Physical Activity Ameliorates Impaired Hippocampal Neurogenesis in the Tg4-42 Mouse Model of Alzheimer’s Disease

Anna-Lina Gerberding¹, Silvia Zampar¹, Martina Stazi¹, David Liebetanz², and Oliver Wirths¹

Abstract

There is growing evidence from epidemiological studies that especially midlife physical activity might exert a positive influence on the risk and progression of Alzheimer’s disease. In this study, the Tg4-42 mouse model of Alzheimer’s disease has been utilized to assess the effect of different housing conditions on structural changes in the hippocampus. Focusing on the dentate gyrus, we demonstrate that 6-month-old Tg4-42 mice have a reduced number of newborn neurons in comparison to age-matched wild-type mice. Housing these mice for 4 months with either unlimited or intermittent access to a running wheel resulted in a significant rescue of dentate gyrus neurogenesis. Although neither dentate gyrus volume nor neuron number could be modified in this Alzheimer’s disease mouse model, unrestricted access to a running wheel significantly increased dentate gyrus volume and granule cell number in wild-type mice.

Keywords

physical activity, dentate gyrus, Alzheimer’s disease, mouse model, neurogenesis

Received September 3, 2019; Revised October 6, 2019; Accepted for publication October 27, 2019

Introduction

Alzheimer’s disease (AD) is the most common form of dementia. There is accumulating evidence that in addition to genetics, other lifestyle and environmental factors may have a share in the individual risk of getting AD, which opens new opportunities for prevention (Mattson, 2015). Risk factors include diabetes mellitus, midlife obesity and hypertension, smoking, depression and dyslipidemia (Mayeux and Stern, 2012), and many of these factors are considered potentially modifiable (Lifton et al., 2017). Physical activity has especially been associated with a reduced dementia risk in a variety of epidemiological studies (Scarmeas et al., 2001; Santos-Lozano et al., 2016) and has been recently shown to exert beneficial effects on cognition and AD pathology, even in individuals with genetically driven autosomal dominant AD (Müller et al., 2018). It has been predicted that ~13% of AD cases can be attributed to physical inactivity and that a 25% reduction in physical inactivity has the potential to prevent nearly 1 million cases worldwide (Barnes and Yaffe, 2011).

One potential weakness of many epidemiological studies is that they mostly have to rely on self-reported exercise frequencies, due to the fact that activity profiles of the participating individuals can only be assessed in retrospect. The use of transgenic AD mouse models could overcome this problem as the lifespan of rodents is considerably shorter and housing conditions can be adapted, ensuring better comparability and reproducibility. Indeed, a vast literature reports on the beneficial effects of physical activity, mainly embedded in enriched environment (EE) paradigms, with regard to improved cognitive performance in, for example, hippocampus-dependent tasks (Jankowsky et al., 2005; Nithianantharajah and Hannan, 2006). In addition to
an amelioration of behavioral deficits, also changes on the brain structural level, such as increased hippocampal volume (Hüttenrauch et al., 2016b), enhanced synaptic plasticity (Fattoretti et al., 2018) or increased neurogenesis (van Praag et al., 1999; Mustroph et al., 2012), have been reported upon augmented physical exercise levels. The latter is of particular importance as it has been widely accepted that hippocampal neurogenesis, at least in rodents, plays a crucial role in the maintenance of learning and memory and that newborn neurons become integrated into functional neuronal networks (Deng et al., 2010), which can be investigated using behavioral tasks depending on spatial memory (Snyder et al., 2005) as well as contextual fear memory (Saxe et al., 2006) or recognition memory (Jessberger et al., 2009). The data on neurogenesis in humans are conflicting, with recent studies reporting either a sharp drop to undetectable levels in adults (Sorrells et al., 2018) or abundant neurogenesis up to old age in healthy individuals with a progressive decline in AD patients (Moreno-Jiménez et al., 2019a). In most transgenic mouse models of AD, neurogenesis is and has been associated with other AD-related pathological hallmarks, such as extracellular amyloid plaque deposition, increased neuroinflammation, or altered behavior (Mu and Gage, 2011; Wirths, 2017). Although a detrimental role of Aβ in neurodegeneration and impaired neuronal progenitor proliferation is well-accepted, a substantial influence of mutant transgenic amyloid precursor protein (APP) overexpression or APP-derived proteolitical fragments is most likely (Wirths, 2017). Effects on neurogenesis might depend on transgene, APP mutations, and promoters used for transgene expression as, for example, in young J20 mice, enhanced neurogenesis rates have been reported (Jin et al., 2004), while unchanged neurogenesis was detected in a model with APP expression restricted only to mature projection neurons (Yetman and Jankowsky, 2013).

