Neurobiology of Disease 104 (2017) 50–60

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Neuron and neuroblast numbers and cytogenesis in the dentate gyrus of aged $APP_{swe}/PS1_{dE9}$ transgenic mice: Effect of long-term treatment with paroxetine

Louise Ørum Olesen [a], Mithula Sivasaravanaparan [b], Maurizio Severino [b], Alicia A. Babcock [b], Elena V. Bouzinova [a], Mark J. West [c], Ove Wiborg [a,1], Bente Finsen [b,*,1]

[a] Department of Clinical Medicine, Aarhus University, Denmark
[b] Department of Neurobiological Research, Institute of Molecular Medicine, University of Southern Denmark, Denmark
[c] Department of Biomedicine, Health, Aarhus University, Denmark

ARTICLE INFO

Article history:
Received 31 December 2016
Revised 7 April 2017
Accepted 27 April 2017
Available online 28 April 2017

Keywords:
Neurogenesis
Hippocampus
APP/PS1 mice
SSRI
Paroxetine
Spontaneous alternation behaviour
Granule cells
Amyloid plaque load

ABSTRACT

Altered neurogenesis may influence hippocampal functions such as learning and memory in Alzheimer's disease. Selective serotonin reuptake inhibitors enhance neurogenesis and have been reported to reduce cerebral amyloidosis in both humans and transgenic mice. We have used stereology to assess the longitudinal changes in the number of doublecortin-expressing neuroblasts and number of granular neurons in the dentate gyrus of $APP_{swe}/PS1_{dE9}$ transgenic mice. Furthermore, we investigated the effect of long-term paroxetine treatment on the number of neuroblasts and granular neurons, hippocampal amyloidosis, and spontaneous alternation behaviour, a measure of spatial working memory, in transgenic mice. We observed no difference in granular neurons between transgenic and wild type mice up till 18 months of age, and no differences with age in wild type mice. The number of neuroblasts and the performance in the spontaneous alternation task was reduced in aged transgenic mice. Paroxetine treatment from 9 to 18 months of age reduced hippocampal amyloidosis without affecting the number of neuroblasts or granular neurons. These findings suggest that the amyloidosis affects the differentiation of neuroblasts and spatial working memory, independent of changes in total granular neurons. Furthermore, while long-term paroxetine treatment may be able to reduce hippocampal amyloidosis, it appears to have no effect on total number of granular neurons or spatial working memory.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that primarily affects the medial temporal lobe and associative neocortical areas (Terry et al., 1991; West, 1993). During physiological aging there is only a minor or no neuronal loss in the hippocampus in humans, while there is a substantial loss of pyramidal neurons in regio superior hippocampus (CA1) in AD (Bobinski et al., 1997; Bobinski et al., 1996; Gomez-Isla et al., 1996; Kordower et al., 2001; Price et al., 2001; Simic et al., 1997; West, 1993; West et al., 1994). In comparison to the very dramatic changes in CA1, there is overall no (Bobinski et al., 1996; Korbo et al., 2004; West et al., 1994; West et al., 2004) or at most a minor (<30%) loss of granule cells in the hippocampal dentate gyrus (DG) (Bobinski et al., 1997). The apparent protection of the DG is particularly interesting considering that the DG is an integrated part of the hippocampal circuitry and the site of adult hippocampal neurogenesis. Adult hippocampal neurogenesis is known to contribute to the processing and the storage of new information (Leuner et al., 2006; Shors et al., 2001), and impaired neurogenesis might therefore affect aspects of learning and memory processes in AD (Haughey et al., 2002; Hernandez-Rabaza et al., 2009), independent of the functional impairment resulting from the pyramidal cell loss in CA1.

The granule cell layer in the DG is maintained by hippocampal neurogenesis throughout life. During this process precursor cells in the subgranular zone of the DG proliferate to generate neuroblasts that differentiate into granular neurons (Cameron and McKay, 2001; Eriksson et al., 1998; Kempermann et al., 2003), which become functionally incorporated into the hippocampal formation (van Praag et al., 2002). Neurogenesis decreases as part of natural aging processes (Heine et al., 2004; Kempermann et al., 2006; Kuhn et al., 1996; van Praag et al., 2005). Although, molecular data has suggested hippocampal neurogenesis to be affected beyond the effects of normal aging (Winner et al., 2011), morphometric data has failed to show evidence of altered neurogenesis in AD (Boekhoorn et al., 2006; Haughey et al., 2002; Heine et al., 2004; B. Li et al., 2008). Most studies of hippocampal neurogenesis in transgenic

