. The resulting sample sizes for the acquisition phase were 16 Wt SH, 13 Wt RW, 11 Tg SH, and 12 Tg RW. Since two Tg animals died after the acquisition phase, but before T10 (4 weeks after acquisition), and some animals jumped or fell from the platform, sample sizes of the final probe trial were 14 Wt SH, 12 Wt RW, 9 Tg SH, and 10 Tg RW.

2.2.2. Corticosterone metabolites in faecal samples

All faeces voided during the 24 h video observation were collected and frozen at −30° C until they were assayed for corticosterone metabolites (corticosterone is the major glucocorticoid in mice [39]). Faeces of animals whose behaviour could not be recorded because recordings were limited to four animals at a time were also collected. The animals were treated in the same way as the animals whose behaviour was recorded. The collected faecal samples were analysed for immunoreactive corticosterone metabolites (CM) using a 5α-pregnane-3β,11β,21-triol-20-one enzyme-immunoassay (EIA). Details regarding development, biochemical characteristics and physiological validation of this assay have been described previously [46,47]. Before EIA analysis, the faecal samples were dried (2 h at 80 °C), homogenized and aliquots of 0.05 g were extracted with 1 ml of 80% methanol. A detailed description of assay performance has been published [46]. The intra- and interassay coefficients of variation were 9.1 and 14.0%, respectively.

2.2.3. Neuropathological analysis

Aβ plaque burden was quantified for 10 mice of the SH and 11 mice of the RW condition, because 2 mice did not reach the age of 150 days.

Animals were deeply anesthetized and transcardially perfused with 0.9% saline. The brains were removed and bisected in the mid-sagittal plane. One hemisphere was fixed overnight in 4% buffered paraformaldehyde and transferred to 30% sucrose/PBS (not used in this study). The other hemisphere was cut longitudinally into two segments; the medial segment was fixed overnight in 4% buffered formaldehyde, followed by paraffin-embedding, and used for Aβ-immunohistochemistry, whereas the lateral segment was snap-frozen and stored at −80 °C (not used in this study).

For Aβ staining three pairs of 2 μm sagittal brain sections of each transgenic animal were pretreated with formic acid and automatically stained in a TechMate Instrument (DakoCytomation, Hamburg, Germany) with 6F/3D anti-Aβ monoclonal antibody to residues 8–17 (1:100, Dako, Hamburg, Germany) followed by the Dako StreptABC complex-horseradish peroxidase conjugated "Duet" anti mouse/rabbit antibody kit and development with 3,3′-diaminobenzidine (DAB). Counterstaining was performed with hematoxylin. The pairs of sections (10 μm distance) were situated between 100 and 300 μm lateral from the mid-sagittal fissure. Each staining was performed in two consecutive procedures making sure that brains of both experimental groups were equally distributed in all procedures. To quantify Aβ plaque burden, neocortices and hippocampi of all stained sections were digitized (Olympus BX50, ColorView II, CCD camera, Olympus, Hamburg, Germany) under constant light and filter settings. Morphometry was conducted by using analySIS 5 software (Soft Imaging System, Münster, Germany). Colour images were converted to grayscale by extracting blue to gray values to obtain best contrast between positive immunoreactivity and background. A constant threshold was chosen for all images to detect immunoreactive staining. Plaque number, size and total area were determined automatically in total neocortex and hippocampus. Absolute values of plaque burden were related to the investigated area (compare [3]).

2.3. Statistical analysis

All data sets were checked for normal distribution by descriptive analysis of the histogram, skewness and kurtosis as well as by Kolmogorov–Smirnov and Shapiro–Wilk test. When necessary, raw data were square-root-, or log-transformed and analysed using a two-way ANOVA with genotype and housing as between subject factors. To establish group differences, a standard post-hoc test (Bonferroni) was applied. Two independent samples were compared with the *t*-test.

For data sets that were not normally distributed and could not be transformed into normally distributed data (exploration time in the object recognition test, average daily running distance, wheel-running during 24 h video observation) non-parametric tests were conducted [38]. Two independent samples were compared using the Mann–Whitney *U*-test. Differences between two paired samples (exploration time of the known versus the novel object in the object recognition task) were assessed using the Wilcoxon-test. To evaluate the strength of association between two variables, Spearman's rank correlation coefficient was calculated. For descriptive analysis of the Barnes maze, the first two trials on the first test day (T1, T2) were depicted separately, while at the following test days (D2–D4) the two trials were averaged to obtain a clear learning curve. For analysis of memory acquisition the area under the curve from T2 to D4 was calculated for each animal and group comparison performed as described above. The Binomial test was used to analyse probe trial performance. All tests were applied two-tailed except for the learning and memory tasks. Tests were calculated using the software package SPSS (version 12.0 for Windows). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Spontaneous behaviour in the home cage

3.1.1. Activity

Activity patterns of Tg mice animals deviated from what is normally described for laboratory mice and what was found in Wt mice. Tg mice were active throughout the complete dark phase and were characterised by an additional activity peak at the end of the light phase, while Wt mice were characterised by low activity levels throughout the complete light phase [for further information on activity changes in TgCRND8 mice see reference 2].

The ANOVA showed a general genotype effect on total activity in the home cage (two-way ANOVA, $F_{1,37} = 92.485$, $p < 0.001$). Further post-hoc analysis revealed that Tg SH mice

