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Research report

Targeting neurogenesis ameliorates danger assessment in a mouse model of Alzheimer's disease

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HIGHLIGHTS

- 3xTgAD mice show impaired danger assessment and reduced neurogenesis.
- Overexpressing Wnt3a in the ventral hippocampus dentate gyrus improves their behavior.
- The behavioral improvement is neurogenesis dependent.
- Neurogenesis may be a therapeutic target for alleviating behavioral deficits in AD patients.

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ABSTRACT

Alzheimer's disease (AD) affects 13% of the population over the age of 65. Behavioral and neuropsychiatric symptoms are frequent and affect 80% of patients. Adult hippocampal neurogenesis, which is impaired in AD, is involved in learning and memory. It remains unclear, however, whether increasing adult neurogenesis improves behavioral symptoms in AD. We report that in the 3xTgAD mouse model of AD, chronic Wnt3a overexpression in the ventral hippocampus dentate gyrus (DG) restored adult neurogenesis to physiological levels. The restoration of adult neurogenesis led to full recovery of danger assessment impairment and the effect was blocked by ablation of neurogenesis with X-irradiation. Finally, using a bed nucleus of stria terminalis (BNST) mRNA expression array, we found that the expression of the 5-HT_{1A} receptor in 3xTgAD mice is selectively decreased and normalized by Wnt3a overexpression in the ventral hippocampus DG, and this normalization is neurogenesis dependent. These findings indicate that reestablishing a functional population of hippocampal newborn neurons in adult AD mice rescues behavioral symptoms, suggesting that adult neurogenesis may be a promising therapeutic target for alleviating behavioral deficits in AD patients.

1. Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder in the world [1]. It is characterized by cognitive, motor, and behavioral symptoms [2]. The behavioral symptoms are extremely common and often much more troubling than the amnestic ones. AD patients exhibit a wide range of behavioral manifestations, including depression, disinhibition, delusions, hallucinations, agitation, anxiety, and aggression [3,4]. Inhibition and disinhibition may differ from one patient to another. Some AD patients are excessively inhibited because of anxiety, whereas others display a loss of inhibitory control, often accompanied by irritability and hostility [5,6]. Currently there is no beneficial treatment for the behavioral symptoms of the disease and the most successful interventions are symptom-specific.

Impairment of adult neurogenesis was reported in a variety of models relevant to behavioral and neuropsychiatric diseases such as anxiety, depression, schizophrenia, and AD [7–9]. New neurons are generated in mammals throughout life in two distinct neurogenic niches: the subventricular zone and the subgranular zone in the hippocampal dentate gyrus (DG) [10]. Newborn neurons exhibit unique electrophysiological characteristics and form a specific cell population particularly inclined to undergo activity-dependent plasticity [11–13]. Therefore, the incorporation of even a small number of functional adult-generated neurons into existing neural networks creates a higher capacity for plasticity that affects memory, learning, and behavior. Thus, targeting adult neurogenesis as a therapy for diseases associated with cognitive and behavioral impairments, as in the case of AD, can be beneficial.

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Indeed, several studies were able to demonstrate improvement in cognitive deficits in AD models by modifying neurogenesis [14–16], but the effect of targeting adult neurogenesis on the behavioral symptoms of AD remains unexplored.

Until recently the hippocampus was regarded as a homogenous formation but now is viewed as a functionally heterogeneous structure along its axis [17–19]. The dorsal hippocampus is involved in learning and memory, whereas the ventral hippocampus regulates emotional behavior. These differences originate from differences in the afferent and efferent connectivity of the hippocampus along the longitudinal axis [20–22]. Moreover, neurogenesis in the dorsal and ventral DG contributes differently to learning and to regulation of emotion [23–28]. One study reported differential effects of chronic mild stress on dorsal and ventral DG proliferation [29]. Recent studies have suggested that antidepressants regulate behavior by selectively increasing ventral hippocampal neurogenesis [30,31]. Thus, selectively stimulating neurogenesis in the ventral hippocampus may affect the behavioral symptoms of AD patients.

Several studies with AD mouse models have reported that although proliferation of progenitor cells in the subgranular zone is increased, most of these cells die and do not become mature neurons [32]. The neurogenic deficit occurs before the development of AD pathology and clinical symptoms [14]. Thus, the preferred approach to improve neurogenesis in AD models is to improve the differentiation and survival of the proliferating cells in the early stage of the disease.

In the present study we overexpressed Wnt3a in the ventral hippocampus DG of 3-months old 3xTgAD mice to investigate this approach. Wnt proteins are extracellular factors that play important roles in the developed and mature central nervous system. They regulate the proliferation of neural progenitor cells and their differentiation to neurons in the DG [33–37]. Moreover, the Wnt signaling pathway is an obligate component of neural progenitor cell differentiation into neurons [38]. We found that behavior response to environmental stimuli in 3xTgAD mice is impaired and that enhanced neurogenesis can reverse this impairment.