* Corresponding author at: Department of Neurobiology Research, University of Southern Denmark, J.B. Winsløws Vej 25, DK-5000 Odense C, Denmark.
E-mail address: bfinsen@health.sdu.dk (B. Finsen).
[1] These authors contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.nbd.2017.04.021
0969-9961/

mouse models of AD report a decrease in neurogenesis (Dong et al., 2004; Donovan et al., 2006; Faure et al., 2011; Haughey et al., 2002; Rodriguez et al., 2008; Zhang et al., 2007), however, increased neurogenesis has also been reported (Chevallier et al., 2005; Jin et al., 2004; Yu et al., 2009). The $APP_{swe}/PS1_{dE9}$ mouse model of AD is well characterised with regard to Aβ pathology (Garcia-Alloza et al., 2006; Jankowsky et al., 2004) and behavioural deficits (Lalonde et al., 2004; Lalonde et al., 2005; O'Leary and Brown, 2009; Reiserer et al., 2007). A fairly consistent finding in this mouse model is a decrease in proliferation and/or survival of the new-born granular neurons in the DG of 2- to 15-month-old transgenic mice (Demars et al., 2010; Hamilton and Holscher, 2012; D. Li et al., 2008; Taniuchi et al., 2007; Verret et al., 2007; Yu et al., 2009).

A dysfunction of the serotonergic system has been implicated both in adult hippocampal neurogenesis, as it has been shown to be stimulated with selective serotonin reuptake inhibitors (SSRIs) in some (Dong et al., 2004; Imoto et al., 2015; Kobayashi et al., 2010; Malberg et al., 2000), but not all (Marlatt et al., 2013) studies, and in the symptoms and pathology of AD (Hendricksen et al., 2004; Trillo et al., 2013; Vermeiren et al., 2014). The $APP_{swe}/PS1_{dE9}$ mouse model also exhibits a progressive loss of serotonergic afferents in the hippocampus, along with reduced levels of 5-HT and a loss of serotonergic neurons in the raphe nuclei that correlates with the amount of Aβ deposits (Liu et al., 2008). In AD, post mortem studies have demonstrated reductions in the density of serotonergic neurons in the raphe nuclei (Hendricksen et al., 2004; Lyness et al., 2003), which correlate with reduced levels of serotonin (5-HT) in various brain areas (reviewed in (Garcia-Alloza et al., 2005, Rodriguez et al., 2012). Treatment with SSRIs has been found to improve cognitive symptoms in AD (Hasselbalch et al., 2008) and to reduce Aβ load in humans and in the $APP_{swe}/PS1_{dE9}$ mouse model, by mechanisms involving reduced Aβ production (Cirrito et al., 2011; Sheline et al., 2014).

The $APP_{swe}/PS1_{dE9}$ mouse develops Aβ pathology similar to that seen in humans (Babcock et al., 2015; Garcia-Alloza et al., 2006; Jankowsky et al., 2004), and exhibits cognitive and behavioural deficits related to amyloidosis (Lalonde et al., 2004; Lalonde et al., 2005; O'Leary and Brown, 2009; Reiserer et al., 2007). While, there is evidence suggesting that the process of neurogenesis taking place in the $APP_{swe}/PS1_{dE9}$ mouse mirrors neurogenesis as it takes place in AD (Demars et al., 2010; Hamilton and Holscher, 2012; D. Li et al., 2008; Taniuchi et al., 2007; Verret et al., 2007), there are still no studies showing whether or not aged $APP_{swe}/PS1_{dE9}$ mice exhibit alterations in cytogenesis in the subgranular zone or in the total number of neuroblasts or granular neurons in the DG. Here, we used a stereological approach, a general cell stain and differentiation stage specific markers to study the process of neurogenesis in $APP_{swe}/PS1_{dE9}$ mice of different ages. Furthermore, we examined the effect of long-term treatment with paroxetine on the neurogenic process, and on spontaneous alternation behaviour, and hippocampal Aβ plaque load in the $APP_{swe}/PS1_{dE9}$ mice. Paroxetine treatment was initiated at 9 months of age, which is prior to any detectable loss of the serotonergic neurons, and terminated at 18 months of age, when significant numbers of the serotonergic neurons are lost in the $APP_{swe}/PS1_{dE9}$ mice (Liu et al., 2008). Hippocampal dependent learning and memory has previously been shown to be associated with adult neurogenesis (Thuret et al., 2009). Spontaneous alternation behaviour is a measure of spatial working memory (Lalonde, 2002), and has not previously been reported for aged $APP_{swe}/PS1_{dE9}$ mice or for aged mice treated for 9 months with paroxetine. We therefore examined the performance of $APP_{swe}/PS1_{dE9}$ mice in the spontaneous alternation test prior to, and after, 9 months of treatment with paroxetine.

