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Interaction of Aβ dimers with the serotonin receptor 5-HT1β influences adult neurogenesis in tgD mice

Dissertation

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Zusammenfassung

Die Alzheimer-Krankheit ist mit mehr als 50 Millionen Betroffenen weltweit die am weitesten verbreitete neurodegenerative Erkrankung. Sie stellt daher eine enorme medizinische, finanzielle und soziale Belastung für die Betroffenen und ihre Familien dar. Seit kurzem ist bekannt, dass lösliche Amyloid-beta-Dimere und -Oligomere stärker mit der Manifestation und dem Schweregrad der Erkrankung korrelieren als unlösliche Aggregate. Darüber hinaus werden strukturelle Veränderungen im Gehirn der Patienten zunehmend mit einer Dysregulation von Neurotransmittern wie Acetylcholin, Dopamin und Serotonin in Verbindung gebracht. Es gibt starke Hinweise darauf, dass lösliche Oligomere die Kognition bereits vor dem Einsetzen der Neurodegeneration beeinflussen. TgD Mäuse produzieren durch Disulfidbrücken stabilisierte Amyloid-beta-Dimere, die weder Plaques bilden noch Anzeichen von neurofibrillären Tangles oder Neurodegeneration zeigen. Dennoch zeigen TgD Mäuse ein vermindertes räumliches Gedächtnis und nicht-kognitive Störungen wie Angst und depressives Verhalten, was den Symptomen der frühen Alzheimer-Erkrankung entspricht und mit einem Ungleichgewicht der Transmitter Serotonin, Dopamin und Acetylcholin einhergeht. Das TgD Mausmodell ist daher ideal, um funktionelle Veränderungen und mögliche therapeutische Ansätze für die Behandlung der frühen Alzheimer-Erkrankung zu untersuchen.
Unsere Experimente zeigen, dass Amyloid-beta zu einer signifikanten Reduktion neuronaler Vorläuferzellen in TgD Mäusen führt, während Überleben, Migration und Differenzierung unbeeinflusst bleiben. Diese Veränderung ist reversibel durch Modulation eines Serotoninrezeptors mit einem 5-HT1β-Rezeptorantagonisten, was uns vermuten lässt, dass Amyloid-beta-Dimere mit dem 5-HT1β-Rezeptor interagieren.
Weitere Forschung ist erforderlich, um die genauen Auswirkungen auf die Alzheimer-Krankheit und die damit verbundenen kognitiven Defizite zu untersuchen. Das Verständnis dieser Mechanismen könnte in Zukunft zur Entwicklung von therapeutischen Strategien zur Förderung der adulten Neurogenese und damit zur Verbesserung des Outcomes von Alzheimer-Patienten führen.

Abstract

Alzheimer's disease remains the most common neurodegenerative disease, affecting more than 50 million people worldwide. This represents an enormous medical, financial and social burden for patients and their families. Recently, there has been increasing evidence that soluble amyloid beta dimers and oligomers, rather than insoluble aggregates, correlate more closely with the manifestations and severity of Alzheimer's disease and, critically, that structural brain changes are associated with dysregulation of neurotransmitters such as glutamate, acetylcholine, dopamine and serotonin, which modulate various cognitive and behavioural processes.

There is strong evidence that soluble oligomers affect cognition prior to neurodegeneration, supported by the tgD mouse model. TgD mice produce stable amyloid beta dimers without plaques, tangles or neuroinflammation. Nevertheless, tgD mice show reduced spatial memory and non-cognitive impairments such as anxiety and depression-related behaviours that mimic early AD symptoms associated with neurotransmitter imbalances in serotonin, dopamine and acetylcholine. TgD mice are therefore an excellent model to study functional changes and potential therapeutic treatments that target the early stages of Alzheimer's disease.

Our experiments show that amyloid beta dimers significantly affect the proliferation of neuronal precursor cells in the hippocampus of tgD mice, while sparing their survival, migration and differentiation. Modulation of 5-HT1β receptors partially reversed the progenitor cell reduction, suggesting an interplay between amyloid beta dimers and serotonin signalling.

Abbreviation

3, 3'-diaminobenzidine	**DAB**
5’Bromo-2‘-deoxyuridine	**BrdU**
5-hydroxyindoleacetic acid	**5-HIAA**
5-hydroxytryptamine/ Serotonin	**5-HT**
acetylcholine	**ACh**
Alzheimer disease	**AD**
amyloid beta	**Aβ**
amyloid-β precursor protein	**APP**
behavioural and psychological signs and symptoms of dementia	**BPSD**
brain derived neurotrophic factor	**BDNF**
bromdesoxyuridin	**BrdU**
cyclic of adenosine monophosphate	**cAMP**
CNS	**central nervous system**
dentate gyrus	**DG**
deoxyribonucleic Acid	**DNA**
disease modifying therapeutics	**DMT**
dopamine	**DA**
doublecortin	**DCX**
Food and Drug Administration	**FDA**
gamma-aminobutyric acid	**GABA**
glial cell line-derived neurotrophic factor	**GDNF**
glomerular layer	**GL**
glutamate	**Glu**
G-protein-coupled receptors	**GPCRs**
granule cell layer	**GCL**
Heinrich-Heine-Universität	**HHU**
hydrochloric acid	**HCL**

hydrogen peroxide	**H_2O_2**
intraperitoneally	**i.p.**
knockout	**KO**
lateral ventricle	**LV**
microtubule Associated Protein Tau	**MAPT**
microtubule-associated protein 2	**MAP2**
messenger ribonucleic acid	**mRNA**
natriumchloride	**NaCl**
neuronal nuclear protein	**NeuN**
neuronal stem/ progenitor cells	**NPC**
N-methyl-D-aspartat	**NMDA**
olfactory bulb	**OB**
parvalbumin-expressing interneuron-specific diphtheria toxin receptor	**PC/DTR**
phosphate buffered saline	**PBS**
polysialylated neuronal cell adhesion molecule	**PSA-NCAM**
presenilin-1	**PS-1**
rostral migratory stream	**RMS**
selective serotonin reuptake inhibitors	**SSRIs**
serotonin reuptake transporter	**SERT**
standard error of the mean	**SEM**
subgranular zone	**SGZ**
subventricular zone	**SVZ**
suprachiasmatic nucleus	**SCN**
swedish and cysteine mutation	**Aβ-S8C**
triple transgenic mice	**3xTg-AD**
tryptophan hydroxylase 2/ serotonin transporter enhancer protein	**Tph2Pet1 CKO**
Phosphate buffered saline with Triton-X 100	**PBS-T**
tryptophan hydroxylase	**TPH**

wild-type	**WT**
Zentrale Einrichtung für Tierforschung und wissenschaftliche Tierschutzaufgaben	**ZETT**

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1. Introduction

1.1 Alzheimer disease

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder, impacting over 50 million individuals globally (reviewed by Ali & von Gall, 2022; Scheltens et al., 2021). The progression of AD increases proportionally with age, affecting individuals over the age of 65 and reaching its highest incidence around the age of 80 (Caroline dos Santos Picanço et al., 2018). Several risk factors have been associated with AD, including genetic factors (Scheltens et al., 2021) and increasing age, but also depression (reviewed by Kumar et al., 2021) and circadian disruption (Erik S. Musiek, 2017). AD accounts for the majority of dementias and is associated with major impairment of quality of life for patients and their relatives (reviewed by Levy et al., 1999). AD is classified into preclinical stages, mild cognitive impairment and AD dementia, which is characterised by substantial progressive cognitive impairment (reviewed by Kumar et al., 2021). In addition to cognitive symptoms, up to 90% of patients with dementia experience behavioural and psychological signs and symptoms (Cerejeira et al., 2012).