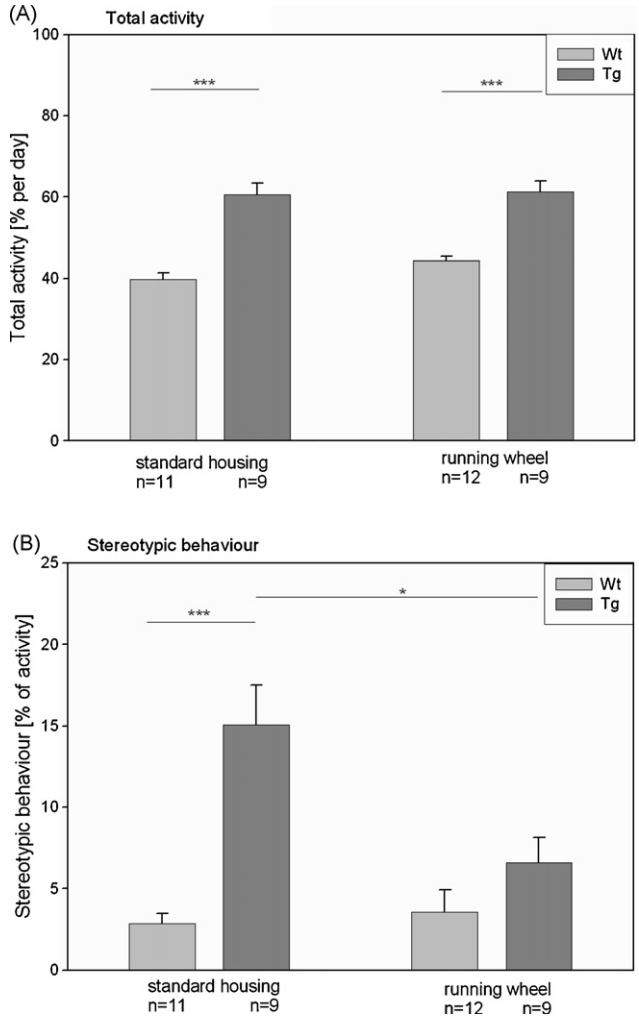

Fig. 1. Spontaneous behaviour in the home cage. (A) The daily activity levels of wildtype (Wt) and transgenic (Tg) mice of both housing conditions. (B) The amount of stereotypic behaviour related to the daily activity. Data are presented as means + S.E.M. Statistics: two-way ANOVA (genotype, housing), Bonferroni post-hoc test, $*p \leq 0.05$, $***p \leq 0.001$.

displayed higher total activity levels than Wt SH mice (Bonferroni post-hoc test, $p < 0.001$, Fig. 1A). Tg animals were also more active comparing the running wheel groups (Bonferroni post-hoc test, $p < 0.001$, Fig. 1A). Contrarily, there was no effect of housing condition (two-way ANOVA, $F_{1,37} = 1.807$, ns) or of genotype by housing (two-way ANOVA, $F_{1,37} = 0.973$, ns) on total activity in the home cage. Exclusion of non-runners from the analysis did not influence these findings.

3.1.2. Stereotypies

Out of the 41 observed mice, 39 animals performed some kind of stereotypic behaviour. Most commonly performed stereotypies were *jumping*, *climbing* and *bar-chewing*. Other stereotypies like *hanging, circling* or *looping* were limited to one or two individuals. Whereas nearly all stereotypy forms occurred in both Wt and Tg mice, only Tg mice performed *jumping*.

Concerning the total amount of stereotypic behaviour, a significant main effect of genotype was found (two-way ANOVA, $F_{1,37} = 27.340$, $p < 0.001$). The ANOVA revealed also a main

effect of housing (two-way ANOVA, $F_{1,37} = 5.215$, $p = 0.028$) and an interaction effect of genotype and housing (two-way ANOVA, $F_{1,37} = 5.378$, $p = 0.026$).

Further pair-wise comparisons revealed that Tg mice in standard housing conditions performed more stereotypic behaviour than Wt SH mice (Bonferroni post-hoc test, $p < 0.001$, Fig. 1B). In animals housed with RW, there was no difference in the amount of stereotypic behaviour between Wt and Tg mice (Bonferroni post-hoc test, $p = 0.269$, ns). Regarding the comparison of the housing condition, Wt mice housed with and without running wheel did not differ in the amount of stereotypic behaviour (Bonferroni post-hoc test, $p = 0.999$, ns), while Tg SH mice showed more stereotypies than Tg RW mice (Bonferroni post-hoc test, $p = 0.024$). Exclusion of non-runners from the analysis did not influence these findings.

3.1.3. Wheel-running

Due to the great variability in wheel-running activity in both, Tg and Wt mice, they did not differ in wheel-running activity, neither in average daily running distance (Mann–Whitney *U*-test; $U = 82$, $p = 0.716$), nor in running activity during the 24 h video observation (Mann–Whitney *U*-test; $U = 51$, $p = 0.862$). The mean daily running distance was 1.40 km/day for Wt mice and 1.35 km/day for Tg mice. It ranged from 0.00 to 5.39 km/day.

Tg mice reached their plateau running distance after approximately 2 weeks. From the second week onwards, daily wheel-running activity remained more or less stable throughout the complete observation period. Contrarily, Wt mice increased their average running distance during 8 weeks until they reached their plateau running distance. However, no significant differences in wheel-running activity over time were found between Wt and Tg mice (*U*-test, $38 \leq U \leq 64$, ns, Fig. 2).

In Tg mice a significant negative correlation between wheel-running activity and stereotypic behaviour during the 24 h video observation was found (Spearman's rank correlation; $r_s = -0.705$, $p = 0.034$, Fig. 3). In Wt mice there was no significant relationship between wheel-running and stereotypies. (Spearman's rank correlation; $r_s = -0.377$; $p = 0.227$).

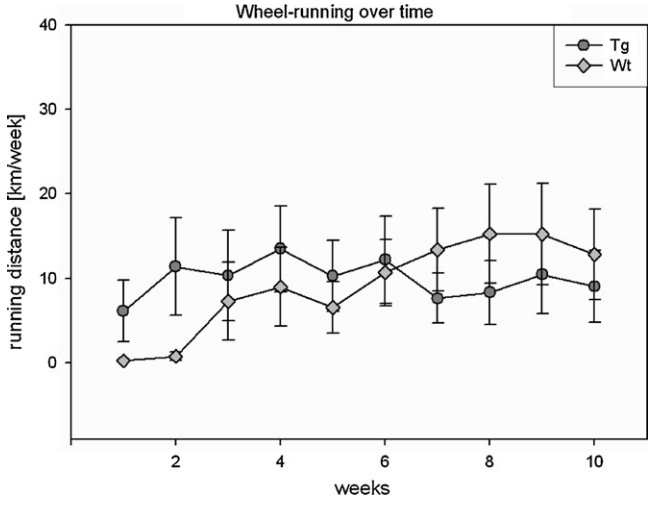

Fig. 2. Wheel-running activity over time in transgenic (Tg) and wildtype (Wt) mice. Mice had access to a running wheel for 10 weeks, starting at the age of 80 days. Data are presented as average running distances per week ± S.E.M. Statistics: Mann–Whitney *U*-test, ns.

