Original Paper

American Society for Neurochemistry

ASN Neuro
Volume 11: 1–9

DOI: 10.1177/1759091419892692
journals.sagepub.com/home/asn
SAGE

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

Anna-Lina Gerberding[1], Silvia Zampar[1], Martina Stazi[1], David Liebetanz[2], and Oliver Wirths[1]

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 (Livingston 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

[1]Department of Psychiatry and Psychotherapy, Molecular Psychiatry, University Medical Center (UMG), Georg-August-University, Göttingen, Germany
[2]Department of Clinical Neurophysiology, University Medical Center (UMG), Georg-August-University, Göttingen, Germany

Corresponding Author:
Oliver Wirths, Department of Psychiatry and Psychotherapy, Molecular Psychiatry, University Medical Center (UMG), Georg-August-University, Von-Siebold-Str. 5, 37075 Göttingen, Germany.
Email: oliver.wirths@med.uni-goettingen.de

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 Aβ4-42 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 (Tg4-42^{hom}) 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 Tg4-42^{hom} 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 Tg4-42^{hom} 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 Aβ4-42 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 (Landeamt 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 × 16 cm × 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 mediosagittal 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 quickly 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 neurogensis 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 existance 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 Tg4-42^{hom} as well as age-matched WT mice which were housed under standard conditions ($n = 5$–7 per group). Six-month-old Tg4-42^{hom} 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 Tg4-42^{hom} mice revealed in general an atrophic appearance with much shorter and less branched dendrites compared to WT mice (Figure 1).

Next, Tg4-42^{hom} 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 Tg4-42^{hom} or WT mice, a genotype effect became evident, with WT-FWI mice (9.133 ± 1.886 km/day) running significantly more than Tg4-42^{hom}-FWI mice

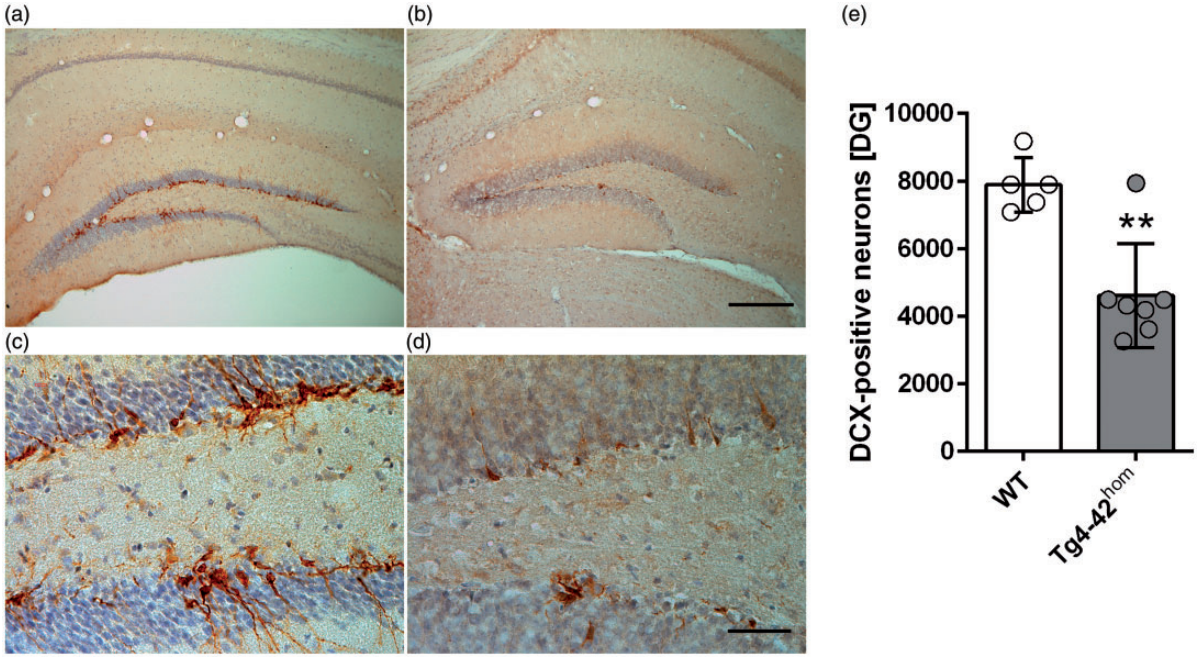

Figure 1. Decreased neurogenesis in the dentate gyrus of 6-month-old Tg4-42^{hom} mice. In comparison to age-matched WT mice (a and c), the number of DCX-positive cells in the DG of Tg4-42^{hom} mice (b and d) is significantly reduced (e) ($n = 5$–7 per group). $^{**}p < .01$. Graph shows mean ± *SD*. Scale bar: (a) and (b): 200 μm; (c) and (d): 33 μm. DCX = doublecortin; DG = dentate gyrus; WT = wild type.

