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Late running is not too late against Alzheimer's pathology

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ABSTRACT

In the last decade a vast number of animal studies have produced overwhelming evidence that exercise not only compensates for memory loss by increasing brain plasticity and cognitive reserve but also directly counteracts Alzheimer-like pathology when provided before disease onset or in early disease stages. But so far, there is little knowledge about therapeutic effects of training when started in advanced disease stages. In the present study we show that following seven months of sedentary life style five months of wheel running, started four months after disease onset was still able to mitigate at least some aspects of the full-blown Alzheimer's pathology in TgCRND8 mice. Late running had mild but significant effects on structural plasticity by increasing the dendritic complexity. It further reduced beta-amyloid (Aβ) plaque burden and enhanced Aβ clearance across the blood-brain barrier, along with attenuating microgliosis, inflammation, oxidative stress, and autophagy deficits, resulting in better memory performance and less agitation. However, unlike early exercise, late running did not affect abnormal amyloid precursor protein metabolism, tau pathology, or angiogenesis. These results allow concluding that it is never too late to counteract Alzheimer's disease with physical training but the earlier the intervention starts, the more pronounced is the therapeutic potential.

1. Introduction

A growing body of epidemiological evidence indicates that an active lifestyle, defined by premorbid physical and cognitive activities, counteracts Alzheimer's disease-related cognitive decline and decreases the risk of developing dementia (Craik et al., 2010; Fratiglioni et al., 2004; Friedland et al., 2001; Okonkwo et al., 2014; Wilson et al., 2002).

More than two decades ago, the hypothesis of the cognitive reserve has been postulated, claiming that high diversity and intensity of mental performance increases synaptic complexity/brain plasticity, therefore leading in upgraded resistance against neurodegenerative diseases such as Alzheimer's disease (Katzman, 1993). Since then, various animal studies supported the cognitive reserve hypothesis as they have demonstrated that environmental enrichment, imitating a physically and cognitively active lifestyle, improves neuroplasticity, due to up-regulation of neurotrophic factors, increase of dendritic and synaptic density, and enhancement of hippocampal neurogenesis in healthy and Alzheimer's disease-affected animals (Herring et al., 2009; Hu et al., 2010; Nithianantharajah and Hannan, 2006; Rodriguez et al., 2011; van Praag et al., 2000; Wolf et al., 2006).

About one decade ago, the first animal studies provided evidence that environmental enrichment (in form of exclusive physical training or as a combination of both physical and cognitive stimulation) not only acts compensatively by induction of the cognitive reserve, but also directly intervenes with Alzheimer's disease as it mitigates beta-amyloid (Aβ) pathology (Adlard et al., 2005; Ambree et al., 2006a, 2006b; Lazarov et al., 2005). What followed was a plethora of investigations substantiating that enrichment antagonizes almost every known feature of Alzheimer's disease-like pathology; it ameliorates tauopathy (Lahiani-Cohen et al., 2011; Ohia-Nwoko et al., 2014), normalizes immune response (Ambree et al., 2006a, 2006b; Arranz et al., 2011; Beauquis et al., 2013; Nichol et al., 2008; Parachikova et al., 2008), counteracts neurovascular dysfunction (Herring et al., 2008), reduces cerebral oxidative stress via anti-oxidant mechanisms (Bo et al., 2014; Garcia-Mesa et al., 2015; Herring et al., 2010; Marques-Aleixo et al., 2012), prevents Aβ-triggered impairment of hippocampal long-term potentiation (Li et al., 2013), and delays hippocampal and amygdalar neurodegeneration (Lin et al., 2015), resulting in reduced cognitive disturbance (Berardi et al., 2007; Chao et al., 2015; Cho et al., 2015; Costa et

Abbreviations: Aβ, beta-amyloid; APP, amyloid precursor protein; BBB, blood-brain barrier; CTFβ, C-terminal fragment β of APP; sAPPα, soluble APP α fragment.

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al., 2007; Jankowsky et al., 2005; Polito et al., 2014; Tapia-Rojas et al., 2015; Wang et al., 2013) and anxiety (Gortz et al., 2008; Pietropaolo et al., 2014).

Additional studies demonstrated that the time point when enrichment starts dictates the pathways that conduct resistance to Alzheimer's disease pathology. At the earliest possible time, in utero stimulation via maternal running during pregnancy mitigates multiple attributes of Alzheimer's disease-related pathology in the offspring as it diminishes amyloidogenic processing of the amyloid precursor protein (APP) and the load of soluble Aβ peptides and insoluble Aβ plaques, improves neurovascular function, reduces oxidative stress and inflammation and induces structural plasticity (Herring et al., 2012). Postnatal environmental stimulation provided before disease onset (during preadolescence) alters APP processing, increases Aβ degradation and reduces cerebral levels of soluble Aβ, whereas stimulation after disease onset in an early stage of Alzheimer's disease-like pathology seems to enhance the anti-oxidative capacity and to disturb Aβ aggregation (Herring et al., 2011), with both treatment paradigms being capable of Aβ plaque reduction. Furthermore, three to nine months of exercise or combined physical and cognitive stimulation started in a moderate disease stage decreases cerebral levels of synaptotoxic Aβ oligomers, diminishes tau pathology, improves cognition and reduces oxidative stress (Blazquez et al., 2014; Garcia-Mesa et al., 2015; Garcia-Mesa et al., 2012). Until now, only very few studies have examined the effects of exercise in late disease stages, whereby only memory performance and Aβ plaque pathology were considered. It has been shown that short-term physical exercise for three to five weeks experienced in a full-blown stage of disease is still capable of producing some cognitive improvements but ineffective to influence Aβ plaque pathology (Ke et al., 2011; Nichol et al., 2007; Parachikova et al., 2008). Data on long-term effects of late exercise on Alzheimer's disease-related pathology are completely lacking.