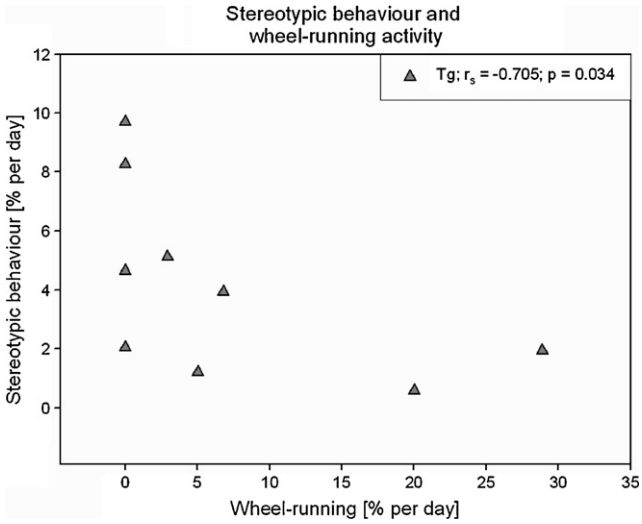

Fig. 3. Correlation between stereotypic behaviour and wheel-running of transgenic mice during the 24 h video observation. Wheel-running data are presented as percent of time mice ran in the wheel during day 113 ± 3 when video observations were conducted. Statistics: Spearman's rank correlation.

3.2. Learning and memory

3.2.1. Object recognition test

For the analysis of object recognition memory in a novel object paradigm, the time spent exploring the known versus the novel object in the choice phase of the test was investigated. Wt mice of the SH condition spent significantly more time exploring the novel than the known object on both test days (day 1, D1; day 2, D2; Wilcoxon-test, $Z_{D1} = -1.726$, $p = 0.045$, $Z_{D2} = -1.726$, $p = 0.045$, Fig. 4). In contrast, Wt mice of the RW condition did discriminate between the objects on day 2 only (Wilcoxon-test, $Z_{D1} = -1.079$, $p = 0.151$, $Z_{D2} = -1.846$, $p = 0.033$, Fig. 4).

In Tg mice of the SH condition no significant difference between the exploration time of the novel and the known object was found, neither on day 1, nor on day 2 (Wilcoxon-test, $Z_{D1} = -0.816$, $p = 0.223$, $Z_{D2} = -0.623$, $p = 0.281$). Tg mice of the RW condition did discriminate between the objects on day 1, while no significant difference between the exploration time of the known and the novel object was found on day 2 (Wilcoxon-test, $Z_{D1} = -2.118$, $p = 0.017$, $Z_{D2} = -0.549$, $p = 0.311$, Fig. 4). Similarly, the analysis of object recognition memory, including only mice that ran in the wheel, did not reveal consistent effects of wheel-running on exploration time of the novel versus the known object.

3.2.2. Barnes maze test

Concerning the acquisition phase of the Barnes maze (area under the curve from trial 2 to day 4), the ANOVA revealed a significant main effect of genotype on both parameters, the latency to enter the correct hole (two-way ANOVA, $F_{1,48} = 10.288$, $p = 0.002$) and the path travelled on the platform (two-way ANOVA, $F_{1,48} = 28.213$, $p < 0.001$). Further post-hoc analysis