2. Materials and methods

2.1. Animals

Double transgenic $APP_{SWE}/PS1_{dE9}$ (Tg) mice, and littermate wild type (Wt) mice were bred on a B6C3 hybrid background (C57BL/6 × C3H/HeN), either in the Biomedical Laboratory, University of Southern Denmark (n = 38) or at Taconic A/S, Denmark (n = 54). Animals that survived to 18 months of age were transferred to TNU, Aarhus University Hospital just before they turned 9 months of age. Mice were genotyped at Taconic or in house, and animals carrying the rd/rd mutation, leading to reduced vision (Jimenez et al., 1996; Garcia et al., 2004), were excluded. A total of 92 male mice were included in this study. Animals were individually housed from 9 months of age, and kept under standard laboratory conditions, i.e. food and water ad libitum, 12-h light/dark cycle (lights on at 6:00 p.m.) with constant temperature (21 ± 2 °C) and humidity (52 ± 2%). All mice had enriched environments that consisted of wood splints bedding, chewing sticks, access to bedding material, and a mouse house. Cages were from Tecniplast, type III (425 × 266 × 155 mm). The Danish National Committee for Ethics in Animal Experimentation approved all animal procedures (J. no. 2011/561-1950 and J. no. 2013-15-2934-00814).

2.2. Paroxetine treatment

Paroxetine (Seroxat® oral solution 2 mg/ml, GSKline) was administered in the drinking water initially at a dose of 30 mg/kg/day, which was reduced to 10 mg/kg/day and finally 5 mg/kg/day, corresponding to reported intraperitoneal dosing of 3 × TgAD mice (Nelson et al., 2007). Tap water was used as the vehicle. Treatment was initiated at 9 months of age. In order to confirm that blood concentrations of paroxetine were within therapeutic range, serum paroxetine concentrations were assessed by HPLC every second month, as described in Olesen et al. (2016).

2.3. Spontaneous alternation behaviour

All experiments were carried out during the first half of the 12-h light cycle. Between experiments, the maze was cleaned thoroughly with water wetted paper towels and dried with cloth. The mice were tested prior to treatment starting at 9 months of age and again at 18 months of age after 9 months of treatment. At 9 months of age, a total of 72 (32 Tg and 40 Wt) mice were included. At 18 months of age, a total of 52 (11 TgVeh; 5 TgPrx; 21 WtVeh; 15 WtPrx) mice were included in the analysis.

The Y-maze had three arms (40 cm long × 8 cm wide), surrounded by clear Plexiglas walls (16 cm high). The mice were individually placed at the end of one arm, facing the end of the arm, and were allowed to explore all the arms freely for the 8 min testing period. The sequence of entries and the total number of entries were recorded by a trained observer. A correct alternation triplet was defined as a visit to all three consecutive arms in one sequence. The total number of arm entries minus 2 provides the maximum number of correct alternation triplets. The alternation rate was calculated as the number of correct alternation triplets divided by the maximum number of correct alternation triplets. Animals with <10 entries were excluded from analysis.

2.4. BrdU administration

To label proliferating cells, the thymidine analogue 5-bromo-2′ deoxyuridine (BrdU) was administered intraperitoneally. Four injections of BrdU (100 mg/kg dissolved in phosphate buffered saline (PBS) to a final concentration of 20 mg/ml, pH 7.4) were given at 2 hour intervals during one day. The animals were sacrificed 16 h after the last injection.

2.5. Tissue processing

The mice were anaesthetized by an overdose of sodium pentobarbital and transcardially perfused with 10 ml of Sorensens phosphate buffer, pH 7.4 followed by 20 ml of 4% paraformaldehyde (PFA) (pH = 7.2–