In the present study we, for the first time, tested whether late and prolonged wheel running for five months, started in a full-blown disease stage, i.e. from seven to twelve months of age, is still sufficient to impact different parameters of Alzheimer's disease-related pathology, i.e. memory deficits and agitation, faulty structural plasticity, Aβ and tau pathology, neurovascular dysfunction, inflammation, oxidative stress and autophagy defects.

2. Materials and methods

2.1. General

All data presented in this paper were generated in blind-coded experiments, in which the person who collected the data was unaware of the specific genotype and treatment of mice. Protein, peptide and mRNA levels were quantified individually in duplicates or triplicates in separate brain areas.

2.2. Experimental housing

Female TgCRND8 mice (transgenic, hemizygously carrying and overexpressing a double-mutant human APP 695 transgene [*hAPP*+/−] harbouring the “Swedish” and “Indiana” mutations [KM670/671NL & V717F] under the control of the hamster Prion protein promoter) (Chishti et al., 2001) as well as wildtype (C57BL/6-C3H/HeJ hybrid background) littermates were used. In this mouse model multiple plaque deposits are present in most mice at 65 and in all mice by 90 days of age. At the age of 210 days the neocortex and hippocampus is infested with numerous diffuse and core plaques, with increasing plaque burden during ageing (see Fig. 1B for plaque ontogeny). To test the influence of late and prolonged physical stimulation on Alzheimer's disease-like pathology, transgenic and wildtype mice were kept in groups of three to four in standard housing cages (Makrolon type III) without access to physical stimulation until postnatal day 210 (P210). From P210 until P360 in a full-blown stage of Aβ pathology, animals were kept single in standard housing cages (nine mice per genotype) or in cages equipped with running wheels (six transgenic mice; nine wildtype mice) (for experimental housing setup see Fig. 1A). Mice had access to running wheels for 24 h per day, seven days per week, for five months. Running performance was individually monitored by a bicycle computer. To minimize a biased effect of the parental genotype on the phenotype, equal numbers of transgenic and wildtype mice per litter were attributed to standard housing or running wheel cages. Due to increased aggressive behaviour when housed in single-sex groups male mice were excluded from this study.

2.3. Behavioural phenotyping

Mice were behaviourally phenotyped between P361 and P366. Prior to testing they had been adapted to the inverted day/night cycle for one week. At P361, mice were tested in the Open Field for exploratory behaviour and general activity according to (Kilic et al., 2010). The Open Field arena (52 × 52 × 30 cm) was located 72 cm above the floor on a circular platform. Each mouse was placed near the wall and observed for 10 min. The test arena was divided into one center (31.2 × 31.2 cm), four border (each 10.4 × 31.2 cm) and four corner (each 10.4 × 10.4 cm) areas. Number of entries, latencies until first time entries, duration and distance covered in each area as well as velocity were automatically tracked and analysed by Video Mot 3D software (TSE, version 7.0.1). From P362 until P366, hippocampus-associated spatial memory and learning performance was assessed by the Barnes Maze according to (Sunyer et al., 2007). The Barnes Maze arena consisted of a circular platform (92 cm diameter, 120 cm above the ground) with 20 equally distributed holes (5 cm diameter, 7.5 cm distance between holes) located at the border. One hole (escape hole) was connected to a box (15.5 × 9.5 × 6 cm), allowing to escape from the Barnes Maze platform, whereas the other 19 holes were closed (error holes). 24 h before tests started, mice were habituated to the setup for two trials, each lasting for 3 min. Tests were performed between 10:00 am and 6:00 pm. Each mouse was placed in a black cylinder located in the middle of the platform for 10 s. During that time, red light was switched to bright light (180 lx) and the cylinder was lifted, defining the start of a 3 min trial. During each trial, primary errors, total errors, primary latency, total latency, path length covered and velocity were automatically recorded. Primary errors and latencies were defined as the number and duration of approximations to error holes until approaching the escape hole for the first time. Total errors and latencies were defined as the number and duration of approximations to error holes and to the escape hole until final escape. Once a mouse escaped, it was allowed to stay in the escape box for 1 min before being transferred to the home cage. If a mouse did not escape during the 3 min interval, it was gently guided to the escape hole until the mouse escaped; otherwise it was placed directly into the escape box for 1 min. On test day 1, each mouse was tested in four trials. On test days 2, 3, 4 and 5, each mouse was tested in two trials. Inter-trial intervals lasted for 15 min. Following the four test days, the escape hole was blocked at day 5 (probe trial). Mice were allowed to explore the platform for 90 s per trial. Before and after each test, the Open Field and Barnes Maze arenas were cleaned with 70% ethanol.

2.4. Blood and tissue sampling

Following behavioural testing, blood was collected at P367 from retrobulbar venous plexus of each transgenic animal to determine baseline circulating plasma Aβ levels (time: 0 min = t_0). In order to quantify Aβ efflux efficacy across the blood-brain barrier (BBB), transgenic mice received an intravenous (tail vein) injection of an Aβ stabilizing anti-Aβ antibody (HJ5.1, 150 μg/animal) (purchased from Dr. David M. Holtzman's lab, St. Louis, Missouri (Castellano et al., 2012)) that only marginally enters brain parenchyma and does not affect the Aβ-brain-