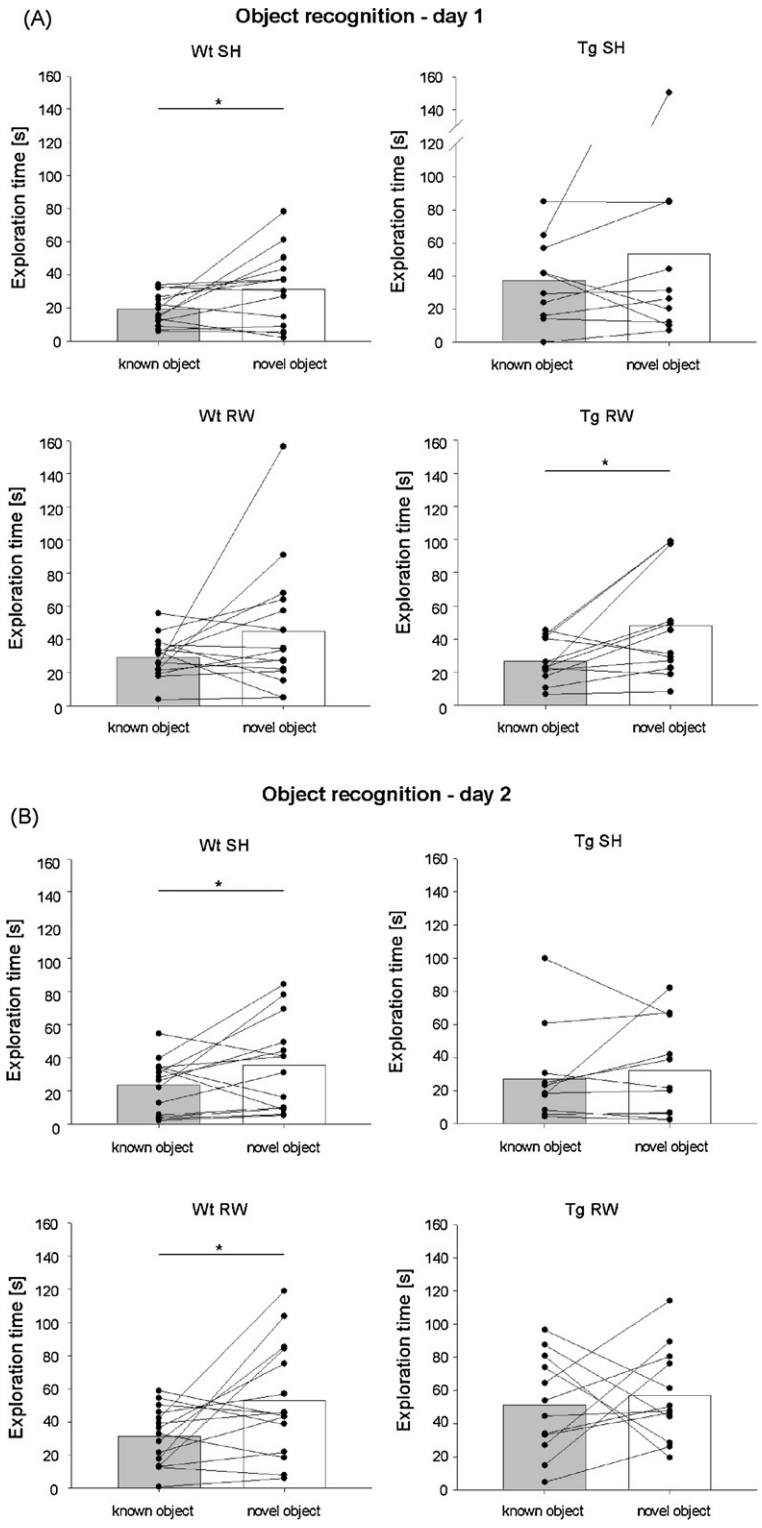

Fig. 4. Paired data of the exploration time of known and novel objects. Higher exploration times of the novel object indicate the presence of an object recognition memory. (A) Data from day 1, (B) data from day 2, presented as means and paired individual values (Wt, wildtype; Tg, transgenic; SH, standard housing; RW, running wheel). Statistics: Wilcoxon-test, $*p \leq 0.05$.

showed that, independent of the housing condition, Tg mice covered greater distances on the platform than Wt mice (Bonferroni post-hoc test: SH, $p = 0.001$; RW, $p = 0.005$). Concerning the latency to enter the correct hole, differences between Wt and Tg mice did not reach significance in pair-wise comparisons (Bonferroni post-hoc test: SH, $p = 0.069$; RW, $p = 0.369$, ns).

Furthermore, the housing condition did neither effect the latency to enter the correct hole (two-way ANOVA,

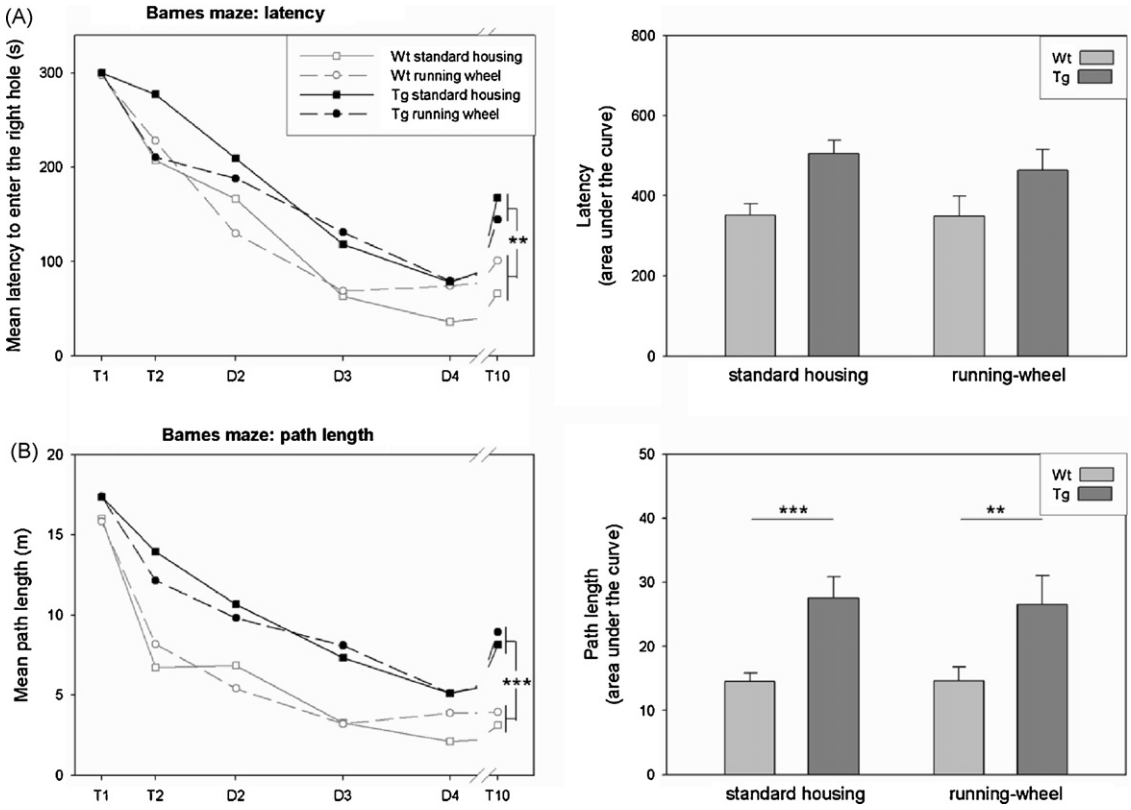

Fig. 5. (A) Latency to enter the right hole and (B) path length in the Barnes maze. Depicted are the learning curve (left) of the acquisition phase and re-test (trial 10 (T10), 4 weeks after day 4: D4) as well as the area under the curve (right, calculated from T2 to D4) for statistical comparison of the acquisition phase. Data are given as means for the learning curves, and means + S.E.M. for the calculated area under the curve. Statistics: two-way ANOVA (genotype, housing), Bonferroni post-hoc test, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

$F_{1,48} = 0.277$, $p = 0.601$) nor the path travelled on the platform (two-way ANOVA, $F_{1,48} = 0.309$, $p = 0.581$). An interaction effect was not found either (two-way ANOVA, path: $F_{1,48} = 0.050$, $p = 0.823$; latency: $F_{1,48} = 0.221$, $p = 0.641$).

In the probe trial (on day 5, not depicted in Fig. 5) all groups showed evidence of spatial memory as indicated by a higher percentage of time spent in the target area than expected by chance (about 16.67%, Binomial-test, Wt SH: $p = 0.001$; Wt RW: $p < 0.001$; Tg SH: $p = 0.001$; Tg RW: $p < 0.001$). However, the ANOVA did not reveal a significant effect of genotype and/or housing on the percentage of time spent in the target area (two-way ANOVA, genotype: $F_{1,48} = 1.092$, $p = 0.301$, ns; housing: $F_{1,48} = 0.579$, $p = 0.451$, ns; genotype*housing; $F_{1,48} = 0.032$, $p = 0.859$, ns).

