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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.

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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

machine (Siemens, Erlangen, Germany). A detailed description of this procedure can be found in Supplementary Information.

2.8. RNA extraction

RNA was extracted using a 5 PRIME PerfectPure RNA Cell and Tissue Kit (5 Prime GmbH, Hamburg, Germany). Total RNA (20 μg) was used to synthesize cDNA using a SuperScript III First-Strand Synthesis System for the RT-PCR kit (Invitrogen).

2.9. mRNA expression array

Taqman custom-made array (Applied Biosystems, Foster City, CA, USA) was used. Thirty genes of interest were chosen in addition to two housekeeping genes, Hypoxanthine guanine phosphoribosyl transferase 1 (HPRT1) and ribosomal protein S18, and run on 96-well microplates using StepOnePlus™ Real-Time PCR Systems (Applied Biosystems). Analysis of gene expression was determined by DataAssist (Applied Biosystems). We performed the mRNA expression array in the BNST and amygdala. The genes tested in the array were: Adra1b, Adra2a, Adra2c, Adrb1, Adrb2, Adrbk1, Avp, Bdnf, Comt1, Crh, Crhbp, Drd1a, Drd2, Fkbp5, Gabra2, Gabra3, Gabra5, Gabrd, Galr1, Htr1a, Htr2c, Htr7, MHC, Npy, Nr3c1, Nr3c2, Oprm1, Oxt, Slc6a2, slc6a3. The mRNA expression of 5-HT_{1A} receptor was tested in the prefrontal cortex, hippocampus, paraventricular nucleus of the hypothalamus and periaqueductal grey using the Taqman assay.

2.10. Statistical analysis

Data were analyzed using SPSS software (SPSS, Chicago, IL, USA). Values are presented as mean ± s.e.m. Statistical analyses were performed using one-way analysis of variance (ANOVA) on behavioral tests, cell proliferation and differentiation, and mRNA expression array experiments; two-way ANOVA with repeated measures on the novel object test; and two-way ANOVA without repeated measures on the X-irradiation study. The ANOVAs were followed by Tukey or Sidak (for two-way ANOVA) *post hoc* comparisons. Differences between the two groups were compared using a two-tailed *t* test. The results were considered significant at $p < 0.05$.

3. Results

3.1. Long-term expression of Wnt3a in the mouse brain

We first tested the time course of Wnt3a expression after stereotaxic hippocampal injection of high-titer LV-Wnt3a or LV-GFP as negative control in non-Tg mice. We used a lentiviral-based system expressing mouse Wnt3a under a recently designed and constructed [39] cytomegalovirus promoter (Supplementary Fig. S1A). Mice injected with LV-Wnt3a showed a significant threefold increase in Wnt3a mRNA expression after 16 weeks (Supplementary Fig. S1B). As the Wnt3a construct also contains an HA tag, we confirmed the overexpression of Wnt3a in the DG of the ventral hippocampus by immunofluorescence using anti-HA antibody after 16 weeks (Supplementary Fig. S1C). To further determine whether an increased level of Wnt3a is also reflected in increased Wnt signaling, we measured the protein level of active β-catenin in the DG of the ventral hippocampus (Supplementary Fig. S2). Active β-catenin levels were decreased in the DG of 3xTgAD mice but completely recovered after Wnt3a overexpression.

3.2. Hippocampal LV-Wnt3a injection reverses danger assessment impairment in 3xTgAD mice

To examine whether LV-Wnt3a injections improve behavioral symptoms, we injected the LV-Wnt3a or LV-GFP control virus into the DG of the ventral hippocampus regions of 3xTgAD mice at age 3 months. Multiple studies have recently demonstrated that 3xTgAD model mice show behavioral symptoms at age 7 months [42–44]. Thus, we designed hippocampal injections at age 3 months as a presymptomatic treatment (Fig. 1A). Injections were followed by behavioral tests at age 7 months.

Behaviors related to anxiety in rodents have been studied primarily by measuring avoidance responses to potentially threatening situations such as open and brightly lit environments, which expose mice to predators. Anxiety-related behaviors were first tested using the emergence test, where the tendency to exit a reassuring cylinder (emergence) is considered to be an index of anxiety-like behavior in mice. 3xTgAD mice showed significantly shorter latency to emerge from the cylinder and time spend on risk assessment than did WT (Fig. 1B). 3 × TgAD mice also showed a tendency toward significant shorter time spent in the protective cylinder. Note that the latency to emerge from the cylinder and risk assessment time were reversed by Wnt3a overexpression.