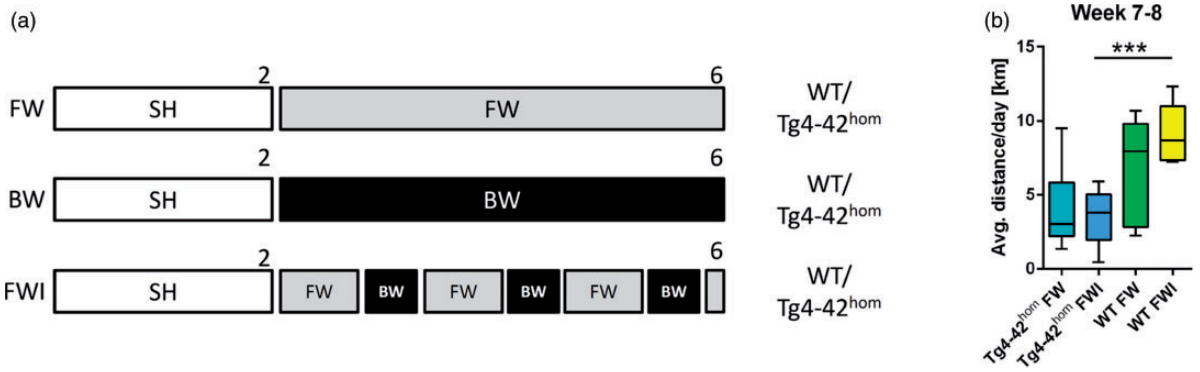

Figure 2. Scheme illustrating the physical activity paradigm. Following 2 months of group SH, Tg4-42^{hom} and WT mice were assigned to single cages with FW, BW, or FWI running wheels (a). In the middle of the paradigm at Weeks 7 to 8, the average daily running distance was analyzed among the different groups (b). $^{***}p < .001$. Graph shows mean ± *SD*. SH = standard housing; WT = wild type; FW = free wheel; BW = blocked wheel; FWI = intermittent free/blocked wheel.

(3.554 ± 1.844 km/day; $p < .001$). WT-FW mice (7.181 ± 3.357 km/day) also traveled a longer distance than Tg4-42^{hom}-FW mice (3.939 ± 2.830 km/day) which however did not reach statistical significance (Figure 2(b)).

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^{hom} compared to WT mice housed under BW conditions (~30%, $p < .001$; Figure 3(g)). However, in Tg4-42^{hom} mice,