Concerning long-term retrieval (Fig. 5A and B T10, 4 weeks after D4) a significant main effect of genotype was found on both, latency (two-way ANOVA, $F_{1,41} = 8.272$, $p = 0.006$) and path length (two-way ANOVA, $F_{1,41} = 12.449$, $p = 0.001$). Again, the housing condition had no effect on latency (two-way ANOVA, $F_{1,41} = 0.750$, $p = 0.392$, ns) and path length (two-way ANOVA, $F_{1,41} = 0.958$, $p = 0.333$, ns) and we did not find any interaction effects (two-way ANOVA, path: $F_{1,41} = 0.856$, $p = 0.360$; latency: $F_{1,41} = 0.121$, $p = 0.729$). However, further post-hoc analysis did not reveal significant group differences, neither for latency to enter the correct hole (Bonferroni post-hoc test, SH: $p = 0.062$, ns; RW: $p = 0.999$, ns) nor for path travelled on the platform (Bonferroni post-hoc test, SH: $p = 0.054$, ns; RW: $p = 0.181$, ns). Similarly, the analysis of spatial memory without non-runners did not reveal an effect of housing condition on spatial learning performance either.

3.3. Faecal corticosterone metabolites

Genotype significantly affected the faecal corticosterone metabolites (two-way ANOVA, $F_{1,50} = 101.514$, $p < 0.001$). Post-hoc analysis revealed that, regardless of the housing condition, Tg mice were characterised by higher concentrations of faecal corticosterone metabolites when compared to Wt mice (Bonferroni post-hoc test, SH: $p < 0.001$; RW: $p < 0.001$, Fig. 6). The housing condition had no significant main effect on corticosterone metabolite concentrations (two-way ANOVA, $F_{1,50} = 0.874$, ns), but the ANOVA revealed a significant interaction effect (two-way ANOVA, $F_{1,50} = 6.982$, $p = 0.011$). Exclusion of non-runners from the analysis did not influence these findings.

3.4. Aβ plaque burden

The number of Aβ plaques in the neocortex and hippocampus did not differ significantly between Tg mice of the SH and those of the RW condition (t-test; $t = -0.899$, $p = 0.383$, Fig. 7A). Additionally, no significant differences in Aβ positive

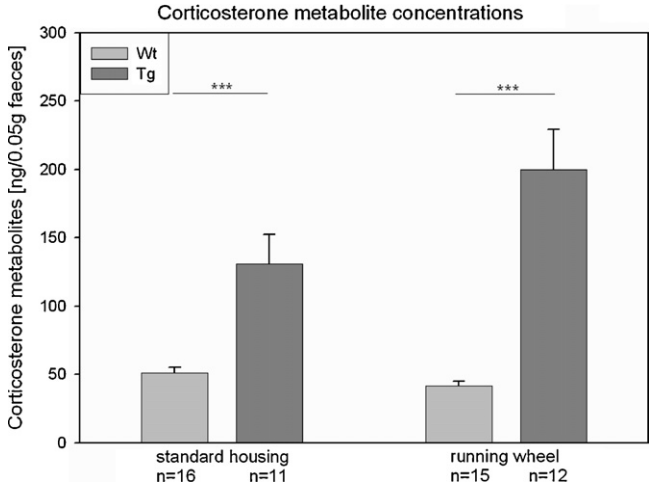

Fig. 6. Concentrations of faecal corticosterone metabolites measured in wild-type (Wt) and transgenic (Tg) mice of both housing conditions. Data are presented as means + S.E.M. Statistics: two-way ANOVA (genotype, housing), Bonferroni post-hoc test, $^{***}p \leq 0.001$.

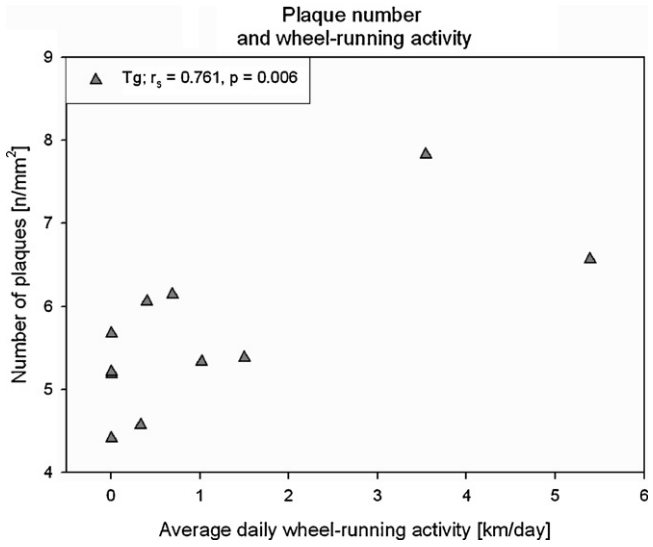

Fig. 8. Correlation between the number of plaques and the average daily running distance an individual covered in the wheel during the 70 days of access to a wheel. Wheel-running activity is presented in km/day. Statistics: Spearman's rank correlation.

area were found between mice of different housing conditions (*t*-test; $t = -0.227$, $p = 0.823$, Fig. 7B).

Analysing the relationship between plaque burden and daily running distance revealed that total plaque load was positively correlated with wheel-running activity (Spearman's rank correlation; number of plaques: $r_s = 0.761$, $p = 0.006$, Fig. 8; plaque area: $r_s = 0.734$, $p = 0.010$).

No correlation existed between plaque load and stereotypic behaviour in both the SH group (Spearman's rank correlation coefficient; plaque number: $r_s = 0.381$, $p = 0.352$; plaque area: $r_s = 0.524$, $p = 0.183$) and the RW group (Spearman's rank correlation; plaque number: $r_s = -0.381$, $p = 0.352$; plaque area: $r_s = 0.095$, $p = 0.823$).