In addition, anxiety-related behavior was tested using the EPM. Again, avoidance of the threatening area was largely decreased in 3xTgAD mice; these animals spent more time in the open arms and emerged into the open arms faster (Fig. 1C). We then explored whether Wnt3a expression is capable of reversing this behavior. Similarly to the emergence test, the tendency of 3xTgAD mice to emerge quickly into the open arms was reversed by Wnt3a expression.

Next, we used the open field test to examine anxiety-related behavior and locomotor activity. 3xTgAD mice showed increased time spent in the new unprotected center (Fig. 1D). We found that Wnt3a expression again reduced the amount of time spent in the center. The behavior did not depend on a non-specific modification of locomotor activity. 3xTgAD mice showed no change in total distance traveled. Moreover, Wnt3a expression did not affect mobility.

The results of these experiments suggest that 3xTgAD mice show a decreased anxiety-related phenotype that is responsive to Wnt3a expression in the ventral DG. But the time spent in novel open areas is a function of the integration of two opposite motivational drives: avoidance of potential predators and exploration of novel areas for foraging sources. To test the relative contribution of the two factors to the time spent in novel open areas we tested the defensive reaction of the mice to predator exposure using the rat exposure test. The time spent in a protective cylinder, which is considered an index of fear of predators, was much shorter in 3xTgAD mice than in WT (Fig. 1E). 3xTgAD mice also showed longer latency to escape into the cylinder. Importantly, Wnt3a expression significantly shortened the latency to escape. To further examine novelty seeking under the overexpression of Wnt3a, we subjected the mice to the novel-object test. Exploration of a novel object in a non-threatening environment did not indicate differences between three groups (Fig. 1F). These data further suggest that 3xTgAD mice show impaired judgment in assessing danger, which is responsive to Wnt3a expression in the ventral DG.

3.3. Enhanced neurogenesis in 3xTgAD mice injected with LV-Wnt3a

As Wnt signaling was shown to enhance endogenous neurogenesis in the subgranular zone of the DG [35,36]. We examined the effect of LV-Wnt3a on neurogenesis (Supplementary Fig. S3). Wnt3a overexpression in the DG of WT mice resulted in a significant increase in neurogenesis. We then examined the effect of