Non-cognitive symptoms are the main cause of significant loss of quality of life for patients and their families, increased costs of care and early institutionalisation of patients (Lyketsos et al., 2011; Petrovic et al., 2007). In AD, the behavioural and psychological signs and symptoms of dementia (BPSD) often manifest at a very early stage and continue to worsen in parallel with cognitive dysfunction (Mathys et al., 2018). BPSD are classified into four symptoms: hyperactivity (including agitation, aggression, euphoria, disinhibition, irritability and abnormal motor activity), psychosis (including hallucinations and delusions), mood liability (including depression and anxiety) and instinctual drift (including appetite disturbance, sleep disturbance and apathy) (Mathys et al., 2018; Milán-Tomás & Shapiro, 2018; Van Erum et al., 2018).

The symptoms tend to worsen with age (Holtzman et al., 2011), leading to progressive disability that ends with the inevitable death of the patient. Therefore, AD represents a major medical, financial and social burden for patients and their families (Brodaty & Donkin, 2009).

The underlying cause of the pathological changes in Alzheimer's disease remains unclear. Different insoluble and soluble forms of amyloid-beta (Aβ) have been shown to induce synaptotoxic effects and neurodegeneration. Recently, there has been increasing evidence that soluble Aβ dimers/oligomers, rather than insoluble aggregates, appear to be more correlated with AD manifestations and severity. Soluble Aβ oligomers can lead to synaptic defects, resulting in neurotransmitter uptake/release imbalances and altered synaptic plasticity, in addition to cytoskeletal abnormalities, changes in receptor cellular localisation and disruption; factors that can lead to cognitive deficits.

According to the amyloid cascade hypothesis (reviewed by Dennis, 1999), the formation of (soluble) Aβ dimers and oligomers is the first step in a cascade of events leading to the formation of (insoluble) Aβ aggregates/plaques, neurofibrillary tangles of hyperphosphorylated tau protein, neuroinflammation (gliosis), cerebral angiopathy, and extensive neuronal loss and gross atrophy of certain brain areas (e.g. hippocampus) (Fig.1). Neuroinflammation and gliosis in response to abnormal Aβ are associated with increased oxidative stress and production of other neurotoxic factors that exacerbate neuronal dysfunction (Mc Donald et al., 2010; reviewed by Sinyor et al., 2020). Importantly, the presence of soluble Aβ dimers and oligomers correlates more strongly with cognitive impairment in AD patients than the presence of insoluble aggregates/plaques (Shankar et al., 2008). Furthermore, soluble Aβ oligomers from AD patients affect memory as well as spine density and synaptic plasticity in rats (Jack et al., 2018). Thus, soluble rather than insoluble forms of Aβ affect behaviour as well as structural and functional neuronal plasticity. It is crucial to define the biomarkers and behavioural profiles that best predict progression from preclinical to clinical stages of mild cognitive impairment and AD dementia (Dubois et al., 2016; Sperling et al., 2011).

Furthermore, for any curative approach, detection of the long preclinical phase of AD is extremely important, as early intervention before massive structural changes occur may offer the best therapeutic effect (Dubois et al., 2016; Sperling et al., 2011; Wirths, 2017).

However, the underlying pathomechanisms for the various symptoms of early AD are still unclear.

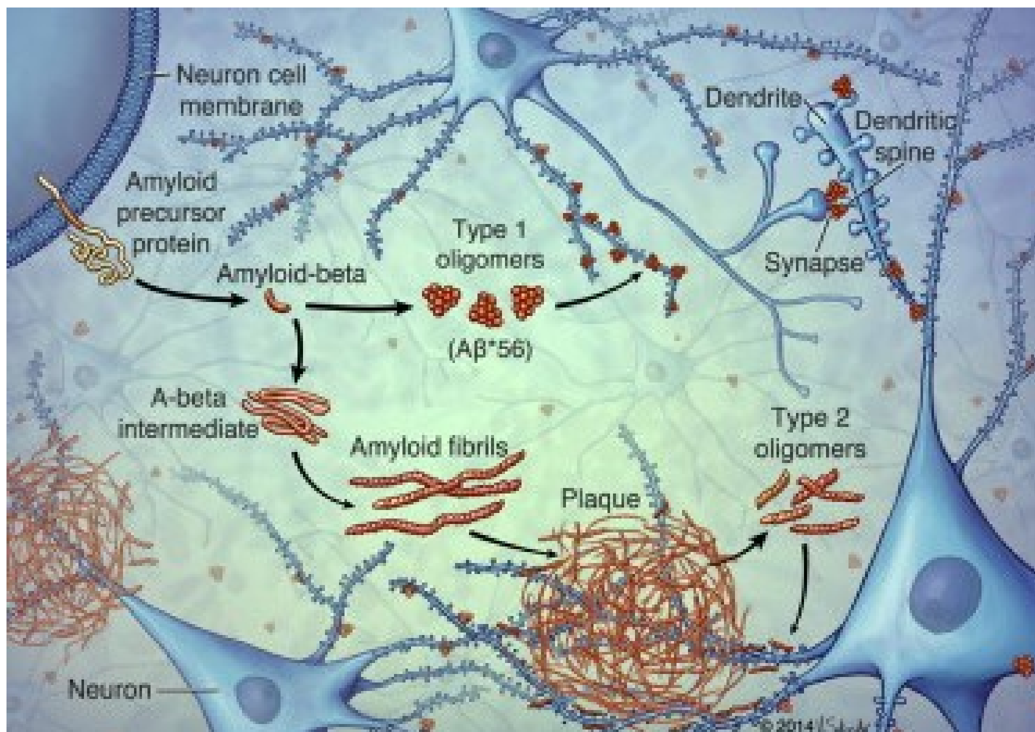


Fig. 1: Classification and impact of AβO subtypes on memory function and amyloid plaque association

AβOs can be classified into two groups based on their temporal, spatial, and structural relationship to amyloid plaques and their impact on memory function. Type 1 AβOs are associated with memory impairment, whereas type 2 AβOs are not associated with memory impairment. Amyloid plaques are exclusively associated with type 2 AβOs (reviewed by Cline et al., 2018). Reprinted from, Cell Reports Vol. 11, (Liu et al., 2015), 'Quaternary Structure Defines a Large Class of Amyloid-β Oligomers Neutralized by Sequestration', pp. 1760–1771, under the terms of Creative Commons Attribution-Non-Commercial-No Derivatives License (CC BY NC ND). Abbreviations: AβO – amyloid beta oligomers.

Unfortunately, there is currently no effective treatment to delay the onset of AD or stop its progression (Holtzman et al., 2011). To date, two classes of drugs improve cognitive function in AD patients, including cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) antagonists, but they are not able to prevent or cure the disease. In addition, disease-modifying therapeutics (DMTs) that act on multiple neuropathological mechanisms are still in clinical trials because their efficacy has not been proven when administered in the advanced stages of AD (Breijyeh & Karaman, 2020). However, scientific advances in genetic, biochemical, and imaging techniques offer hope that if AD is diagnosed before the onset of overt pathological and cognitive changes, targeted treatments of the underlying pathogenesis may be effective (Holtzman et al., 2011).

Importantly, the functional and structural changes in the brain are associated with dysregulation of several neurotransmitter systems, including glutamate (Glu), acetylcholine (ACh), dopamine (DA) and serotonin (5-HT), which modulate various cognitive processes and behaviours (Francis, 2005). Drugs that improve the various symptoms of Alzheimer's disease target different neurotransmitter systems. ACh is an important transmitter in cognitive processes, and cholinesterase inhibitors are most commonly used to improve memory, attention, and language. Animal studies have suggested a link between the deficiency of cholinergic neurons and increased deposition of amyloid-β and tau pathology, which contributes to cognitive impairment (Ramos-Rodriguez et al., 2013). Specifically, targeted lesions of cholinergic neurons in the basal forebrain of rodent models with features of AD have been observed to result in increased deposition of Aβ and increased levels of hyperphosphorylated tau in the hippocampus and cortex (reviewed by Hampel et al., 2018).