4. Discussion

We studied the effects of physical exercise on cognition, spontaneous behaviour, corticosterone levels, and amyloid deposition in male TgCRND8 mice, a murine model of Alzheimer's disease. There were genotype-dependent differences in all parameters, confirming Alzheimer-like pathology in this model. Tg mice were characterised by higher activity, more stereotypic behaviour, cognitive deficits in object recognition memory and spatial learning, and elevated basal corticosterone concentrations, when compared to Wt mice. Exposure of the animals to a running wheel from 80 days to 5 months of age resulted in genotype-dependent changes in behavioural parameters. In Tg mice, access to a running wheel led to a decrease of stereotypic behaviour, whereas activity levels, cognitive abilities, corticosterone secretion and amyloid deposition were not affected. Furthermore, a positive correlation was found between plaque burden and wheel-running in Tg mice. In Wt mice, an effect of access to a running wheel on object recognition memory was found. While standard housed mice showed evidence of object

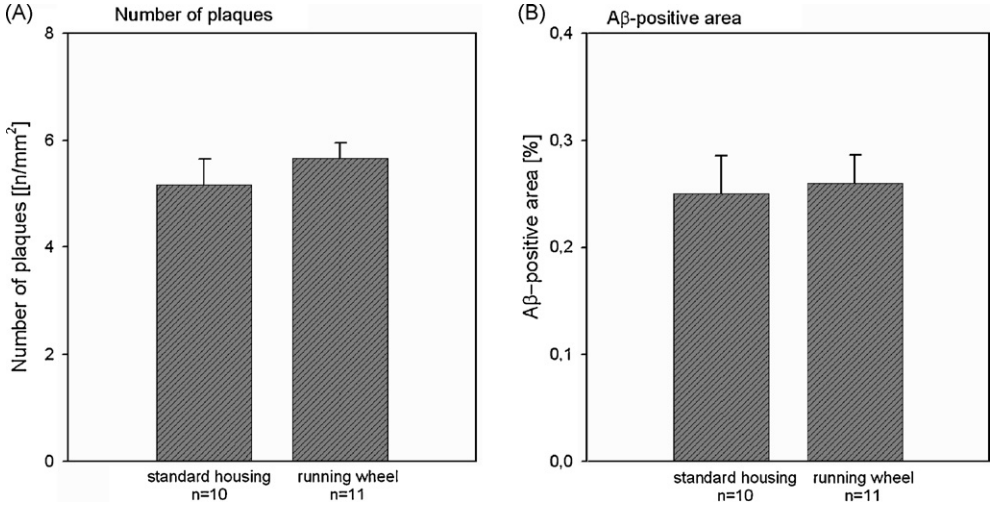

Fig. 7. (A) Number of plaques in the total neocortex and hippocampus and (B) the percentage of Aβ-positive area related to the investigated area (neocortex and hippocampus). Data are presented as means + S.E.M. Statistics: *t*-test, ns.

recognition memory, Wt mice with a running wheel discriminated between the familiar and the novel objects only on day 2 of the test.

4.1. Alzheimer-related differences between the genotypes

Tg mice (∼4 months of age) were characterised by cognitive deficits in an object recognition task as well as in spatial memory performance, including long-term retrieval, in the Barnes maze test. These findings are in line with previous studies reporting cognitive deficits in TgCRND8 mice using various learning paradigms, including the Morris water maze [9,23,24], conditioned taste aversion [25], a six-arm radial water maze [30] and a Y-maze [23]. Cognitive deficits in these mice correlate with onset and progression of an Alzheimer-like pathology, indicating an association between the aggregation of Aβ and learning impairments [23].

Analysis of the spontaneous behaviour revealed that Tg mice were characterised by altered daily activity rhythms, displayed higher activity levels in the home cage, and spent more time engaging in stereotypies than their Wt littermates. Furthermore, faecal corticosterone metabolites were significantly elevated in Tg mice as compared to Wt animals. These findings are in line with previous studies reporting activity changes and marked stereotypic behaviour as well as a hyperactivity of the HPA-axis in Tg mice of this line compared to Wt animals [2,48]. Several other studies also showed that overexpression of APP or elevated Aβ-levels lead to disrupted activity patterns [22,43,49,52]. Disturbances of circadian rhythms and sleep, as well as sundowning are common symptoms in human AD and correspond to alterations in daily activity patterns in TgCRND8 mice [35].

Concerning stereotypic behaviour, similar behaviour was observed in APP23 mice [27]. Although this finding cannot be linked directly to humans, it seems that dopaminergic mechanisms are involved in the expression of both, stereotypic behaviour in TgCRND8 mice [2,4] and non-cognitive behavioural symptoms in Alzheimer patients [42]. A recent study suggests that TgCRND8 mice develop a dopamine deficit in the hippocampus and in contrast higher levels of this neurotransmitter in the neostriatum. This can be a compensation for the reduced levels in the hippocampus and thereby eliciting higher levels of stereotypic behaviour [4]. Another link between TgCRND8 mice and AD patients exists regarding elevated corticosterone metabolites indicating a hyperactivity of the HPA-axis. High glucocorticoid levels are common in aging people and Alzheimer patients [12,21] and may contribute to neurodegeneration via the glucocorticoid cascade as hypothesized by Sapolsky and colleagues [37]. In summary, the comparison of TgCRND8 and Wt mice revealed a number of differences at the cognitive, non-cognitive and endocrine level, pointing to a strong Alzheimer-like phenotype in these mice.

4.2. Effects of access to a running wheel on Alzheimer-like symptoms and pathology

Various environmental factors are known to influence the risk and development of Alzheimer's disease throughout lifetime. Simple lifestyle changes may be sufficient to slow the onset and progression of the disease. Some studies describe an educational and occupational influence [5,8,40,53]; others report an influence of intellectual or physical activity. In one epidemiological study for example, AD patients were characterised by reduced activities in midlife compared to healthy control-group members [19]. Laurin et al. [28] reported protective effects of physical activity against the development of cognitive impairment. In mouse models of Alzheimer's disease, effects of physical exercise in terms of wheel-running are inconsistent. Adlard et al. [1] reported a decrease of amyloid load and improved water maze learning by voluntary exercise in TgCRND8 mice, whereas Wolf and co-workers [55] did not find any beneficial effects of wheel-running on spatial learning and plaque load in APP23 mice.