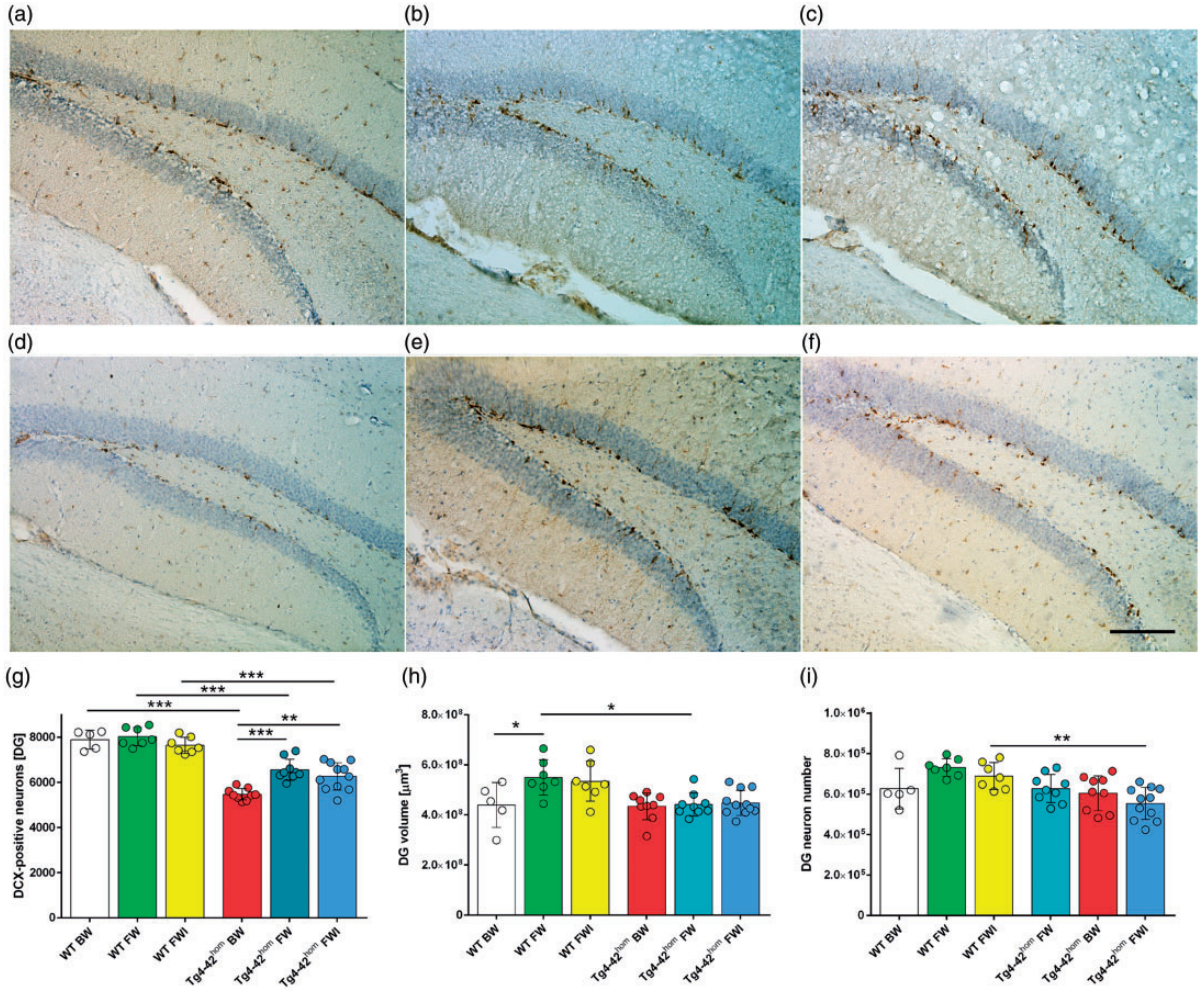

Figure 3. Analysis of neurogenesis, DG volume and neuron number. Representative images of WT (a–c) and Tg4-42^{hom} mice (d–f) housed in either BW (a and d), FW (b and e) or FWI (c and f) conditions. Tg4-42^{hom}-BW mice showed a reduced number of DCX-positive cells in DG compared to WT-BW mice, while 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^{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.

housing 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^{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^{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^{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^{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^{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 ± 99978) 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

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^{hom} and WT mice at the age of 6 months revealed a significantly reduced number of newborn neurons in the animals expressing the A$\beta_{4\text{-}42}$ 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/PS1KI (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., 2013). This is quite interesting, as it has been shown that, for example, APP/PS1ΔEx9 mice show a reduced number of BrdU- and DCX-positive cells at the age of 9 months in comparison to age-matched WT control mice, while no such difference could be detected at an age of 5 months in the absence of amyloid pathology (Taniuchi et al., 2007). A related finding showed that 12- to 14-month-old APP transgenic mice harboring numerous amyloid deposits in the hippocampal formation have 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 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 results in decreased hippocampal neurogenesis (Naumann et al., 2010), while a replacement of endogenous APP with human APP carrying the Swedish mutant did not cause detrimental effects on neurogenesis (Zhang et al., 2007).