Psychiatric symptoms are usually treated with antidepressants, anxiolytics and/or antipsychotics, while sleep disturbances are usually treated with benzodiazepines. Among others, selective serotonin reuptake inhibitors (SSRIs), the first line of treatment for major depressive disorder, are useful in the treatment of BPSD (Lyketsos et al., 2011; Milán-Tomás & Shapiro, 2018).

However, little is known about the pathomechanisms that lead to the disruption of the complex interplay of neurotransmitters in AD.
Many of these drugs have severe side effects. Therefore, better identification of the underlying mechanisms that cause the various cognitive and non-cognitive impairments in AD may help to improve therapeutic strategies (Martorana et al., 2010).

1.2 Adult neurogenesis

In the adult mammalian brain, the generation of new neurons continues throughout life and has important functional aspects. The process of adult neurogenesis involves a multi-step progression, including the proliferation of neuronal stem/progenitor cells (NSPCs), migration of neuroblasts, differentiation into mature neurons, and their functional integration into pre-existing neuronal circuits (Ali & von Gall, 2022). Adult neurogenesis occurs primarily in specific regions of the brain known as 'neurogenic niches'. These niches are found in the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ) of the lateral ventricle (Ali & von Gall, 2022).

The SGZ is characterised by the proliferation of radial glia-like cells, commonly referred to as type 1 cells. The type 1 cells generate intermediate precursors, or type 2 cells, which then form neuroblasts expressing doublecortin (DCX), also known as type 3 cells. (Fig.3). The neuroblasts go through a short migration to their final destination within the DG, where they turn into premature neurons. Some neuroblasts survive and develop into granule cells. These cells express NeuN, a mature neuron marker and these cells integrate into the hippocampal neuronal network by extending dendrites into the molecular layer and axons toward the CA3 area (Fig.2) (Kempermann et al., 2015; Ming & Song, 2011). Newly created hippocampal neurons play a vital role in various functions like learning, cognition, stress response, and emotional regulation (Cameron & Glover, 2015; Toda et al., 2019).

Radial-like neural progenitor cells positioned along the ventricle wall in the subventricular zone (SVZ) become activated and proliferate. This proliferation generates transient amplifying cells, which then produce neuroblasts. In rodents, these neuroblasts migrate through the rostral migratory stream (RMS) with astrocyte support and guidance from neurotrophic factors. Eventually they reach the olfactory bulb (OB). There, premature cells develop into mature interneurons, crucial for olfactory bulb function (Ming & Song, 2011; Takahashi et al., 2018).

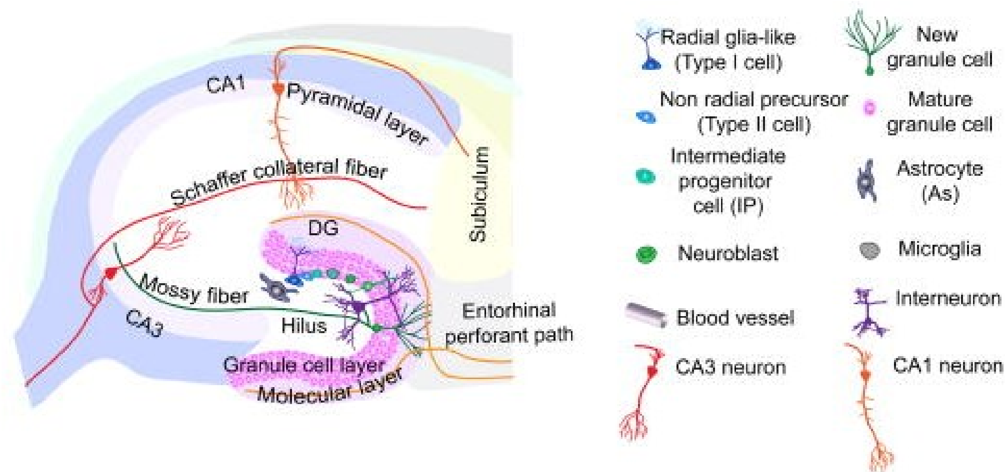


Fig. 2: Adult Neurogenesis in the Dentate Gyrus: five developmental Stages

Overview of the adult hippocampal neurogenesis process: '(1) activation of radial glia-like cell in the subgranular zone; (2) proliferation of non-radial precursor and intermediate progenitors; (3) generation of neuroblasts; (4) integration of immature neurons; (5) maturation of adult-born dentate granule cells' (reviewed by Ming & Song, 2011). Reprinted from Neuron, Vol. 70, Ming & Song, Author(s), 'Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions', pages 687-702, 2011, with permission from Elsevier.

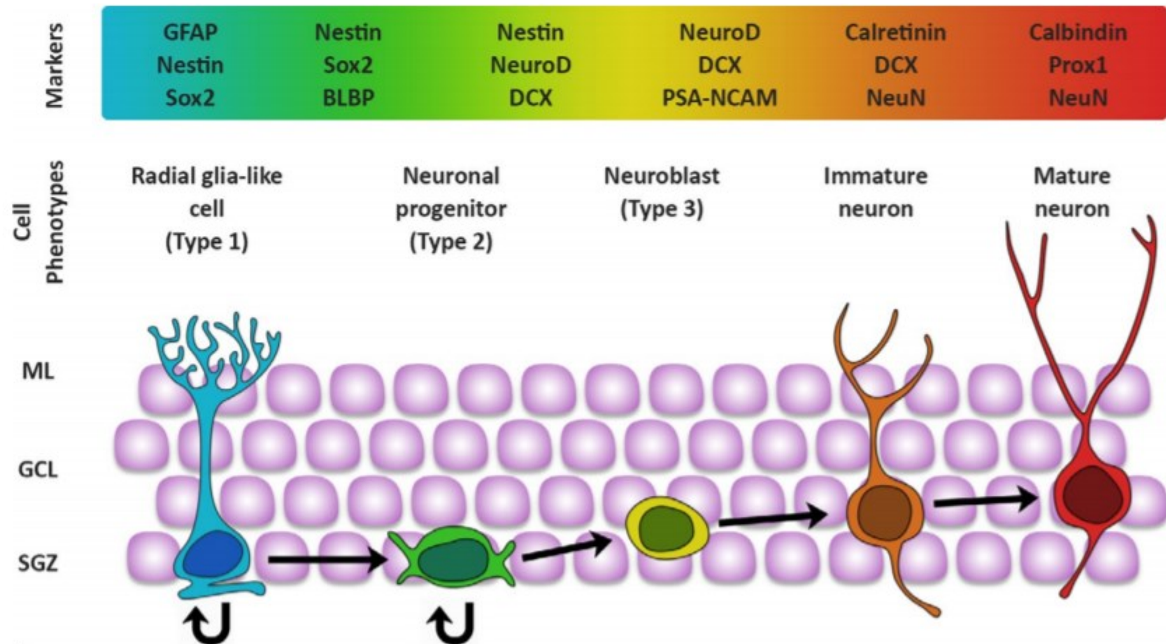


Fig. 3: Stages of Hippocampal Neurogenesis: Illustration of the Neurogenic Process

Type 1 stem cells, which resemble radial glia-like cells, self-renew and produce type 2 progenitor cells with similar markers but different morphology. Type 2 cells rapidly proliferate, express neuronal fate markers, and give rise to type 3 neuroblasts. These neuroblasts enter early survival and extend processes towards the molecular layer. Only mature and functionally connected newborn neurons persist during the late survival stage. The somata of granule neurons are shown in purple (reviewed by Kozareva et al., 2019). Reprinted from 'Born this way: Hippocampal neurogenesis across the lifespan' reviewed by Kozareva et al., 2019, published in Aging Cell/ Volume 18, Issue 5/ e13007, under the terms of the Creative Commons Attribution Non-Commercial No Derivatives License (CC BY 4.0 DEED).