Present data revealed an effect of access to a running wheel only on stereotypic behaviour. We found a reduction of stereotypic behaviour in Tg RW mice, but not in Wt RW mice. Wt mice of this model display only low levels of stereotypic behaviour, making it difficult to find a potential beneficial effect on stereotypy level. Additionally, this may also explain why a negative correlation between wheel-running and stereotypic behaviour was found only in Tg mice. Thus, although data from Tg mice confirmed previous findings showing a reduction in stereotypy levels by a running wheel-enrichment [36], no general conclusions about the effectiveness of a running wheel can be drawn from this study.

Moreover, a running wheel-enrichment did neither influence Aβ pathology, overall activity levels and corticosterone metabolite concentrations, nor benefit cognitive performance. These findings are in contrast to other studies on the effects of wheel-running. For example, in several species of mammals, plasma glucocorticoid levels rise with moderate to exhaustive exercise [10,20,41,45] and, in rodents, several studies on wheel-running reported improved cognitive abilities, usually focusing on spatial learning tasks [50,51]. Furthermore, a previous study on wheel-running mice reported an increase of overall activity levels [14]. Several reasons may account for the discrepant results of the present study in comparison to previously described data.

One main difference influencing the outcome of behavioural testing may be the genetic backgrounds of the tested animals [34]. TgCRND8 mice on the hybrid C57/C3H background may be less susceptible to physical activity than other mouse strains that are frequently used in research. In addition, the time point of providing a wheel and the period of time with free access to a running wheel seems to play another crucial role regarding the effects of wheel-running. Both parameters also differ in comparison to the study by Adlard et al. [1] who equipped cages with a running wheel already in the presymptomatic phase and reported reduced amyloid load and improved spatial memory after 5 months of wheel-running. Our mice of the same model had access to a running wheel for 3 months from 80 days of age onwards, the time when plaques first occur in the brain. This intervention failed to improve cognitive deficits or pathology.

In summary, the study by Adlard et al. [1] suggests that increased physical activity, i.e. running, at an early, presymptomatic time point may indeed have protective and beneficial effects on the development of Alzheimer-like symptoms and

pathology. If, however, access to a running wheel is provided after the onset of plaque deposition, physical activity does not seem to counteract further cognitive decline and plaque formation. Since a positive correlation between wheel-running and plaque load was found in our study, we conclude that running in this phase can be interpreted as symptom of pathological brain function. Furthermore, this may offer the possibility of using wheel-running as a behavioural marker for Alzheimer's pathology.

4.3. Wheel-running: a stereotypy?

Access to a running wheel led to a strong reduction of stereotypic behaviour in Tg mice. At first glance, this finding supports the view that a running wheel in laboratory housing systems satisfies some behavioural needs and hence contributes to good welfare [6]. However, access to a running wheel did not improve learning and memory, decrease corticosterone levels or reduce plaque burden. Since wheel-running correlated negatively with stereotypic behaviour in Tg mice, stereotypic behaviour may have been substituted by wheel-running, indicating that wheel-running may be another form of stereotypic behaviour. It also complies with the definition of stereotypic behaviour, i.e. repetitive, invariant and without any obvious goal or function [32,33]. Hence, improving housing conditions for laboratory mice requires more than access to a running wheel.

Acknowledgement

This study was supported by the German Federal Ministry of Education and Research (BMBF Grant 01GW0520 to W.R.S. and N.S.).

References

[1] Adlard PA, Perreau VM, Pop V, Cotman CW. Voluntary exercise decreases amyloid load in a transgenic mouse model of Alzheimer's disease. J Neurosci 2005;25:4217–21.
[2] Ambrée O, Touma C, Görtz N, Keyvani K, Paulus W, Palme R, et al. Activity changes and marked stereotypic behavior precede Aβ pathology in TgCRND8 Alzheimer mice. Neurobiol Aging 2006;27:955–64.
[3] Ambrée O, Leimer U, Herring A, Görtz N, Sachser N, Heneka MT, et al. Reduction of amyloid angiopathy and Aβ plaque burden after enriched housing in TgCRND8 mice. Am J Pathol 2006;169:544–52.
[4] Ambrée O, Richter H, Sachser N, Lewejohann L, Dere E, de Souza Silva MA, Herring, A, Keyvani K, Paulus W, Schäbitz WR. Levodopa ameliorates learning and memory deficits in a murine model of Alzheimer's disease. Neurobiol Aging, in press, doi:10.1016/j.neurobiolaging.2007.11.010.
[5] Andel R, Vigen C, Mack WJ, Clark LJ, Gatz M. The effect of education and occupational complexity on rate of cognitive decline in Alzheimer's patients. J Int Neuropsychol Soc 2006;12:147–52.
[6] Balcombe JP. Laboratory environments and rodents' behavioural needs: a review. Lab Anim 2006;40:217–35.
[7] Barnes CA. Memory deficits associated with senescence: a neurophysiological and behavioural study in the rat. J Comp Physiol Psychol 1979;93:74–104.
[8] Bennett DA, Wilson RS, Schneider JA, Evans DA, Mendes de Leon CF, Arnold SE, et al. Education modifies the relation of AD pathology to level of cognitive function in older persons. Neurology 2003;60:1909–15.
[9] Chishti M, Yang D, Janus C, Phinney AL, Horne P, Pearson J, et al. Early-onset amyloid deposition and cognitive deposits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. J Biol Chem 2001;276:21562–70.
[10] Coleman MA, Garland T, Marler CA, Newton SS, Swallow JG, Carter PA. Glucocorticoid response to forced exercise in laboratory house mice (*Mus domesticus*). Physiol Behav 1998;63:279–85.
[11] Cotman CW, Berchtold NC. Exercise: a behavioural intervention to enhance brain health and plasticity. Trends Neurosci 2002;25:295–301.
[12] Davis KL, Davis BM, Greenwald BS, Mohs RC, Mathé AA, Johns CA, et al. Cortisol and Alzheimer's disease. I. Basal studies. Am J Psychol 1986;143:300–5.
[13] Dere E, Huston JP, De Souza Silva MA. The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev 2007;31:673–704.
[14] De Visser L, Van den Bos R, Spruijt BM. Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. Behav Brain Res 2005;160:382–8.
[15] Ehninger D, Kempermann G. Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex. Cereb Cortex 2003;13:845–51.
[16] 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.
[17] Ennaceur A, Michalikova S, Bradford A, Ahmed S. Detailed analysis of the behaviour of Wistar rats in anxiety, object recognition and object location tasks. Behav Brain Res 2005;159:247–66.
[18] Finkel SI. Behavioral and psychological symptoms of dementia. Clin Geriatr Med 2003;19:724–99.
[19] 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.
[20] Girard I, Garland T. Plasma corticosterone response to acute and chronic voluntary exercise in female housed mice. J Appl Physiol 2002;92:1553–61.
[21] Hatzinger M, Z'Brun A, Hemmeter U, Seifritz E, Baumann F, Holsboer-Trachsler E, et al. Hypothalamic-pituitary-adrenal system function in patients with Alzheimer's disease. Neurobiol Aging 1995;16:205–9.
[22] Huitrón-Reséndiz S, Sánchez-Alavez M, Gallegos R, Berg G, Crawford E, Giacchino JL, et al. Age-independent and age-related deficits in visuospatial learning, sleep–wake states, thermoregulation and motor activity in PDAPP mice. Brain Res 2002;928:126–37.
[23] Hyde LA, Kazdoba TM, Grilli M, Lozza G, Brussa R, Zhang Q, et al. Age-progressing cognitive impairments and neuropathology in transgenic CRND8 mice. Behav Brain Res 2005;160:344–55.
[24] Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, et al. Aβ peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 2000;408:979–82.
[25] Janus C, Welzl H, Hanna A, Lovasic L, Lane N, George-Hyslop P, et al. Impaired conditioned taste aversion learning in APP transgenic mice. Neurobiol Aging 2004;25:1213–9.
[26] Jung AP, Curtis TS, Turner MJ, Lightfoot JT. Influence of age of exposure to a running wheel on activity in inbred mice. Med Sci Sports Exerc 2006;38:51–6.
[27] Lalonde R, Dumont M, Sturchler-Pierrat C, Staufenbiel M, Strazielle C. Spatial learning, exploration, anxiety, and motor coordination in female APP23 transgenic mice with the Swedish mutation. Brain Res 2002;956:36–44.
[28] Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K. Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 2001;58:498–504.
[29] Lawlor B. Managing behavioural and psychological symptoms in dementia. Br J Psychiatry 2002;181:463–5.
[30] 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.