We have previously shown that housing under conditions of environmental enrichment for 4 months resulted in a significantly increased number of DCX-positive neurons in the DG of homozygous Tg4-42 mice, while on the contrary, no alterations in DG neurogenesis were detected in heterozygous Tg4-42 mice housed under EE conditions for a prolonged period of 11 months (Hüttenrauch et al., 2016a). The latter observation likely reflects the general age-related drop in the rate of neurogenesis in rodents (Kuhn et al., 1996), which became also evident in the previously observed ~7-fold decreased DCX-positive neuron number of 12-month-old heterozygous Tg4-42 in comparison to homozygous 6-month-old Tg4-42 mice (Hüttenrauch et al., 2016a).

The effect of increased physical activity with regard to disease progression and severity has been intensely studied in preclinical animal models of AD. Several reports have demonstrated that exercise decreased extracellular amyloid pathology (Adlard et al., 2005; Lazarov et al., 2005; Yuede et al., 2009), while others found stable amyloid plaque load (Cotel et al., 2012; Marlatt et al., 2013; Hüttenrauch et al., 2017) or even exacerbated amyloid plaque formation (Jankowsky et al., 2003). Housing under EE conditions led to an improvement of spatial memory deficits as well as an amelioration of CA1 neuron loss in Tg4-42^{hom} mice at 6 months of age without changes in the CA1 volume (Hüttenrauch et al., 2016a). In this study, we investigated whether solely physical activity without further social or environmental stimulation results in beneficial effects on neurogenesis or DG organization and whether this is influenced by periods of inactivity.

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 could be likely attributed to a motor phenotype in the Tg4-42 mice, that becomes evident in tasks addressing motor coordination, such as balance beam, or in general motor performance and motor learning, which have been assessed using the rotarod task (Hüttenrauch et al., 2016a; Wagner et al., 2019). 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^{hom} 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^{hom} 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^{hom} 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

References

Adlard, P. A., Perreau, V. M., Pop, V., & Cotman, C. W. (2005). Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *J Neurosci*, *25*, 4217–4221.

Antonios, G., Borgers, H., Richard, B. C., Brauß A, Meißner, J., Weggen, S., Pena, V., Pillot, T., Davies, S. L., Bakrania, P., Matthews, D., Brownlees, J., Bouter, Y., & Bayer, T. A. (2015). Alzheimer therapy with an antibody against N-terminal Abeta 4-X and pyroglutamate Abeta 3-X. *Sci Rep*, *5*, 17338.

Barnes, D. E., & Yaffe, K. (2011). The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol*, *10*, 819–828.

Biggio, F., Mostallino, M. C., Talani, G., Locci, V., Mostallino, R., Calandra, G., Sanna, E., & Biggio, G. (2019). Social enrichment reverses the isolation-induced deficits of neuronal plasticity in the hippocampus of male rats. *Neuropharmacology*, *151*, 45–54.

Bouter, Y., Dietrich, K., Wittnam, J. L., Rezaei-Ghaleh, N., Pillot, T., Papot-Couturier, S., Lefebvre, T., Sprenger, F., Wirths, O., Zweckstetter, M., & Bayer, T. A. (2013). N-truncated amyloid beta (Abeta) 4-42 forms stable aggregates and induces acute and long-lasting behavioral deficits. *Acta Neuropathol*, *126*, 189–205.

Cotel, M. C., Bayer, T. A., & Wirths, O. (2008). Age-dependent loss of dentate gyrus granule cells in APP/PS1KI mice. *Brain Res*, *1222*, 207–213.

Cotel, M. C., Jawhar, S., Christensen, D. Z., Bayer, T. A., & Wirths, O. (2012). Environmental enrichment fails to rescue working memory deficits, neuron loss, and neurogenesis in APP/PS1KI mice. *Neurobiol Aging*, *33*, 96–107.

Couillard-Despres, S., Winner, B., Schaubeck, S., Aigner, R., Vroemen, M., Weidner, N., Bogdahn, U., Winkler, J., Kuhn, H. G., & Aigner, L. (2005). Doublecortin expression levels in adult brain reflect neurogenesis. *Eur J Neurosci*, *21*, 1–14.

Deng, W., Aimone, J. B., & Gage, F. H. (2010). New neurons and new memories: How does adult hippocampal neurogenesis affect learning and memory? *Nat Rev Neurosci*, *11*, 339–350.

Dong, H., Goico, B., Martin, M., Csernansky, C. A., Bertchume, A., & Csernansky, J. G. (2004). Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. *Neuroscience*, *127*, 601–609.