The highly dynamic process of adult neurogenesis is influenced by several extrinsic and intrinsic factors in both neurogenic niches. These include an enriched environment (Leal-Galicia et al., 2007), social interaction (Holmes, 2016; Kempermann et al., 1997), physical exercise (Ma et al., 2017), ageing (Apple et al., 2017), stress (Du Preez et al., 2021), clock genes (Ali et al., 2019; Ali et al., 2015), various neurotransmitters (Platel et al., 2010) and diet (Poulos et al., 2017).
While most studies on the effects on adult neurogenesis have been conducted in rodents, some studies in humans show promising results.

Epidemiological studies suggest that increased physical activity in older adults is associated with a reduced risk of developing dementia. Neuroimaging studies confirm that seniors with higher aerobic fitness have larger hippocampal volumes and perform better on spatial memory tasks. Longitudinal studies of cognitively normal adults suggest that increased physical activity in earlier years is associated with greater grey matter volume in several brain regions, including the hippocampus. This may ultimately reduce the risk of cognitive impairment. Despite a large body of evidence supporting the efficacy of physical activity in reducing the risk of Alzheimer's disease, the exact mechanisms underlying this relationship remain unknown (reviewed by Yau et al., 2014).

In mice, neuroinflammation can have an impact on adult neurogenesis in two different ways. Acute inflammation has a proneurogenic effect that promotes suprachiasmatic nucleus (SCN) repair. Chronic inflammation leads to long-term damage that has an antineurogenic effect (Fuster-Matanzo et al., 2013). Multiple signalling pathways, including Notch, Hedgehog and Wnt signalling, growth and neurotrophic factors, cytokines, transcription factors and epigenetic modifications, exert their modulatory effects on the different stages of adult neurogenesis (reviewed by Gonçalves et al., 2016).

Interestingly, region-specific neurogenesis is a key player in human neural plasticity (reviewed by Kempermann et al., 2018). It has been shown that increased levels of neurogenesis in mice lead to improved performance in learning and memory tasks. This suggests that newborn hippocampal neurons are essential for learning and memory (Braun & Jessberger, 2014; Toda et al., 2019).

The hippocampus plays a critical role in memory processes related to spatial and object recognition in rodents (Jessberger et al., 2009). Furthermore, the hippocampus is involved in pattern segregation, a function associated with the DG, which facilitates the formation of unique and non-overlapping memories from similar experiences (Nakashiba et al., 2012).

Further research indicates that adult hippocampal neurogenesis is involved in the stress response and emotional regulation in mice (reviewed by Cameron & Glover, 2015; Snyder et al., 2011).

Newly generated interneurons in the OB of the rodent brain are crucial in the process of olfactory discrimination (reviewed Takahashi et al., 2018). There is a hypothesis suggesting that adult neurogenesis may be a distinctive feature of Alzheimer's disease. The hypothesis is based on the reduced numbers of progenitor cells in the post-mortem SVZ of individuals with AD.

Altered neurogenesis is associated with several human pathologies and with age-related decline in cognitive function. Adult neurogenesis is also reduced in several neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, and Huntington's disease (Terreros-Roncal et al., 2021). In general, age-dependent accumulation of Aβ correlates with impaired neurogenesis in most studies and is associated with cognitive deficits (Wirths, 2017). However, studies of adult neurogenesis in different animal models of AD show different results depending on the experimental conditions and do not fully explain the intracellular (tau-mediated) and extracellular (amyloid-mediated) effects of AD pathology in the stem cell niche (Winner & Winkler, 2015).

Mice with mutations in the Aβ precursor protein (APP) showed a reduction in adult neurogenesis in the hippocampus, with no change in neuronal fate determination (Donovan et al., 2006). In addition, triple transgenic mice (3xTg-AD) carrying three mutant genes (APP, presenilin-1 (PSEN1), and tau) showed age-dependent reduced proliferation in the DG that correlated with the accumulation of Aβ plaques (Rodríguez et al., 2008). Other data have shown that impaired adult hippocampal neurogenesis is an early hallmark of AD that precedes amyloid plaque deposition (Krezymon et al., 2013).

In addition, studies suggest that Aβ disrupts the homeostasis of several neurotransmitters in the hippocampus. Aβ-mediated dysregulation of gamma-aminobutyric acid (GABA) neurotransmission or an imbalance between inhibitory GABAergic and excitatory glutamatergic neurotransmission in the hippocampus affects the input to adult hippocampal neurogenesis in AD (Schinder & Morgenstern, 2009; Sun et al., 2009).

APP expression in NPCs also appears to play a critical role in impaired neurogenesis. Mice with an amyloid-rich environment and overexpression of APP in mature neurons, but not in NPCs, showed unaltered neurogenesis (Yetman & Jankowsky, 2013). While conventional APP overexpression was associated with decreased proliferation in several mouse models, APP knockout mice showed increased proliferation in the DG without affecting neuronal differentiation (Wang et al., 2014).

Exercise ameliorates deterioration and exposure to an enriched environment results in cognitive improvement and is associated with an increase in mature adult neurons, which had an increased number, dendritic length and number of projections to CA3 (Valero et al., 2011).

Alterations in neurogenesis occur at a very early presymptomatic stage of AD, suggesting that adult neurogenesis is an important part of AD pathology. In post-mortem AD patients, neurogenesis markers were reduced in both neurogenic niches. In AD mouse models, altered neurogenesis has been reported to be associated with other AD-typical pathological features such as extracellular plaque deposition, behavioural deficits or neuroinflammation, and Aβ has been shown to play a toxic role in impaired neuronal progenitor proliferation (Table 1) (Wirths, 2017).

Stimulating endogenous neurogenesis through physical activity or an enriched environment may promote regenerative processes and modulate disease progression in early stages.

This mainly results from increased release of neurotrophic factors and enhanced angiogenesis, both of which facilitate increased neuro- and synaptogenesis (Wirths, 2017).

Transgenic mouse model	**Mutation APP**	**Mutation PS1**	**Promoter**	**Plaque onset**	**Neuron loss**	**Neuro-genesis**	**Effect of PA/EE on NG**	**Reference**
Tg2576	Swedish	-	Hamster Prion Protein	12m	No	↓	↑	23,16
3xTg-AD	Swedish	M146V	Thy1 (APP, Tau) PS1 knock-in	6m	n.a.	↓	↑	17,24
APP_{751SL}/PS1KI	Swedish, London	M233T, L235P	Thy1 (APP) PS1 knock-in	2m	✓(6m)	↓	↔	18, 25, 32, 33
Tg4-42	-	-	Thy1 (A*β*4-41)	-	✓(5m)	n.a.	↑	26, 35
TTA/APP	Swedish, Indiana	-	CaMKII*α*-TTA	< 6m	n.a.	↔	n.a.	27
APP-wt	-	-	PDGF	-	n.a.	↓	↑	37
Ts65Dn	trisomic	-	-	-	n.a.	↓	↑	42, 41

Table 1: Different Alzheimer's disease mouse models with impaired neurogenesis

Adapted from Neurogenesis (Austin), Vol. 4, e1327002, Wirths, 2017, 'Altered neurogenesis in mouse models of Alzheimer disease'.

There is increasing evidence that the severity of AD pathology is not necessarily related to amyloid plaque burden. TgD mice carry the APP gene with the familial Swedish mutation (APP-K670N/M671L) and the Aβ dimer stabilising mutation (APP-S679C), termed the Aβ-S8C mutation. The Swedish mutation results in a 7-fold overexpression of APP, whereas the Aβ-S8C mutation stabilises soluble Aβ dimers through an intermolecular disulfide bridge.

The APP-Swedish mutation does not alter the Aβ42/total Aβ ratio compared to wild-type APP. The results consistently show a constant Aβ42/total Aβ ratio of 20% in tgD mice throughout their lifespan, similar to the levels observed in wild-type mice. Thus, the high levels of soluble Aβ dimers were not associated with deposition of insoluble Aβ or plaque formation (van Gerresheim et al., 2021).