The aim of this study was to investigate whether physical activity has beneficial effects on neurogenesis and dentate gyrus (DG) structure in the recently developed Tg4-42 mouse model of AD (Bouter et al., 2013) and age-matched wild-type (WT) littermates. Tg4-42 mice overexpress Aβ42-42 peptides, which are among the most abundant Aβ species in human AD brain, in the absence of APP overexpression and without AD-related mutations. These mice develop an age- and gene dose-dependent loss of CA1 pyramidal neurons which becomes obvious at 6 months of age in homozygous (Tg4-42hom) animals (Antonios et al., 2015), correlating with deficits in memory tasks such as the Morris water maze (Hüttenrauch et al., 2016a), but in the absence of overt extracellular amyloid plaque pathology. It has been shown that the running wheel represents the major stimulus present in EE paradigms, which seems sufficient to exert neurogenic effects (Kobilo et al., 2011; Mustroph et al., 2012). To assess whether continuous or intermittent physical exercise is needed to exert beneficial effects on hippocampal neurogenesis, a paradigm comprising isolation housing of WT and Tg4-42hom mice for a period of 4 months was employed. In contrast to group housing, this experimental set-up allows to monitor exercise levels of individual animals. In this study, we were able to demonstrate that 6-month-old Tg4-42hom mice have a reduced number of newborn neurons in the subgranular zone (SGZ) of the DG in comparison to age-matched WT mice, which could be rescued by both continuous as well as intermittent physical activity. In addition, unrestricted physical activity resulted in a significantly increased DG volume in WT mice compared to littermates which had only access to blocked running wheels.

Material and Methods

Transgenic Mice

The generation of the Tg4-42 mouse model has been described previously (Bouter et al., 2013). In brief, Tg4-42 mice were generated and maintained on a C57Bl/6J genetic background and express the human Aβ42-42 peptide sequence. The peptide is expressed under the control of the neuron-specific murine Thy1-promoter and has been combined with the thyrotropin-releasing hormone signal peptide sequence to ensure secretion through the secretory pathway. Animals were handled according to the German guidelines for animal care and experiments were approved by the local animal care and use committee (Landamts für Verbraucherschutz und Lebensmittelsicherheit [LAVES], Lower Saxony).

Housing Conditions

Female mice were housed in groups under standard conditions until the age of 2 months. For the exercise paradigm, mice were assigned randomly to individual cages (22 cm x 16 cm x 14 cm) equipped with either a free (FW), blocked (BW), or temporarily blocked (FWI) running wheel until the age of 6 months. While in the FW or BW condition the wheel was either free or blocked for the entire period, the FWI condition consisted of an alternating paradigm with 3 weeks free wheel access followed by 2 two weeks blockage over the entire period, in order to assess whether continuous activity is needed for beneficial effects. Food and water were provided ad libitum in all conditions. A rotation sensor connected to the running wheel axis transmitted running activity with a resolution of 1/16 revolution and with a sampling rate of 1/0.48 s to a customized recording device (Boenig und Kallenbach oHG, Dortmund, Germany). From these raw data, the average weekly running distance (km)
was calculated and visualized using a custom-designed Matlab (The MathWorks, Inc., Natick, MA) program (Hüttenrauch et al., 2016a).