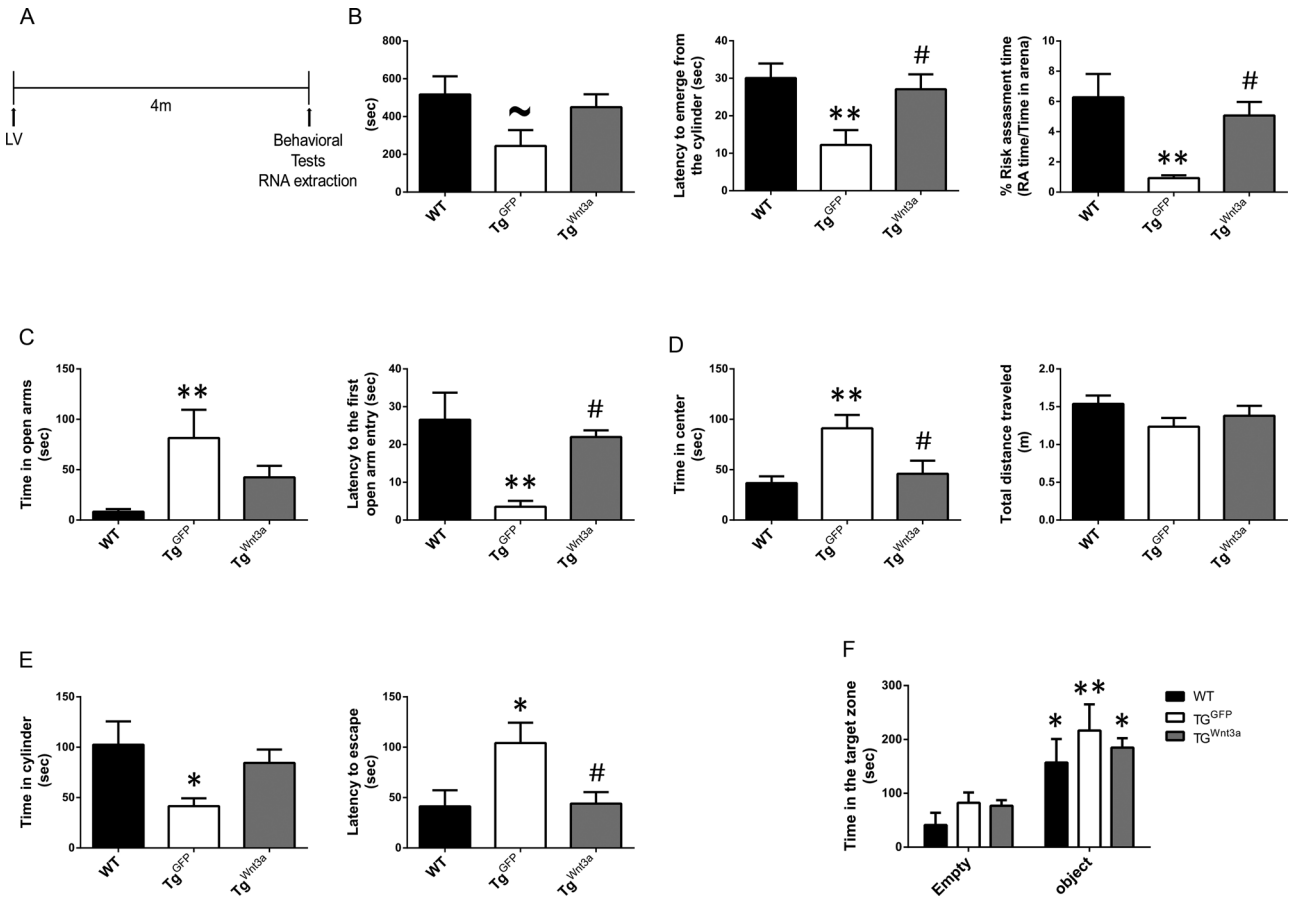

Fig. 1. Wnt3a overexpression improves behavioral symptoms in 3 × TgAD mice. |(A) Schematic representation of the experimental procedure for treatment. 3 × TgAD mice aged 3 months were injected with LV-Wnt3a or LV-GFP. (B) Anxiety-related behavior of 3 × TgAD mice, measured by the emergence test. Time in cylinder, latency to emerge from the cylinder, and risk assessment time were evaluated. (**C**). Anxiety-related behavior of 3xTgAD mice, measured by EPM. Time in open arms and latency to the first arm entry were evaluated. (D) Anxiety-related behavior of 3xTgAD mice, measured by the open-field test. Time in center and total distance traveled were evaluated. (E) Harm avoidance of 3xTgAD mice, measured by the rat exposure test. Time in cylinder and latency to escape were evaluated. Results are expressed as mean ± s.e.m. (n = 12–13 per group). Significance was determined using a one-way analysis of variance (ANOVA) with Tukey's *post hoc* tests. ∼P < 0.1, $^{*}P$ < 0.05, $^{**}P$ < 0.01, compared with the WT group. $^{\#}P$ < 0.05, compared with the Tg^{GFP} group. (F) Novelty-seeking of 3xTgAD mice, measured by the novel object test. Time in the target zone was evaluated. Results are expressed as mean ± s.e.m. (n = 12–13 per group). Significance was determined using a two-way ANOVA with repeated measures with Sidak's *post hoc* tests. $^{*}P$ < 0.05, $^{**}P$ < 0.01, compared with the compatible group.

LV-Wnt3a on neurogenesis in 3xTgAD mice (Fig. 2A). Analysis of Dcx, a marker for newly generated premature neurons, revealed that the number of Dcx^{+} cells in the DG of 3xTgAD mice injected with LV-GFP was significantly reduced (Fig. 2B). These mice showed a moderate but significantly increased number of Dcx^{+} cells in the DG after LV-Wnt3a injection, suggesting an enhancement of neuronal differentiation by Wnt3a gene expression. To determine the effect of LV-Wnt3a on neuronal survival, mice were injected with the proliferating cell marker EdU four weeks before tissue excision, making it possible to detect new neurons (Fig. 2A and C). Remarkably, the total EdU^{+} cell counts in the DG were increased in LV-Wnt3a-injected mice (Fig. 2D). Similarly, the number of EdU^{+} cells that formed mature neurons ($EdU^{+}/NeuN^{+}$) significantly increased in 3xTgAD mice injected with LV-Wnt3a (Fig. 2C). These data demonstrate that neural stem cell differentiation and survival were reduced in 3xTgAD mice and significantly restored to WT levels by LV-Wnt3a injections.

3.4. Reversal of behavioral symptoms in 3xTgAD mice is mediated by neurogenesis

To assess whether adult neurogenesis is required for Wnt3a-mediated reversal of the 3xTgAD phenotype in several behavioral tasks, we performed ablation of neurogenesis. Animals were first exposed to sham or X-irradiation to induce ablation of hippocampal neurogenesis [41]. At 3 months after exposure, animals were injected with EdU to examine the long-term effectiveness of X-irradiation in blocking neurogenesis (Fig. 3A). We found that X-irradiation dramatically reduced the number of proliferating cells in the DG, which confirmed long-term ablation of hippocampal neurogenesis (Fig. 3B and C). Wnt3a overexpression in the dentate gyrus before X-irradiation did not reverse the reduction in neurogenesis (data not shown).