Fabricius, K., Helboe, L., Steiniger-Brach, B., Fink-Jensen, A., & Pakkenberg, B. (2010). Stereological brain volume changes in post-weaned socially isolated rats. *Brain Res*, *1345*, 233–239.

Fattoretti, P., Malatesta, M., Cisterna, B., Milanese, C., & Zancanaro, C. (2018). Modulatory effect of aerobic physical activity on synaptic ultrastructure in the old mouse hippocampus. *Front Aging Neurosci*, *10*, 141.

Faure, A., Verret, L., Bozon, B., El Tannir El Tayara, N., Ly, M., Kober, F., Dhenain, M., Rampon, C., & Delatour, B. (2011). Impaired neurogenesis, neuronal loss, and brain functional deficits in the APPxPS1-Ki mouse model of Alzheimer's disease. *Neurobiol Aging*, *95*, 92–101.

García-Cabezas, M. Á., John, Y. J., Barbas, H., & Zikopoulos, B. (2016). Distinction of neurons, glia and endothelial cells in the cerebral cortex: An algorithm based on cytological features. *Front Neuroanat*, *10*, 107.

Grégoire, C.-A., Bonenfant, D., Le Nguyen, A., Aumont, A., & Fernandes, K. J. L. (2014). Untangling the influences of voluntary running, environmental complexity, social housing and stress on adult hippocampal neurogenesis. *PLoS One*, *9*, e86237.

Gundersen, H. J., & Jensen, E. B. (1987). The efficiency of systematic sampling in stereology and its prediction. *J Microsc*, *147*, 229–263.

Haughey, N. J., Nath, A., Chan, S. L., Borchard, A. C., Rao, M. S., & Mattson, M. P. (2002). Disruption of neurogenesis by amyloid β-peptide, and perturbed neural progenitor cell homeostasis, in models of Alzheimer's disease. *J Neurochem*, *83*, 1509–1524.

Hüttenrauch, M., Brauss, A., Kurdakova, A., Borgers, H., Klinker, F., Liebetanz, D., Salinas-Riester, G., Wiltfang, J., Klafki, H. W., & Wirths, O. (2016a). Physical activity delays hippocampal neurodegeneration and rescues memory deficits in an Alzheimer disease mouse model. *Transl Psych*, *6*, e800.

Hüttenrauch, M., Salinas, G., & Wirths, O. (2016b). Effects of long-term environmental enrichment on anxiety, memory, hippocampal plasticity and overall brain gene expression in C57BL6 mice. *Front Mol Neurosc*, *9*, 62.

Hüttenrauch, M., Walter, S., Kaufmann, M., Weggen, S., & Wirths, O. (2017). Limited effects of prolonged environmental enrichment on the pathology of 5XFAD mice. *Mol Neurobiol*, *54*, 6542–6555.

Ibi, D., Takuma, K., Koike, H., Mizoguchi, H., Tsuritani, K., Kuwahara, Y., Kamei, H., Nagai, T., Yoneda, Y., Nabeshima, T., & Yamada, K. (2008). Social isolation rearing-induced impairment of the hippocampal neurogenesis is associated with deficits in spatial memory and emotion-related behaviors in juvenile mice. *J Neurochem*, *105*, 921–932.

Jankowsky, J. L., Melnikova, T., Fadale, D. J., Xu, G. M., Slunt, H. H., Gonzales, V., Younkin, L. H., Younkin, S. G., Borchelt, D. R., & Savonenko, A. V. (2005). Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J Neurosci*, *25*, 5217–5224.

Jankowsky, J. L., Xu, G., Fromholt, D., Gonzales, V., & Borchelt, D. R. (2003). Environmental enrichment exacerbates amyloid plaque formation in a transgenic mouse model of Alzheimer disease. *J Neuropathol Exp Neurol*, *62*, 1220–1227.

Jessberger, S., Clark, R. E., Broadbent, N. J., Clemenson, G. D., Consiglio, A., Lie, D. C., Squire, L. R., & Gage, F. H. (2009). Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn Memory*, *16*, 147–154.

Jin, K., Galvan, V., Xie, L., Mao, X. O., Gorostiza, O. F., Bredesen, D. E., & Greenberg, D. A. (2004). Enhanced neurogenesis in Alzheimer's disease transgenic (PDGF-APP-Sw, Ind) mice. *Proc Nat Acad Sci*, *101*, 13363–13367.