Tissue Processing and Neuron Counting
Stereological analysis was performed on brain hemispheres of homozygous Tg4-42 and age-matched WT mice. The animals were anaesthetized and transcardially perfused with ice-cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA). The brains were carefully removed, halved following the mediasigittal line and postfixed for 2 hr at 4°C in PFA. The left brain hemispheres were then cryoprotected by immersion in 30% sucrose in PBS at 4°C overnight, followed by quick freezing on dry-ice and storage at −80°C until further processing. The tissues were cut on a cryostat (Leica CM1850UV) into 30 μm thick coronal sections. Every tenth section was mounted on a glass slide and stained with Cresyl violet as previously described (Rutten et al., 2003). Stereological analysis of the DG (Bregma −1.34 to −3.80) neuron number was performed as published previously. The experimenter was blinded to genotype and treatment in all analyses. Neurons were identified based on cytological features of neurons in Nissl-stained sections, such as large round or ovoid nucleoli and visible cytoplasm around the nucleus (García-Cabezas et al., 2016). The volume was calculated by delineating and measuring the area on all analyzed sections. From the obtained data and taking into account the corresponding actual average section thickness after histological preparations, as well as the intersection interval, the total volumes were calculated by means of Cavalieri’s principle (Gundersen and Jensen, 1987; Rutten et al., 2003; Cotel et al., 2008; Hüttenrauch et al., 2016a).

Analysis of Neurogenesis
Using a free-floating staining protocol, a series of every 10th coronal frozen section of 30 μm thickness was processed to quantify the number of newborn neurons. Briefly, sections were rehydrated in PBS, and endogenous peroxidase activity was quenched by immersion in PBS including 0.3% hydrogen peroxide for 30 min. Sections were subsequently washed in PBS including Triton x-100, and unspecific antibody binding was blocked by incubation in PBS including 10% fetal calf serum (FCS) and 4% low-fat dry milk powder. The primary antibody against doublecortin (DCX, 1: 200, Santa Cruz Biotechnology, RRID:AB_2088494) was incubated overnight, followed by incubation with a secondary biotinylated antibody. DCX has been shown to specifically reflect the level of adult neurogenesis and its modulation (Couillard-Despres et al., 2005); however, it should be noted that this marker is expressed during neuron maturation and does not necessarily exactly reflect the existence of mature neurons. Staining was visualized using the ABC method using a Vectastain kit (Vector Laboratories) and DAB as chromogen. Images were analyzed using an Olympus BX51 microscope with a motorized stage. The overall number of newborn neurons was counted in the SGZ of the DG using the meander scan option of Stereoinvestigator 7.0 (MBF Bioscience) to quantify all DCX-positive cells in a given section. The resulting neuron number was multiplied by 10 to obtain the total number of newborn neurons (Cotel et al., 2012).

Statistical Analysis
Differences between groups were tested by unpaired t tests or by two-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test when more than two groups were analyzed. All data were given as mean ± standard deviation (SD). Significance levels were given as follows: ***p < .001; **p < .01; *p < .05. All calculations were performed using GraphPad Prism version 6.07 for Windows (GraphPad Software, San Diego, CA).

Results
Decreased Neurogenesis in the DG of Tg4-42 Mice
The number of newborn DCX-positive neurons in the SGZ of the DG was analyzed in a group of naïve Tg4-42hom as well as age-matched WT mice which were housed under standard conditions (n = 5–7 per group). Six-month-old Tg4-42hom showed a significantly reduced number of DCX-positive cells in the DG (~45%; p < .01; Figure 1). In addition to the reduced number, a qualitative assessment of DCX-positive cells in the Tg4-42hom mice revealed in general an atrophic appearance with much shorter and less branched dendrites compared to WT mice (Figure 1).

Next, Tg4-42hom and WT mice were housed under standard conditions for 2 months and were subsequently randomly assigned to individual cages equipped with a running wheel. During the following 4 months, the running wheels were free (FW) or blocked (BW) for the entire period or free/blocked in alternating intervals (FWI; Figure 2(a)). In the middle of the treatment period, the individual use of the running wheels was monitored and the average distance per day was calculated. Although no difference in the average daily distance between the FW or FWI conditions was detected in either Tg4-42hom or WT mice, a genotype effect became evident, with WT-FWI mice (9.133 ± 1.886 km/day) running significantly more than Tg4-42hom-FWI mice.
### Analysis of Neurogenesis, DG Volume, and Neuron Number