Next, we submitted animals to focal hippocampal X-irradiation before injection with lentiviruses, and again assessed anxiety-related behavior using the emergence test (Fig. 3D). Although X-irradiated WT and 3xTgAD mice did not display any behavioral alterations, 3xTgAD mice were insensitive to Wnt3a injection for latency to emerge from the cylinder and for risk assessment time parameters (Fig. 3E). Our results indicate that ablation of hippocampal neurogenesis on its own has no effect on the emergence test but prevents Wnt3a reversal. Similar results were obtained in the EPM and open-field test. In the EPM, the effect of Wnt3a on 3xTgAD mice was abolished after X-irradiation for time in open arms and for latency in the first arm entry (Fig. 3F). In the open field we found an effect of X-irradiation on time in the center in 3xTgAD mice injected with Wnt3a (Fig. 3G). Taken together, the results indicate

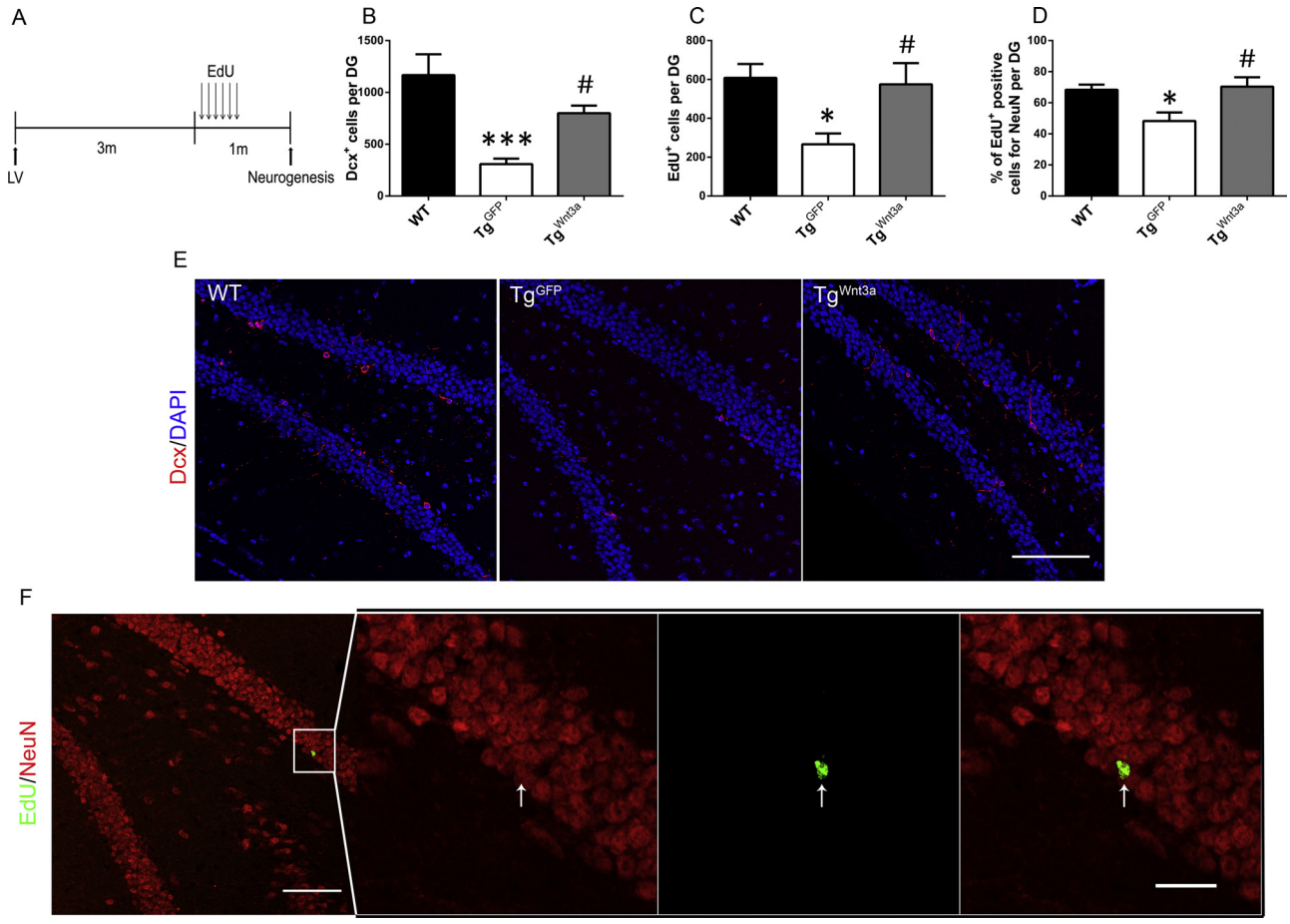

Fig. 2. Wnt3a overexpression enhances neurogenesis in 3xTgAD mice. (A) Timeline of experimental procedures. (B) Number of Dcx$^+$ cells in the DG of the ventral hippocampus. (C) Number of EdU$^+$ cells in the DG of the ventral hippocampus. (D) Percentage of EdU$^+$ cells positive for NeuN. (E) Dcx staining in the DG of WT or 3xTgAD mice. Scale bar, 100 μm. (F) Confocal images showing co-localization of EdU$^+$ and NeuN$^+$ in the DG. Arrows indicate EdU$^+$/NeuN$^+$ double-positive cells. Scale bar, 100 μm (left) 20 μm (right). Results are expressed as mean ± s.e.m. ($n = 6$ per group). Significance was determined using a one-way analysis of variance (ANOVA) with Tukey's *post hoc* tests. $^*P < 0.05$, $^{***}P < 0.001$, compared with the WT group. $^{\#}P < 0.05$, compared with the Tg^{GFP} group.

that hippocampal neurogenesis is required for the recovery effect, although it is not directly involved in anxiety-related behavior.

3.5. *Wnt3a overexpression restored normal levels of the 5-HT$_{1A}$ receptor mRNA in the BNST*

To determine whether the observed behavioral effect following Wnt3a expression in the DG was also reflected in the gene expression profile of brain regions responsible for risk assessment in the environment, we evaluated the expression levels of selected BNST and amygdala genes in WT and 3xTgAD mice overexpressing GFP or Wnt3a in the ventral hippocampus DG. The expression levels of 30 