Interestingly, no evidence of neuroinflammation in the form of activated glial cells, tau hyperphosphorylation, or neurodegeneration was observed in this mouse model. Furthermore, soluble forms of Aβ impair cognition in AD even before neurodegeneration (Cleary et al., 2005). This is supported by the tgD transgenic mouse model generated by Prof. Dr. Carsten Korth (Müller-Schiffmann et al., 2018) (Institute of Neuropathology, HHU), which produces a stable form of Aβ dimers without the formation of plaques, tangles or neuroinflammation. TgD mice show reduced cognitive functions such as spatial memory, non-cognitive impairments including anxiety, and reward and depression-related behaviours, mimicking early AD symptoms (Abdel-Hafiz et al., 2018; Müller-Schiffmann et al., 2018). Thus, tgD mice are an excellent model system to study functional changes and potential therapeutic treatments in early AD. Importantly, the behavioural changes in tgD mice are associated with an imbalance in neurotransmitter systems, including 5-HT, DA, and ACh. While the absolute levels of 5-HT and its metabolite 5-HIAA did not show significant differences between the tgD and wild-type mice, an important finding emerged regarding the ratio of 5-HIAA to 5-HT. In certain brain regions, such as the ventral striatum, hippocampus and amygdala of tgD mice, there was a significant decrease in the 5-HIAA/5-HT ratio (Abdel-Hafiz et al., 2018).

The release of these neurotransmitters can be modulated by presynaptic 5-HT1β homo- and heteroreceptors. Since serotonin also modulates neurogenesis, we focused on the interaction between Aβ, 5-HT and neurogenesis.

1.3 Serotonin Signalling

Serotonin is a neurotransmitter that has a significant impact on multiple physiological functions far beyond mood regulation. It plays a role in several aspects of survival and general well-being, including food intake, reproductive processes, immune responses, neurological functions, and stress management (Leibowitz & Shor-Posner, 1986).

Serotonin is produced exclusively in the brainstem in clusters of cells called raphe nuclei. These nuclei are strategically positioned to send their axons throughout the brain (Hornung, 2003). As a result, the influence of serotonin is widespread and can affect the activity of various neural networks of the central nervous system (CNS).

The hippocampus is one of the brain regions with dense serotonergic innervation. Serotonin modulates the activity of the hippocampal neural network and thus learning and memory. Interestingly, serotonergic axons show a distinct gradient in the hippocampus. The highest concentration is found in the CA3 region, followed by the gyrus, while the CA1 region has the lowest density of serotonergic axons (Berumen et al., 2012).

Serotonin exerts its functions by interacting with receptors. These receptors fall into seven families known as 5-HT1-7 (Fig.4) (Leibowitz & Shor-Posner, 1986).

There is a remarkable diversity in the hippocampus alone, where 14 different subtypes of serotonin receptors are found (Berumen et al., 2012). This complexity of serotonin signalling in the hippocampus highlights the complexity of its role in brain function, particularly in adult neurogenesis (Alenina & Klempin, 2015). This suggests that the effects of serotonin go beyond simple cognitive regulation and extend to various cognitive processes such as memory, body temperature, respiratory rhythms (respiratory system), heart rate (cardiovascular function in general), eating and bowel motility (gastrointestinal system), ejaculatory latency and bladder control, muscle contraction/relaxation and locomotion, sleep, arousal, pain and sensation, emotions, and cognition (Berger et al., 2009; De Matos Feijó et al., 2011; Nichols & Nichols, 2008).

In addition, the diversity of serotonin receptors in this area is exciting for future research and promises to reveal new therapeutic targets (Narboux-Nême et al., 2008).

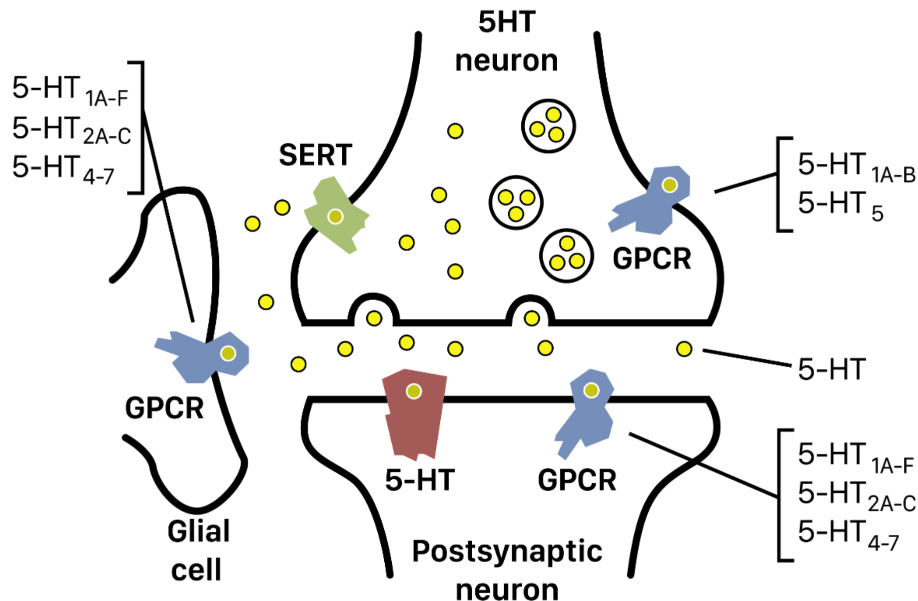


Fig. 4: Serotonin receptor subtypes

*The 5-HT system consists of three main components: 5-HT3 Cys-loop receptors (highlighted in red), 5-HT GPCRs (highlighted in blue) and SERT (highlighted in green). These components are found both pre- and postsynaptically on 5-HT neurons and glial cells. Specific elements of the 5-HT system play critical roles in various CNS disorders, including anxiety and depression (SERT and 5-HT1A-C,2A-C,4,6,7), addiction (5-HT1A-B,2A,2C,3), migraine and nausea (5-HT1A,3), learning and memory (5-HT1A-B,2A,3,4,6,7), and obesity (5-HT1A,2A-C,4) (adapted from Schiøtt, 2016). Abbreviations: 5-HT – 5-hydroxytryptamine; CNS – central nervous system; GPCR – G protein-*coupled *receptor; SERT – serotonin reuptake transporter.*

5-HT1β receptors are localised presynaptically on serotonergic neurons (autoreceptors) and postsynaptically (heteroreceptors) on non-serotonergic neurons (Abdel-Hafiz et al., 2018; Müller-Schiffmann et al., 2018).

Activation of 5-HT1β autoreceptors inhibits 5-HT release and turnover rate (Barnes Nm, 1999; Moret & Briley, 2000), whereas activation of 5-HT1β heteroreceptors decreases DA and ACh release (reviewed by Lesch & Waider, 2012; Rutz et al., 2006).

Serotonin signalling is induced by binding to serotonin receptors, which are classified into 7 families (5-HT1-7) based on their structure and functional properties and include 14 different members of the 5-HT receptor family. All are G-protein-coupled receptors (GPCRs) except the 5-HT3 receptor, which is a ligand-gated ion channel (Amidfar et al., 2018). Serotonin receptors are distributed throughout the brain (Shukla et al., 2014). The 5-HT1 family of receptors are Gi/Go protein-coupled and exert inhibitory presynaptic and postsynaptic effects where they inhibit adenylyl cyclase and reduce cellular levels of cyclic adenosine monophosphate (cAMP) (Amidfar et al., 2018).

5-HT1β receptors are Gi-protein-coupled receptors whose messenger ribonucleic acid (mRNA) sequence is similar in humans and rodents and are widely expressed in the brain, with a high density in the basal ganglia. Involved in sleep, locomotor activity, body temperature regulation, sexual and cognitive functions. Mainly presynaptically distributed at the axonal terminal and thus may act as autoreceptors inhibiting serotonin release or heteroreceptors regulating the release of other neurotransmitters 5-HT1β led to a decrease in serotonin release via reduction of cAMP and downstream processes or possibly by regulating serotonin transporter function (Tiger et al., 2018).