To assess a potential beneficial effect of voluntary exercise on neurogenesis and DG integrity, unbiased design-based stereological analyses of the DG were carried out in all genotypes upon the end of the treatment period (Figure 3(a) to (f)). With regard to neurogenesis, no differences could be detected in WT mice among the three groups. A significant genotype effect was detected using two-way ANOVA, $F(1, 42) = 174.3, p < .0001$, with WT showing significantly higher numbers of DCX-positive cells in all three treatment groups (all $p$ values < .001). The reduced number of newborn neurons found in naïve mice could be confirmed in Tg4-42$^{\text{hom}}$ compared to WT mice housed under BW conditions (~30%, $p < .001$; Figure 3(g)). However, in Tg4-42$^{\text{hom}}$ mice,
housed either in FW (p < .001) or in FWI conditions (p < .01) resulted in a significantly increased number of DCX-positive cells in the DG (Figure 3(g)).

A comparison of WT and Tg4-42\textsuperscript{hom} mice housed in BW conditions did not reveal any differences with regard to DG volume. Although no differences in the DG volume were detected among Tg4-42\textsuperscript{hom} mice housed under BW, FW, or FWI conditions, a significantly increased DG volume became apparent in WT-FW compared to WT-BW mice (p < .05). With regard to a genotype-dependent effect, $F(1, 42) = 12.69$; p = .0009. WT-FW mice showed a significantly increased DG volume compared to Tg4-42\textsuperscript{hom} mice housed under the same conditions (p < .05; Figure 3(h)).

In view of altered DG neuron numbers upon physical activity, no major differences could be noted in Tg4-42\textsuperscript{hom} housed under BW (604346 ± 86566), FW (627529 ± 69080), or FWI (553910 ± 79892) conditions. A significant genotype effect was detected in WT-FWI showing increased DG neuron numbers compared to Tg4-42\textsuperscript{hom} mice house under the same condition (p < .01). Interestingly, WT mice allowed to continuously exercise (WT-FW; 731756 ± 43827) showed higher DG neuron number (+ ~17%) compared to their WT-BW (626900 ± 99789) littermates which were not able to participate in the exercise paradigm; however, this was not significant using two-way ANOVA with Tukey’s multiple comparison test. WT-FWI (689710 ± 66671) also showed

**Figure 3.** Analysis of neurogenesis, DG volume and neuron number. Representative images of WT (a–c) and Tg4-42\textsuperscript{hom} mice (d–f) housed in either BW (a and d), FW (b and e) or FWI (c and f) conditions. Tg4-42\textsuperscript{hom}-BW mice showed a reduced number of DCX-positive cells in DG compared to WT-BW mice, while both housing under FW and FWI conditions resulted in a significantly increased neurogenesis in this genotype (g). No difference in DG volume could be detected in Tg4-42\textsuperscript{hom} mice in either housing condition, while WT mice with a free wheel should a significantly increased DG volume compared to their BW littermates (h). Continuous or intermittent physical activity did not change DG neuron in WT or Tg4-42 mice (i). (n = 5–11 mice per group); *p < .05. **p < .01. ***p < .001. All graphs show mean ± SD. Scale bar: (a)–(f): 100 μm. DCX = doublecortin; DG = dentate gyrus; WT = wild type; FW = free wheel; BW = blocked wheel; FWI = intermittent free/blocked wheel.
higher numbers than WT-BW mice, but this increase was also not statistically significant (Figure 3(i)).