genes previously linked to mood disorders were assessed using a custom-made real-time polymerase chain reaction (PCR) array. In the BNST, we found 5 genes that were changed in the 3xTgAD model (Fig. 4A): brain-derived neurotropic factor, corticotrophin-releasing hormone, and 5-HT$_{1A}$ receptor expression were increased, whereas the Dopamine receptor D$_2$ and GABAA receptor delta-subunit gene were decreased. Only the increase of 5-HT$_{1A}$ receptor gene expression was entirely reversed in 3xTgAD mice injected with LV-wnt3a. In the amygdala, we found an increase only in corticotrophin-releasing hormone expression in the 3xTgAD mice, and the expression level did not change with LV-Wnt3a injection (Supplementary Fig. S4). We next wanted to evaluate whether the 5-HT$_{1A}$ receptor expression increase is specific to the BNST. The expression level of 5-HT$_{1A}$ receptor did not change in the prefrontal cortex, hippocampus, paraventricular nucleus of the hypothalamus, and periaqueductal grey in 3xTgAD model (Supplementary Fig. S5).

To determine whether 5-HT$_{1A}$ receptor expression correlated with behavioral function, we performed a scatter plot analysis between 5-HT$_{1A}$ receptor expression and risk assessment for WT and 3xTgAD mice that received LV-GFP or LV-Wnt3a injections (Fig. 4B). Pearson's correlation analysis indicated a significant correlation between 5-HT$_{1A}$ receptor expression and risk assessment time ($r = -0.6955$). These data suggest that 5-HT$_{1A}$ receptor expression is associated with anxiety-related behavior in this AD mouse model.

To examine whether the changes in 5-HT$_{1A}$ receptor expression in the BNST are neurogenesis dependent, we investigated whether ablation of neurogenesis induces changes in 5-HT$_{1A}$ receptor expression. We found that neurogenesis ablation did not change 5-HT$_{1A}$ receptor expression in WT or 3xTgAD mice (Fig. 4C). Wnt3a overexpression, however, was able to restore 5-HT$_{1A}$ receptor expression in sham 3xTgAD mice to WT levels, but not in X-irradiated 3xTgAD mice. Taken together, the results show that normalization of the 5-HT$_{1A}$ receptor in the BNST is neurogenesis dependent.

4. Discussion

Recent evidence has shown that hippocampal neurogenesis has therapeutic implications for cognitive disorders in AD [14–16]. In the present study we explored the potential link between adult