2. Materials and methods

2.1. Animals

Breeding pairs of triple-transgenic AD mice (3xTgAD, homozygous mice that express human APPswe, tauP301L, and PS1M146V mutations) and their background strain (129/Sv × C57BL/6) were obtained from Jackson Laboratories (Bar Harbor, Maine, USA). The colony was established at the Tel-Aviv University, Israel. The 3xTgAD mice were regularly genotyped to confirm the purity of the colony. Experiments were performed using 3xTgAD and non-Tg males. Mice were maintained under a 12-h light/12-h dark cycle (lights on at 6:00 AM) with continuous access to food and water. Behavioral testing is performed between 8:00 AM and 5:00 PM. All animal studies were approved by the Animal Care and Use Committee of Tel-Aviv University.

2.2. High titer lentiviral preparation

Lentiviral vectors (LVs) were prepared as previously described [39]. To overexpress Wnt3a, we used our previously described LV [39], which expresses the mouse Wnt3a coding sequence (LV-Wnt3a), and an LV that expresses only green fluorescent protein as a control vector (LV-green fluorescent protein (GFP)). The ability of LV-Wnt3a vectors to express functional Wnt3a was assessed by Western blot analysis and functional signaling, as described previously [39].

2.3. Intrahippocampal injections of lentiviral vectors

At the age of 3 months, 3xTgAD mice and their WT littermates were anesthetized with ketamine/xylazine and placed in a stereotactic frame. WT mice received either PBS ($n = 7$) or LV-GFP ($n = 7$), and the 3xTgAD mice were administered either LV-GFP ($n = 13$) or LV-Wnt3a ($n = 13$). Viral preparations in 2-μL volume were injected bilaterally into the DG region of the ventral hippocampus using the following coordinates: ±2.3 mm medial/lateral, −3.2 mm anterior/posterior, 2.6 mm dorsal/ventral from the bregma and according to the atlas of Paxinos and Franklin [40]. In-vivo expression of Wnt3a was validated using real-time PCR of Wnt3a. Immunostaining with anti-hemagglutinin (HA) antibody, as described previously [39], was used to confirm the accuracy of the injection site. Anti-GFP (Sigma) antibody was used to detect GFP fluorescence, as detailed [39]. Wnt signaling activation was assessed by Western blot analysis of active β-catenin (anti-ABC) as previously described [39]. Active β-catenin detects the active dephosphorylated form of β-catenin that accumulates in the cytoplasm and translocates into the nucleus following Wnt signaling activation.

2.4. Behavioral studies

Starting at age 7 months, mice were tested in five behavioral tasks: emergence test, open field, elevated plus maze (EPM), novel object test, and rat exposure test. There was a week's interval between each task. The emergence test, the EPM, and the open-field test were used to assess normal avoidance from novel stimuli. The open-field test was also used to measure general locomotor activity. The rat exposure test was used to evaluate the defensive reaction to predator exposure. The novel object test was used to estimate the exploration of a novel object in a non-threatening environment. A detailed description of these tests can be found in the Supplementary information.

2.5. Brain tissue collection

Immediately after decapitation, the brain was removed and placed in a steel brain matrix, 1.0 mm, coronal (Zivic Instruments, Pittsburgh, PA, USA). The brains were sliced into 2 mm slices using standard razor blades, and were quickly frozen on dry ice. The area of interest was punched out using a microdissecting needle of 14G. The bed nucleus of stria terminalis (BNST), amygdala, prefrontal cortex, hippocampus, paraventricular nucleus of the hypothalamus and periaqueductal were removed using this method. Punches were immediately stored at −80 °C.

2.6. Neurogenesis assessment

A different group of mice treated with LV at the age of 3 months ($n = 6$) was injected with 50 mg/kg 5-ethynyl-2′-deoxyuridine (EdU) (Invitrogen, Carlsbad, CA, USA) for 5 consecutive days at the age of 6 months. Four weeks after the end of EdU administration, brains were assessed for neurogenesis. Neurogenesis in the DG was evaluated by counting of cells that were labeled with doublecortin (Dcx) and co-labeled with EdU/Neuronal Nuclei (NeuN). A detailed description of this procedure and quantification can be found in the Supplementary information.

2.7. X-irradiation

X-ray irradiation was performed using a modified protocol from previously reported studies [41]. All mice ($n = 60$) were anesthetized with ketamine/xylazine, then only X-irradiated mice ($n = 30$) were exposed to focal irradiation using a Stabilipan