Discussion

In the present report, we investigated the effects of voluntary exercise in the absence of confounding other factors implicated in EE paradigms, such as social or environmental stimuli. An analysis of DG neurogenesis in naïve Tg4-42\homo and WT mice at the age of 6 months revealed a significantly reduced number of newborn neurons in the animals expressing the Aβ42 peptides. Decreased neurogenesis is a common feature of transgenic AD mice and has been shown in a variety of models (Mu and Gage, 2011; Wirths, 2017), such as Tg2576 (Krezymon et al., 2013), 3xTg-AD (Rodriguez et al., 2008), APP/PS1IKI (Faure et al., 2011; Cotel et al., 2012), and 5XFAD (Moon et al., 2014). Although most of the other models harbor extracellular amyloid pathology in the hippocampus, no overt extracellular plaque deposition is evident in Tg4-42 mice despite a robust neuron loss in the CA1 pyramidal layer (Bouter et al., 2008), APP/PS1 (Faure et al., 2011; Cotel et al., 2012), and 5XFAD (Moon et al., 2014). Although most of the other models harbor extracellular amyloid pathology, the Tg4-42 model is a lack of APP overexpression. This is an important and often disregarded confounding factor in most other transgenic AD mouse models, as it has been shown that, for example, WT APP overexpression in the hippocampal formation has a reduced neurogenesis rate compared to age-matched WT mice, while no such difference could be detected in young mice which had not yet developed extracellular amyloid pathology (Haughey et al., 2002). Another interesting characteristic of the Tg4-42 model is the presence of a running wheel offers the opportunity to exercise voluntarily, and housing in single cages allows a much better assessment of the activity level in individual animals compared to group housing. However, an analysis of the physical activity level at weeks 7 to 8 in the middle of the treatment period revealed that WT mice in general showed increased levels of physical activity compared to Tg4-42 mice. This might suggest that reduced running distance could account for the observed decrease in hippocampal neurogenesis in Tg4-42 mice. However, a major influence seems to be unlikely, as neurogenesis is also decreased upon standard housing conditions and Tg4-42\homo mice do not show overt alterations in general locomotor activities (Wagner et al., 2019). It has been further shown that physical exercise seems to be the most relevant factor with regard to neurogenesis (Marlatt et al., 2013) and that it represents the crucial factor in EE paradigms (Kobilo et al., 2011). Either unrestricted or intermittent access to the running wheel increased neurogenesis and supports previous findings from a complex EE paradigm. The finding of an unchanged DG granule cell number in all Tg4-42\homo treatment groups does not support previous results showing an increased number in enriched versus standard-housed mice (Hüttenrauch et al., 2016a). This might suggest that other factors such as social stimuli or environmental novelty play an important role in hippocampal structural changes. Indeed, it has been recently
shown that, for example, social enrichment in the absence of further cognitive stimulation has a potent neurogenesis-stimulating potential (Moreno-Jiménez et al., 2019b) and might exert further beneficial effects on hippocampal plasticity. The exercise paradigm utilized in our study resembles social isolation due to housing in single cages, which has been associated with diminished neurogenesis (Dong et al., 2004), reduced DG, CA2/3 or total hippocampal volume (Fabricius et al., 2010), as well as altered neuronal tree arborization in the DG in rats (Biggio et al., 2019). However, the fact that observed DCX-positive neuron numbers in 6-month-old WT and Tg4-42<sup>hom</sup> mice housed either under standard or isolation (BW) conditions were comparable argues against a major detrimental effect and supports studies that report no prolonged impact of social isolation on neurogenesis (Grégoire et al., 2014). The effect of social isolation might be more relevant in WT mice, which showed unchanged but high neurogenesis rates already in the blocked wheel condition. Although increased neurogenesis has been reported in 6-month-old WT mice upon enriched housing (Mirochnic et al., 2009; Cotel et al., 2012), no differences were detected in the three different housing conditions comprising social isolation in this study. This resembles related findings showing that individual housing precludes the positive influence of running on adult neurogenesis (Stranahan et al., 2006; Ibi et al., 2008) and might become apparent in particular when neurogenesis is not compromised per se.

In conclusion, we provide evidence that both continuous and intermitted physical activity results in rescued neurogenic properties in the Tg4-42 transgenic mouse model of AD, thereby supporting epidemiological data on human AD obtained in retrospective studies. The use of a mouse model expressing only Aβ in the absence of mutant APP overexpression further corroborates the critical involvement of this peptide in the regulation of hippocampal neurogenesis.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors acknowledge support by Gerhard Hunsmann Stiftung (to O.W.) and the Open Access Publication Funds of the Göttingen University.

**ORCID iD**

Oliver Wirths https://orcid.org/0000-0002-4115-0334

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