Kobilo, T., Liu Q-R, Gandhi, K., Mughal, M., Shaham, Y., & van Praag, H. (2011). Running is the neurogenic and neurotrophic stimulus in environmental enrichment. *Learn Memory*, *18*, 605–609.

Krezymon, A., Richetin, K., Halley, H., Roybon, L., Lassalle J-M, Francès, B., Verret, L., & Rampon, C. (2013). Modifications of hippocampal circuits and early disruption of adult neurogenesis in the Tg2576 mouse model of Alzheimer's disease. *PLoS One*, *8*, e76497.

Kuhn, H. G., Dickinson-Anson, H., & Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation. *J Neurosci*, *16*, 2027–2033.

Lazarov, O., Robinson, J., Tang, Y. P., Hairston, I. S., Korade-Mirnics, Z., Lee, V. M., Hersh, L. B., Sapolsky, R. M., Mirnics, K., & Sisodia, S. S. (2005). Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell*, *120*, 701–713.

Livingston, G., Sommerlad, A., Orgeta, V., Costafreda, S.G., Huntley, J., Ames, D., Ballard, C., Banerjee, S., Burns, A., Cohen-Mansfield, J., & Cooper, C. (2017). Dementia prevention, intervention, and care. *Lancet*, *390*, 2673–2734.

Marlatt, M. W., Potter, M. C., Bayer, T. A., van Praag, H., & Lucassen, P. J. (2013). Prolonged running, not fluoxetine treatment, increases neurogenesis, but does not alter neuropathology, in the 3xTg mouse model of Alzheimer's disease. *Curr Top Behav Neurosci*, *15*, 313–340.

Mattson, M. P. (2015). Lifelong brain health is a lifelong challenge: From evolutionary principles to empirical evidence. *Ageing Res Rev*, *20*, 37–45.

Mayeux, R., & Stern, Y. (2012). Epidemiology of Alzheimer disease. *Cold Spring Harb Perspect Med*, *2*, a006239.

Mirochnic, S., Wolf, S., Staufenbiel, M., & Kempermann, G. (2009). Age effects on the regulation of adult hippocampal neurogenesis by physical activity and environmental enrichment in the APP23 mouse model of Alzheimer disease. *Hippocampus*, *19*, 1008–1018.

Moon, M., Cha, M. Y., & Mook-Jung, I. (2014). Impaired hippocampal neurogenesis and its enhancement with ghrelin in 5XFAD mice. *J Alzheimers Dis*, *41*, 233–241.

Moreno-Jiménez, E. P., Flor-García, M., Terreros-Roncal, J., Rábano, A., Cafini, F., Pallas-Bazarra, N., Ávila, J., & Llorens-Martín, M. (2019a). Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat Med*, *25*, 554–560.

Moreno-Jiménez, E. P., Jurado-Arjona, J., Ávila, J., & Llorens-Martín, M. (2019b). The social component of environmental enrichment is a pro-neurogenic stimulus in adult c57BL6 female mice. *Front Cell Developm Biol*, *7*, 62.

Mu, Y., & Gage, F. H. (2011). Adult hippocampal neurogenesis and its role in Alzheimer's disease. *Mol Neurodegen*, *6*, 85.

Müller, S., Preische, O., Sohrabi, H. R., Gräber, S., Jucker, M., Ringman, J. M., Martins, R. N., McDade, E., Schofield, P. R., Ghetti, B., & Rossor, M. (2018). Relationship between physical activity, cognition, and Alzheimer pathology in autosomal dominant Alzheimer's disease. *Alzheimer Dement*, *14*, 1427–1437.

Mustroph, M. L., Chen, S., Desai, S. C., Cay, E. B., DeYoung, E. K., & Rhodes, J. S. (2012). Aerobic exercise is the critical variable in an enriched environment that increases hippocampal neurogenesis and water maze learning in male C57BL/6J mice. *Neuroscience*, *219*, 62–71.

Naumann, N., Alpár, A., Ueberham, U., Arendt, T., & Gärtner, U. (2010). Transgenic expression of human wild-type amyloid precursor protein decreases neurogenesis in the adult hippocampus. *Hippocampus*, *20*, 971–979.

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nat Rev Neurosci*, *7*, 697–709.