Heteroreceptors are primarily located on non-serotonergic neurons, such as glutamatergic neurons, but our understanding of their precise function remains rather limited (Tiger et al., 2018). However, recent evidence suggests that these heteroreceptors tend to exert inhibitory effects on neurotransmitter release and influence GABA, Glu, and ACh signalling.
In particular, their influence on DA signalling appears to be more complex, possibly because GABA release indirectly inhibits dopamine activity rather than acting directly. In parallel, serotonin turnover, as measured by the 5-HIAA/5-HAT ratio, is a critical parameter.

Long-acting selective antagonists of the 5-HT1β receptor have been observed to increase serotonin turnover in specific brain regions such as the hypothalamus and hippocampus (Stenfors & Ross, 2002).

Serotonin is believed to play a crucial role in regulating adult neurogenesis. This is because serotonin acts as a neurotransmitter and modulator of the CNS. Specifically, serotonin appears to be a key modulator of the proliferation and differentiation of neural stem cells, which are responsible for the production of new neurons (Brezun & Daszuta, 1999). Furthermore, there is evidence for the active involvement of different 5-HT receptor subtypes in facilitating cell proliferation and neurogenesis in the adult brain via activation of 5-HT transmission. The consistent stimulation of neurogenesis in both neurogenic zones via 5-HT1A activation, coupled with the detectable involvement of 5-HT2C receptors specifically in the SVZ and the OB, suggests the involvement of multiple neuronal circuits in the regulatory mechanisms controlling adult neurogenesis (Table 2) (Banasr et al., 2004).

ND: not determined; ↔: no change

Treatment	**SGL**	**SVZ**
5-HT 1A		
8-OH-DPAT (agonist)	↑	↑
PCPA+8-OH-DPAT	*Reversal/ PCPA*	*ND*
5-HT 1B		
Sumatriptan (agonist)	↔	↓
GR127935 (antagonist)	↔	↑
PCPA+Sumatriptan	*Reversal/ PCPA*	*No reversal/ PCPA*
5-HT 2A/2C		
DOI (antagonist)	↔	↑
Ketansenin (antagonist)	↓	↔
5-HT/2C		
RO600175 (agonist)	↔	↑
SB 206553 (antagonist)	↔	↔

Table 2: 'Effects of agonists and antagonists of 5-HT receptors on cell proliferation in the two neurogenic zones of the adult brain' *(Banasr et al., 2004).*

Adapted from Neuropsychopharmacology (Springer Nature), Vol. 29, 450-460 (2004), Banasr et al., 2004, 'Serotonin-Induced Increases in Adult Cell Proliferation and Neurogenesis are Mediated Through Different and Common 5-HT Receptor Subtypes in the Dentate Gyrus and the Subventricular Zone' with permission from Springer Nature.

Furthermore, studies have demonstrated that serotonin can enhance the production of neurotrophic factors, including brain derived neurotrophic factor (BDNF), which facilitate the development of these newly formed neurons (Leschik et al., 2022). Serotonin has been shown to regulate synaptic plasticity, which is essential for the proper functioning of newly formed neurons (Banasr et al., 2004).
Finally, serotonin is known to play an important role in sleep regulation (Jouvet, 1999), although the relevant relationships are complex (Cespuglio, 2018). Serotonergic neurons constitute the most widely distributed neurochemical network in the vertebrate CNS (Jacobs & Azmitia, 1992), and their activity decreases during slow-wave sleep and completely ceases during REM sleep (Ashford, 2019).

Taken together, this evidence suggests that serotonin is an essential regulator of adult neurogenesis and likely plays a role in the development of new neurons.

1.4 Interaction of Aβ and serotonin signalling

The connection between Aβ dimers and the 5-HT1β receptor is crucial in the context of AD. This is because the activation of the serotonergic system has been demonstrated to prevent neuroinflammation caused by Aβ oligomers (Ledo et al., 2016). The Aβ-induced inflammatory response and associated neuronal cell death play an important role in the pathology of AD (Yang et al., 2021).
In a mouse model of APPswe/PS1 E9, which is associated with excessive Aβ deposition and increased plaque formation, an association was found with degeneration of serotonin-releasing neurons (Liu et al., 2008).

Levels of 5-HT and its metabolite 5-hydroxyindoleacetoacetic acid are also reduced in the post-mortem brains of AD patients (Rodriguez et al., 2012).

The results of another study show a downregulation of 5-HT1β associated with amyloid pathology (Tajeddinn et al., 2015). This reduction was demonstrated both in vitro and in the hippocampus of Tg2576 at 24 months, when these mice have high amyloid pathology (Bilkei-Gorzo, 2014). In addition, 5-HT1β receptor levels were found to increase with age in WT mice, whereas the opposite pattern was observed in Tg2576 mice. These data are consistent with the binding study showing reduced receptor densities in the brains of AD patients (Garcia-Alloza et al., 2004). Finally, the abnormal behaviour and altered balance of the neurotransmitters 5-HT, DA and ACh in tgD mice (Abdel-Hafiz et al., 2018; Müller-Schiffmann et al., 2018) is consistent with an interaction of Aβ dimers with 5-HT1β signalling.

Using an 5-HT1β receptor agonist or antagonist, we observed the interaction of Aβ dimers with the 5-HT1β receptor and its effects on adult neurogenesis in tgD mice.

1.5 Aim of the work

In this study, we used tgD mice that express Aβ dimers without plaque formation, tau pathology or neuroinflammation, mimicking early Alzheimer's disease. We aimed to test the hypothesis that chronic exposure to soluble Aβ dimers affects adult hippocampal neurogenesis through an interaction with 5-HT1β receptor signalling. We hypothesised that Aβ dimers may modulate the functionality of the 5-HT1β pathway and thus influence adult neurogenesis at the molecular level. This interaction is likely to be unidirectional. This study may provide a better understanding of the sole effect of soluble dimers on hippocampal structural plasticity and may help to develop new pharmacotherapeutic strategies for the treatment of early-stage Alzheimer's disease.