[31] Martin P, Bateson P. Measuring behaviour. An introductory guide. 2nd ed. Cambridge: Cambridge University Press; 2002.
[32] Mason GJ. Stereotypies: a critical review. Anim Behav 1991;41:1015–37.
[33] Mason GJ. Stereotypies and suffering. Behav Proc 1991;25:103–15.
[34] McClearn GE. Nature and nurture: interaction and coaction. Am J Med Genet 2004;124B:124–30.
[35] McDonald RJ. Multiple combinations of co-factors produce variants of age-related cognitive decline: a theory. Can J Exp Psychol 2002;56:221–39.
[36] Olsson IA, Sherwin CM. Behaviour of laboratory mice in different housing conditions when allowed to self-administer an anxiolytic. Lab Anim 2006;40:392–9.
[37] Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr Rev 1986;7:284–301.
[38] Siegel S. Nichtparametrische statistische Methoden. Eschborn Frankfurt am Ma Fachbuchhandlung für Psychologie 1987.
[39] Spackman DH, Riley V. Corticosterone concentrations in the mouse. Science 1978;200:87.
[40] Stern Y, Gurland B, Tatemichi TK, Tang MX, Wilder D, Mayeux R. Influence of education and occupation on the incidence of Alzheimer's disease. JAMA 1994;271:1004–10.
[41] Stranahan AM, Khalil D, Gould E. Social isolation delays the positive effects of running on adult neurogenesis. Nat Neurosci 2006;9:526–33.
[42] Tanaka Y, Meguro K, Yamaguchi S, Ishii H, Watanuki S, Funaki Y, et al. Decreased striatal D2 receptor density associated with severe behavioral abnormality in Alzheimer's disease. Ann Nucl Med 2003;17:567–73.
[43] Tate B, Aboody-Guterman KS, Morris AM, Walcott EC, Majocha RE, Marotta CA. Disruption of circadian regulation by brain grafts that overexpress Alzheimer β/A4 amyloid. Proc Natl Acad Sci USA 1992;89:7090–4.
[44] Teitelbaum P, Pellis MP, DeVietti TL. Disintegration of stereotypy induced by drugs or brain damage: a microdescriptive behavioural analysis. In: Cooper SJ, Dourish CT, editors. Neurobiology of stereotyped behaviour. Oxford: Clarendon Press; 1990. p. 169–99.
[45] Tharp GD. The role of glucocorticoids in exercise. Med Sci Sports Exerc 1975;7:6–11.
[46] Touma C, Sachser N, Möstl E, Palme R. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol 2003;130:267–78.
[47] Touma C, Palme R, Sachser N. Analyzing corticosterone metabolites in fecal samples of mice: o noninvasive technique to monitor stress hormones. Horm Behav 2004;45:10–22.
[48] 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.
[49] Van Dam D, D'Hooge R, Staufenbiel M, Van Ginneken C, Van Meir F, De Deyn PP. Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. Eur J Neurosci 2003;17:388–96.
[50] Van Praag H, Christie BR, Sejnowski TJ. Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. Neurobiol 1999;96:13427–31.
[51] Van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 2005;25:8680–5.
[52] Vloeberghs E, Van Dam D, Engelborghs S, Nagels G, Staufenbiel M, De Deyn PP. Altered circadian locomotor activity in APP23 mice: a model for BPSD disturbances. Eur J Neurosci 2004;20:2757–66.
[53] White J, Katzman R, Losonczy K, Salive M, Wallace R, Berkman L, et al. Association of education with incidence of cognitive impairment in three established populations for epidemiologic studies of the elderly. J Clin Epidemiol 1994;47:363–74.
[54] Wiedenmayer C. Effect of cage size on ontogeny of stereotyped behaviour in gerbils. Appl Anim Behav Sci 1996;47:225–33.
[55] 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.
[56] Würbel H, Stauffacher M, Von Holst D. Stereotypies in laboratory mice – quantitative and qualitative description of the ontogeny of 'wire-gnawing' and jumping' in Zur:ICR and Zur:ICR nu. Ethology 1996;102:371–85.