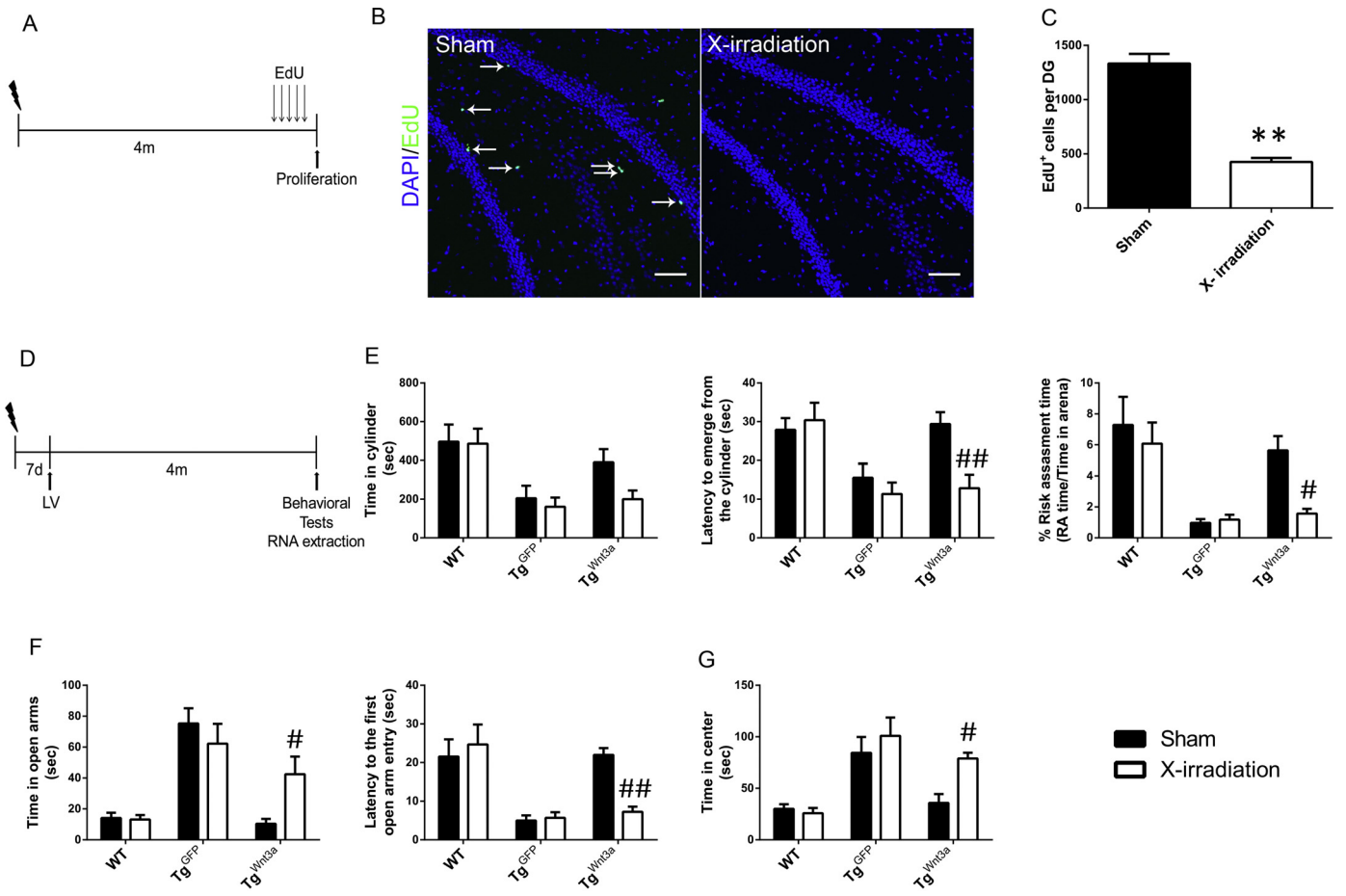

Fig. 3. Hippocampal neurogenesis is involved in mediating the behavioral effect of Wnt3a. (A–C) Ablation of hippocampal neurogenesis by X-irradiation. (A) Timeline of experimental procedures. (B) EdU staining in the DG of sham or X-irradiated mice. Arrows indicate EdU$^+$ cells. Scale bar, 100 μm. (C) Number of EdU$^+$ cells in the DG. $^{**}P < 0.01$. (D) Experimental design. 3xTgAD mice were injected with LV-Wnt3a or LV-GFP at 3 months of age, one week after exposure to the sham procedure or to X-irradiation. (E) Anxiety-related behavior of X-irradiated 3xTgAD mice, measured by the emergence test. Time in cylinder, latency to emerge from the cylinder, and risk assessment time were evaluated. (F) Anxiety-related behavior of X-irradiated 3xTgAD mice, measured by EPM. Time in open arms and latency to the first arm entry were evaluated. (G) Anxiety-related behavior of X-irradiated 3xTgAD mice, measured by the open-field test. Time in center and total distance traveled were evaluated. Results are expressed as mean ± s.e.m. ($n = 10$ per group). Significance was determined using a two-way analysis of variance (ANOVA) with Sidak's *post hoc* tests. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the compatible sham group.

ventral hippocampal neurogenesis and management of behavioral symptoms in the AD mouse model. We showed that increased ventral hippocampal neurogenesis following Wnt3a overexpression in the DG attenuates danger assessment impairment in the 3xTgAD mice and that neurogenesis is critical for the behavioral improvement of this model. Taken together, these results suggest a neurobiological process by which neurogenesis participates in the improvement of behavioral symptoms.