2. Materials and Methods

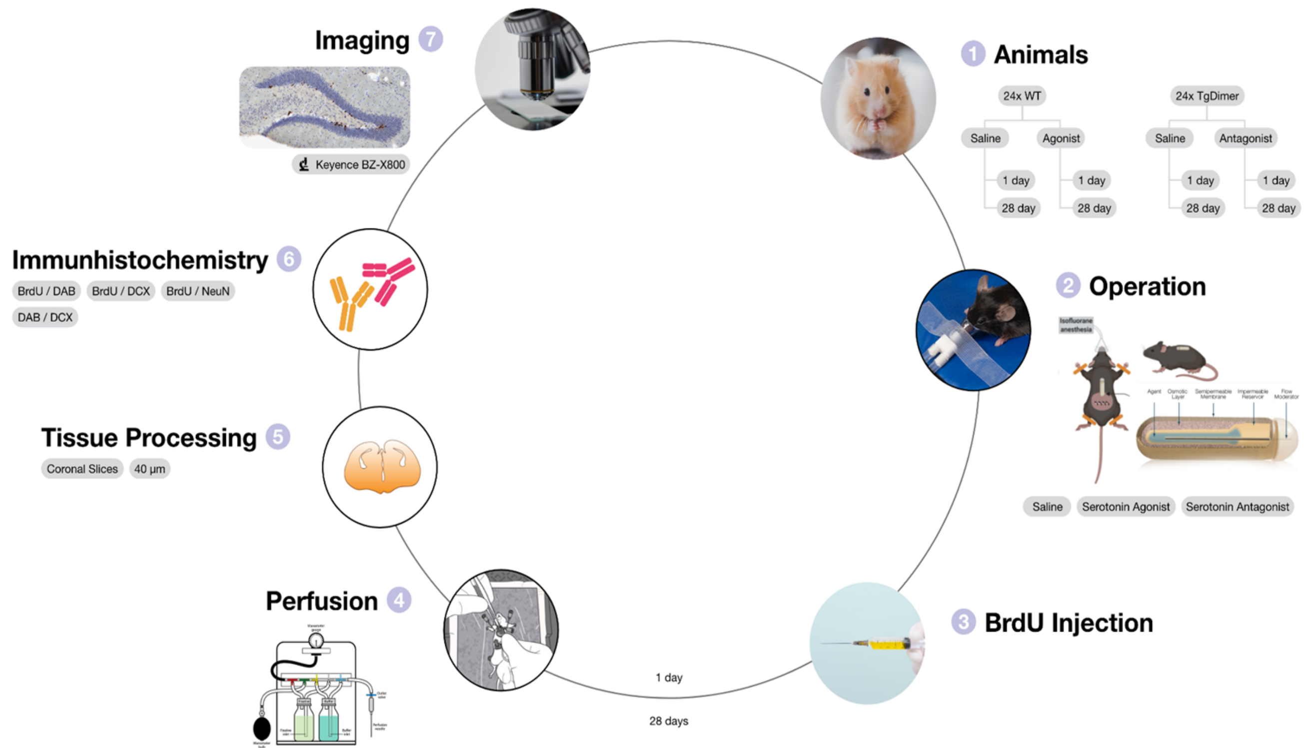


Figure 5: Workflow of experiments

2.1 Animals

Homozygous adult male tgD mice on the C57BL/6N background and wild-type C57BL/6N mice (n=24 per genotype), aged 4-5 months and weighing approximately 30 g, were used. The line was independently bred at the Central Animal Laboratory at the University Hospital Essen.

Animals were housed in sound- and light-proof chambers (Bioscape, Germany) in standard APEC polycarbonate cages. All animals were maintained on a standard photoperiod of 12 hours light and 12 hours dark, with light on at 6 am and off at 6 pm. Light intensity was 300 lux. All animals had ad libitum access to food and water.

The animal experiments were performed at the ‘Zentrale Einrichtung für Tierforschung und wissenschaftliche Tierschutzaufgaben (ZETT)’ and approved by the local government, North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection (LANUV), Germany (approval number: AZ: 81-02.04.2019.A239). All tests conducted in accordance with European Directive 2010/63/EU. Every effort was made to minimise the number of animals used and to minimise their pain and distress.

2.2 Surgery and drug delivery

A mini-osmotic pump (alzet® model 2006) was used to deliver 0.9% saline, 5-HT1β receptor agonist (CP 94253 hydrochloride, Tocris Bioscience, Ellisville, MO, USA) or antagonist (SB 216641 hydrochloride, Tocris Bioscience, Ellisville, MO, USA) to the mice. We used a potent, selective 5-HT1β agonist (K_i values of 89, 2, 860, 49 and 1,600 nM for 5-HT1A, 5-HT1β, 5-HT1C, 5-HT1D and 5-HT2 receptors, respectively) that is centrally active after systemic administration in vivo and a selective 5-HT1β antagonist with approximately 25-fold selectivity over $5\text{-HT}1_D$ and little or no affinity for a range of other receptor types.

The dose of the 5-HT1β agonist is 4 mg/kg/day and has been chosen based on antidepressant-like effect (Tatarczynska et al., 2004) and 5-HT synthesis (Skelin et al., 2012). The dose of the 5-HT1β receptor antagonist is 3 mg/kg/day and has been modified from Stenfors & Ross (Stenfors & Ross, 2002), who reported a counteracting effect on the fluoxetine-induced increase in the 5-HIAA/5-HT ratio at a dose of 4 mg/kg/day due to skin irritation at this concentration.

Each pump consisted of a main body and associated flow moderator. Using a sterile infusion needle and cannula, the substance was injected into the pump without air bubbles. The fill volume was approximately 200µl, which was released continuously over 42 days at a release rate of 3.6µl. The flow moderator was then inserted into the main body of the pump so that no liquid could escape from the pump. Further information is described by Theeuwes and Yun (F Theeuwes, 1976).

The pump was placed in a 5ml tube with the flow moderator facing up. The tube was filled with 0.9% NaCl solution and incubated at 37°C for 72 hours before use.

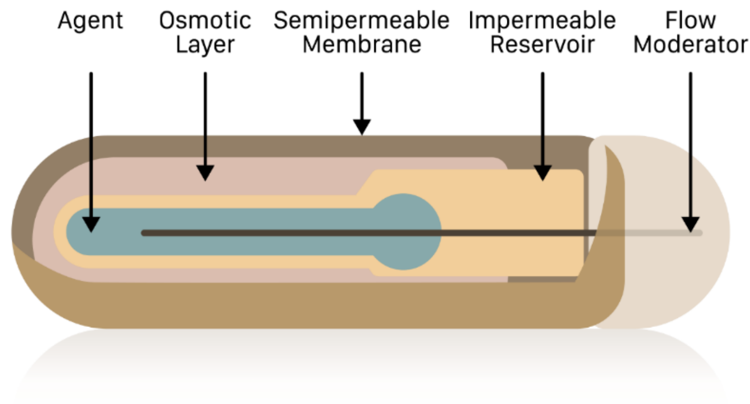


Figure 6: Schematic illustration of the mini-osmotic pump (ALZET)

The minipump operates by utilising the osmotic pressure differential between the osmotic layer inside the pump and the surrounding tissue where the pump is implanted. The higher osmolality of the osmotic layer causes water to enter the pump through a semi-permeable membrane that serves as the pump's outer surface. As water passes through the osmotic layer, it compresses a flexible reservoir, which expels the test solution from the pump at a regulated and predetermined rate (Figure adapted from https://www.alzet.com/products/alzet_pumps/how-does-it-work/).

Animals were first anaesthetised with isoflurane at a flow rate of 1.5-2% in a closed chamber. After checking for loss of pain reflexes, a 1 cm x 2 cm area on the back of the mouse was shaved and disinfected. A 2 cm sagittal incision was made and an artery clamp was used to separate the skin from the subcutaneous fat to create a pocket. The pump with the flow moderator was then placed in this pocket in a caudal direction. The skin was then closed with 4-0 VICRYLTM absorbable sutures (Ethicon®) and covered with silver spray (Selectavet, Dr Otto Fischer GmbH). Mice were injected after surgery and for a further 3 consecutive days and allowed to recover for 2 weeks.

2.3 BrdU injections

A dose of 100 mg/kg 5'-bromo-2'-deoxyuridine (Roche Diagnostics GmbH, Germany) (BrdU) was injected intraperitoneally (i.p.). 10 mg of BrdU was dissolved in 1 ml of 0.9% NaCl and administered to the mice in a weight-adjusted manner. After a recovery period of two weeks post-operatively, the mice were injected twice daily for three consecutive days at 7 am and 6 pm.

2.4 Experimental groups

Wild type and tgD mice were randomly divided into four groups (n=6 mice per group). The first group was implanted with an osmotic minipump releasing NaCl, injected with BrdU for three consecutive days, and perfused one day after the last BrdU injection.

The second group was implanted with an osmotic minipump releasing NaCl, injected with BrdU for three consecutive days and perfused 28 days after the last BrdU injection.

The third group was implanted with an osmotic minipump releasing a selective 5-HT1β agonist (chemical name: 5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo[3,2-b]pyridine hydrochloride) for wild-type mice or a selective 5-HT1β antagonist (chemical name: N-[3-[3-(dimethylamino)ethoxy]-4-methoxyphenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1'-biphenyl]-4-carboxamide hydrochloride) for tgD mice, injected with BrdU for three consecutive days and then perfused 1 day after the last BrdU injection to study the proliferation of NPCs.