Rodriguez, J. J., Jones, V. C., Tabuchi, M., Allan, S. M., Knight, E. M., LaFerla, F. M., Oddo, S., & Verkhratsky, A. (2008). Impaired adult neurogenesis in the dentate gyrus of a triple transgenic mouse model of Alzheimer's disease. *PLoS One*, *3*, e2935.

Rutten, B. P., Wirths, O., Van de Berg, W. D., Lichtenthaler, S. F., Vehoff, J., Steinbusch, H. W., Korr, H., Beyreuther, K., Multhaup, G., Bayer, T. A., & Schmitz, C. (2003). No alterations of hippocampal neuronal number and synaptic bouton number in a transgenic mouse model expressing the beta-cleaved C-terminal APP fragment. *Neurobiol Dis*, *12*, 110–120.

Santos-Lozano, A., Pareja-Galeano, H., Sanchis-Gomar, F., Quindós-Rubial, M., Fiuza-Luces, C., Cristi-Montero, C., Emanuele, E., Garatachea, N., & Lucia, A. (2016). Physical activity and Alzheimer disease: A protective association. *Mayo Clin Proc*, *91*, 999–1020.

Saxe, M. D., Battaglia, F., Wang J-W, Malleret, G., David, D. J., Monckton, J. E., Garcia ADR, Sofroniew, M. V., Kandel, E. R., Santarelli, L., Hen, R., & Drew, M. R. (2006). Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc Nat Acad Sci*, *103*, 17501–17506.

Scarmeas, N., Levy, G., Tang, M. X., Manly, J., & Stern, Y. (2001). Influence of leisure activity on the incidence of Alzheimer's disease. *Neurology*, *57*, 2236–2242.

Snyder, J. S., Hong, N. S., McDonald, R. J., & Wojtowicz, J. M. (2005). A role for adult neurogenesis in spatial long-term memory. *Neuroscience*, *130*, 843–852.

Sorrells, S. F., Paredes, M. F., Cebrian-Silla, A., Sandoval, K., Qi, D., Kelley, K. W., James, D., Mayer, S., Chang, J., Auguste, K. I., Chang, E. F., Gutierrez, A. J., Kriegstein, A. R., Mathern, G. W., Oldham, M. C., Huang, E. J., Garcia-Verdugo, J. M., Yang, Z., & Alvarez-Buylla, A. (2018). Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature*, *555*, 377.

Stranahan, A. M., Khalil, D., & Gould, E. (2006). Social isolation delays the positive effects of running on adult neurogenesis. *Nat Neurosci*, *9*, 526–533.

Taniuchi, N., Niidome, T., Goto, Y., Akaike, A., Kihara, T., & Sugimoto, H. (2007). Decreased proliferation of hippocampal progenitor cells in APPswe/PS1dE9 transgenic mice. *Neuroreport*, *18*, 1801–1805.

van Praag, H., Kempermann, G., & Gage, F. H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci*, *2*, 266–270.

Wagner, J. M., Sichler, M. E., Schleicher, E. M., Franke, T. N., Irwin, C., Löw, M. J., Beindorff, N., Bouter, C., Bayer, T. A., & Bouter, Y. (2019). Analysis of motor function in the Tg4-42 mouse model of Alzheimer's disease. *Front Behav Neurosci*, *13*, 107.

Wirths, O. (2017). Altered neurogenesis in mouse models of Alzheimer disease. *Neurogenesis*, *4*, e1327002.

Yetman, M. J., & Jankowsky, J. L. (2013). Wild-type neural progenitors divide and differentiate normally in an amyloid-rich environment. *J Neurosci*, *33*, 17335–17341.

Yuede, C. M., Zimmerman, S. D., Dong, H., Kling, M. J., Bero, A. W., Holtzman, D. M., Timson, B. F., & Csernansky, J. G. (2009). Effects of voluntary and forced exercise on plaque deposition, hippocampal volume, and behavior in the Tg2576 mouse model of Alzheimer's disease. *Neurobiol Dis*, *35*, 426–432.

Zhang, C., McNeil, E., Dressler, L., & Siman, R. (2007). Long-lasting impairment in hippocampal neurogenesis associated with amyloid deposition in a knock-in mouse model of familial Alzheimer's disease. *Exp Neurol*, *204*, 77–87.