Overall, 3xTgAD mice appear to have decreased anxiety-related behavior which originates from the distorted assessment of risk-related information. The literature on the 3xTgAD strain provides conflicting reports about anxiety-related behavior. Previous studies have reported decreased [45], increased [46,47] and unchanged anxiety-related behaviors [43,46]. There are several general factors that may be responsible for the discrepancy in findings between researchers using this strain. Probably most important, testing procedure and apparatus design varies between the researchers. A different factor that could affect results is the sex of the mice used, as many of the studies that have examined behavior in these mice have used only one sex or both sexes but not analyzed the effect of sex, which could impact the results. The third factor is the strain of mice used as a control. Original control mice of the mouse strain and the control mice commercially available, may behave differently on different tests. Since we found the anxiety-related behavior was consistent throughout a battery of anxiety-related tests, we found these mice to be a reliable model to this behavior. The 3xTgAD mice show risk assessment patterns that are inconsistent with normal defensive behavior. Anxiety process is initially associated with withdrawal and movement arrest, giving way to a crucial and long-lasting risk-assessment stage that provides information leading to either further defensiveness or a return to nondefensive behaviors [48–50]. In AD patients this behavior often manifests itself as risky disinhibited behavior [4].

There has been much discussion about a possible causative role of adult neurogenesis in AD [51,52]. As far as the behavioral symptoms are concerned, our results do not support such a possibility. Consistent with studies in models relevant to behavioral and neuropsychiatric diseases such as stress, anxiety, and depression [53–55], the ablation of adult-generated hippocampal neurons does not cause any behavioral symptoms. We cannot exclude the possibility that a longer period of ablation might reveal behavioral deficits in WT mice to uncover a potential role of basal hippocampal neurogenesis. Alternatively, the functional properties of neurons that are generated in response to Wnt3a stimulation may be different from those of cells generated in baseline conditions. In either case, the ineffectiveness of irradiation on basal behavioral responses in the WT mice suggests that our focal x-ray procedure does not elicit a nonspecific behavioral impairment.

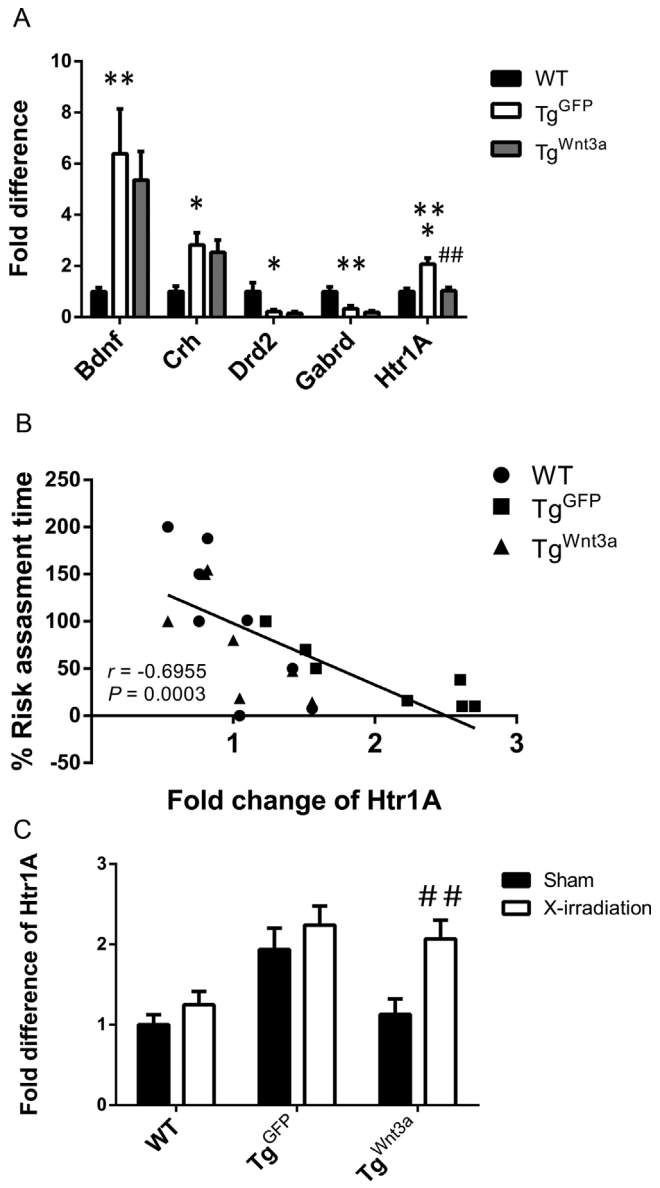

Fig. 4. Hippocampal neurogenesis restores normal 5-HT_{1A} receptor levels in the bed nucleus of stria terminalis (BNST). (A) mRNA levels of genes showed induced changes in the BNST expression array of 3xTgAD compared with WT mice. Results are expressed as mean ± s.e.m. (n = 7–8 per group), expressed as a fold-change compared with the WT. Significance was determined using a one-way analysis of variance (ANOVA) with Tukey's *post hoc* tests. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the WT group. $^{\#\#}P < 0.01$, compared with the Tg^{GFP} group. (B) Correlation between 5-HT_{1A} receptor levels in the BNST and anxiety-related behavior. Pearson correlation analysis shows a significant correlation (r value) between 5-HT_{1A} receptor levels in the BNST and risk assessment time. (C) 5-HT_{1A} receptor levels in the BNST in 3xTgAD mice that were injected with LV-Wnt3a or LV-GFP one week after exposure to the sham procedure or to X-irradiation. Results are expressed as mean ± s.e.m. (n = 7–8 per group). Significance was determined using a two-way analysis of variance (ANOVA) with Sidak's *post hoc* tests. $^{\#\#}P < 0.01$, compared with the compatible sham group. Bdnf, brain derived neurotropic factor; Crh, corticotrophin-releasing hormone; Htr1A, 5-HT_{1A} receptor; Drd2, Dopamine receptor D_2; Gabrd, GABAA receptor delta-subunit gene.

The role of neurogenesis in regulating emotionality is considered controversial. Hippocampal neurogenesis is increased by several categories of antidepressants, and some of the anxiolytic and antidepressant effects of antidepressants require intact hippocampal neurogenesis [53,55]. Neurogenesis has also been shown to modulate the stress response both in baseline conditions and in response to antidepressants and enrichment [7,56,57]. However, some groups suggest that targeting neurogenesis alone is not a sufficient treatment strategy [58,59]. Although psychiatric disorders are linked to decreased hippocampal volume, these conditions are also accompanied by robust circuit-based changes [60]. Thus, the effect of neurogenesis on emotions is influenced by several conditions that affect these connections such as the type of model, source for enhanced neurogenesis and arrangement of the new neurons. We hypothesize that targeting adult neurogenesis to improve connectivity and dysfunction of the dentate gyrus, may be beneficial for the treatment of some emotional state disorders.

The hippocampus is highly interconnected with various brain areas. Unlike the dorsal hippocampus, the ventral hippocampus sends projections to the prefrontal cortex, amygdala, the nucleus accumbens shell, the BNST, and structures associated with the hypothalamic-pituitary-adrenal axis [61–64]. Thus, elevated ventral hippocampal neurogenesis may strengthen hippocampal influence on these brain regions. Here, we found neurogenesis-dependent changes in the expression of the 5-HT_{1A} receptor in the BNST that correlating with behavioral performance. The BNST receives a dense innervation from serotonergic neurons [65], and 5-HT_{1A} receptors are expressed in its neurons [66]. This receptor is thought to play an important role in the etiology in anxiety disorders [67]. Electrophysiological studies have demonstrated that the application of serotonin to BNST neurons elicits inhibitory responses mediated by the activation of the postsynaptic 5-HT_{1A} receptor [68,69] and that local infusion of a 5-HT_{1A} receptor agonist, 5-carboxamidotryptamine (5-CT), into the BNST attenuates the acoustic startle response in rats [68]. Buspirone, a partial 5-HT_{1A} receptor agonist clinically used in the treatment of anxiety, blocks the effects of light-enhanced startle in rats [70,71]. Thus, in response to potentially threatening situations, enhanced levels of the 5-HT_{1A} receptor in the BNST play a critical role in anxiety-related behavior. Given that the majority of BNST neurons express multiple 5-HT receptors that have opposing physiologic actions in the same cell [72], adult neurogenesis may modulate the relative efficacy of the 5-HT_{1A} receptor and thereby affects the response of the BNST to local 5-HT release. Taken together, the BNST might modulate the appropriate behavior response to aversive stimuli. However, further research is needed to establish a causative relationship between 5-HT_{1A} receptor in the BNST and behavioral changes in this model.

In conclusion, the present study demonstrates how presymptomatic enhanced hippocampal neurogenesis restores behavioral symptoms in 3xTgAD mice. Further research is warranted to understand the molecular mechanism of neurogenesis and its therapeutic application with respect to the behavioral aspect of AD.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2013.12.028.

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