The fourth group was implanted with an osmotic minipump releasing a selective 5-HT1β agonist for wild-type mice or a selective 5-HT1β antagonist for tgD mice, injected with BrdU for three consecutive days and perfused 28 days after the last BrdU injection to study survival and neuronal differentiation.

2.5 Tissue processing

The mice were anaesthetised with ketamine/xylazaline (100 mg/10 mg/kg body weight) and then intracardially perfused with 0.9% NaCl followed by 4% paraformaldehyde using a Ministar peristaltic pump (World Precision Instruments, Sarasota, FL, USA). The brains were removed from the skull and postfixed in 4% paraformaldehyde for 24 hours. Subsequently, the brains were cryoprotected by storing them in 20% and then 30% sucrose. The right hemisphere was sliced into 40µm free-floating coronal sections using a cryostat (LEICA CM3050 S).

2.6 Immunohistochemistry

Every sixth section from each brain was used for immunohistochemistry. The sections were first washed in phosphate buffered saline (PBS). The sections were then treated with 0.6% H2O2 for 30 minutes and washed again with PBS. For DNA denaturation, sections were incubated in 2N HCl at 37°C for 30 minutes. For neutralisation, 0.1M boric acid was used for 10 minutes and washed with PBS containing 0.2% Triton-X 100 (PBS-T 0.2%). To minimise non-specific binding of the secondary antibody, sections were incubated in 5% normal goat serum (Vector Laboratories, United States) in PBS-T 0.2% for one hour. Incubation with the primary antibody rat monoclonal anti-BrdU antibody (1:1000, Biorad, Germany) followed overnight at 4°C. After washing the sections with PBS-T 0.2% the next day, they were incubated with the secondary antibody goat anti-rat IgG (1:500, Vector Laboratories, United States) for one hour. After a further wash with PBS-T 0.2%, the sections were incubated with VECTASTAIN® Elite® ABC solution (Vector Laboratories, United States) for one hour at room temperature. The sections were washed with PBS-T 0.2% and incubated with 0.05% 3,3'-diaminobenzinide (Sigma-Aldrich, Germany) for 4 minutes. The sections were then washed again, mounted on slides, counterstained with cresyl violet at 60°C for 3 minutes and then destained in 70% isopropanol for 2 x 1 minute.

This was followed by incubation with 97% isopropanol and 100% isopropanol for one minute each twice and cover slipping with Depex (Serva Electrophoresis GmbH, Germany).

Animals sacrificed one day after BrdU injection were subjected to further immunohistochemistry. The primary antibody was rabbit anti-DCX (Abcam, England) and the secondary antibody was goat anti-rabbit (Vector laboratories. United States). DNA denaturation steps were omitted.

2.7 Immunofluorescence

All brain sections from animals killed one day after BrdU injection were treated with double immunofluorescence antibodies against BrdU/DCX. All brain sections from animals killed 28 days after BrdU injection were treated with double immunofluorescence antibodies against BrdU/NeuN.

The DNA was denatured for BrdU staining according to the method described above. Sections from animals killed one day after the last BrdU injection were incubated with the following primary antibodies: rat monoclonal anti-BrdU (1:500, Biorad, Germany) and rabbit polyclonal anti-DCX (1:1000, Abcam, England), while sections from animals killed 28 days after the last BrdU injection were incubated with anti-BrdU and rabbit polyclonal anti-NeuN (1:1000, Sigma-Aldrich Chemie GmbH, Germany). The sections were washed and then incubated with secondary antibodies Alexa Fluor 488 goat anti-rabbit IgG (1:500, Invitrogen by Thermo Fisher Scientific, United States) and Alexa Fluor 568 goat anti-rabbit IgG (1:500, Thermo Fisher Scientific, United States) for one hour. The sections were then washed again and coverslipped with DAPI Fluoromount-G (Southern Biotech, United States). Slides were stored at 4°C in the dark until use.

2.8 Imaging and quantification

It should be noted that the samples were not blinded in the study performed.

1. Analysis of the number of BrdU-positive cells in the DG

BrdU-labelled cells were counted manually using a 40X objective of the KEYENCE BZ-X800 microscope. The dentate gyrus was divided into 4 zones: the outer third, the middle third, the inner third and the SGZ. The SGZ was defined as the area from the inner third to the hilus, which still contained two nuclei.

Every sixth brain section was stained with peroxidase/diaminobenzidine (DAB) and the cells counted. The resulting number was multiplied by 6 to give the total number of BrdU-positive cells in the entire hippocampus.

2. Analysis of the neuronal phenotype of BrdU-positive cells in the DG

Fifty to sixty BrdU-positive cells were examined for co-labelling with DCX to detect migrating neuroblasts. Twenty to thirty BrdU-positive cells were tested for co-expression of NeuN (marker of neuronal differentiation) to analyse differentiation after 28 days. Fluorescence signals were captured using a KEYENCE BZ-X800 series (Japan) with a 40X objective. Images were analysed using BZ-II analysis software. Identical settings (exposure time, exposure interval, haze reduction) were used for each analysis. Co-localisation was demonstrated using Z-stack and real-time 3D methods.

3. Analysis of the number of DCX-positive cells in the DG

The number of cells recorded on the slides was multiplied by the number of serial sections to obtain a measure of total number. Cells were assigned to the GCL or SGZ according to their location.

2.9 Statistical analysis

Statistical analysis was performed using Graph Pad Prism software. Normal distribution was tested using the Shapiro-Wilk test, and if the data passed the normality test, the parametric one-way ANOVA test was used, followed by the Tukey post hoc test to detect differences between groups. If the data did not pass the normality test, the non-parametric Kruskal-Wallis test was used to identify differences between groups. Values are expressed as mean ± standard error of the mean (SEM). A P value < 0.05 was considered statistically significant.

3. Results

3. 1 Proliferation of neuronal progenitors and neuroblast pool is reduced in tgD mice

To demonstrate the proliferation of NPCs in the SGZ and GCL, we performed a BrdU assay, as the pyrimidine analogue is incorporated into the DNA of proliferating cells. The BrdU-positive (+) cells were found in the DG of tgD and WT mice and had homogeneously stained triangular, longitudinal, or round nuclei. In the SGZ, BrdU+ cells were mainly arranged in clusters, whereas in the CGL, BrdU+ cells were more sporadically distributed. The tgD mice had a significantly reduced number of BrdU+ cells (1369±123.1 cells) compared to WT mice when treated with saline and sacrificed one day after BrdU injection (2133±39.4 cells) by approximately 64.18% (P=0.01) (Fig.7.1).

Consistently, the number of cells immunoreactive for the neural progenitor type 3 marker DCX was significantly higher (P=0.003) in WT (761.3±29.4) than in tgD mice (394.9±48.9) (Fig.7.2). These data suggest that Aβ dimers affect the proliferation of NPCs.

3.2 Reduction of proliferating progenitors in tgD mice reversed by 5-HT1β receptor antagonist

TgD mice treated with 5-HT1β receptor antagonist showed a significant increase in the number of BrdU+ cells one day after the last BrdU injection (1932±217 cells) compared to tgD mice treated with saline and scarified one day after the BrdU injection (1369±123.1) (P = 0.05), showing an approximately 70.86% increase in the number of NPCs, which was comparable to WT mice (Fig. 7.1).

Consistently, we observed that in tgD mice treated with 5-HT1β antagonist, the number of DCX cells increased (662.7±68.3) (P=0.024) and became comparable to the DCX+ cell density in WT mice (Fig.7.2). These data suggest that 5-HT1β antagonist treatment ameliorates the effect of Aβ dimers on NPC proliferation.
The number of BrdU+ cells (Fig.7.1) and the density of DCX+ (Fig.7.2) were not significantly different between WT treated with 5-HT1β agonist or vehicle. These data suggest that 5-HT1β activation does not affect the proliferation of NPCs in the absence of Aβ dimers.

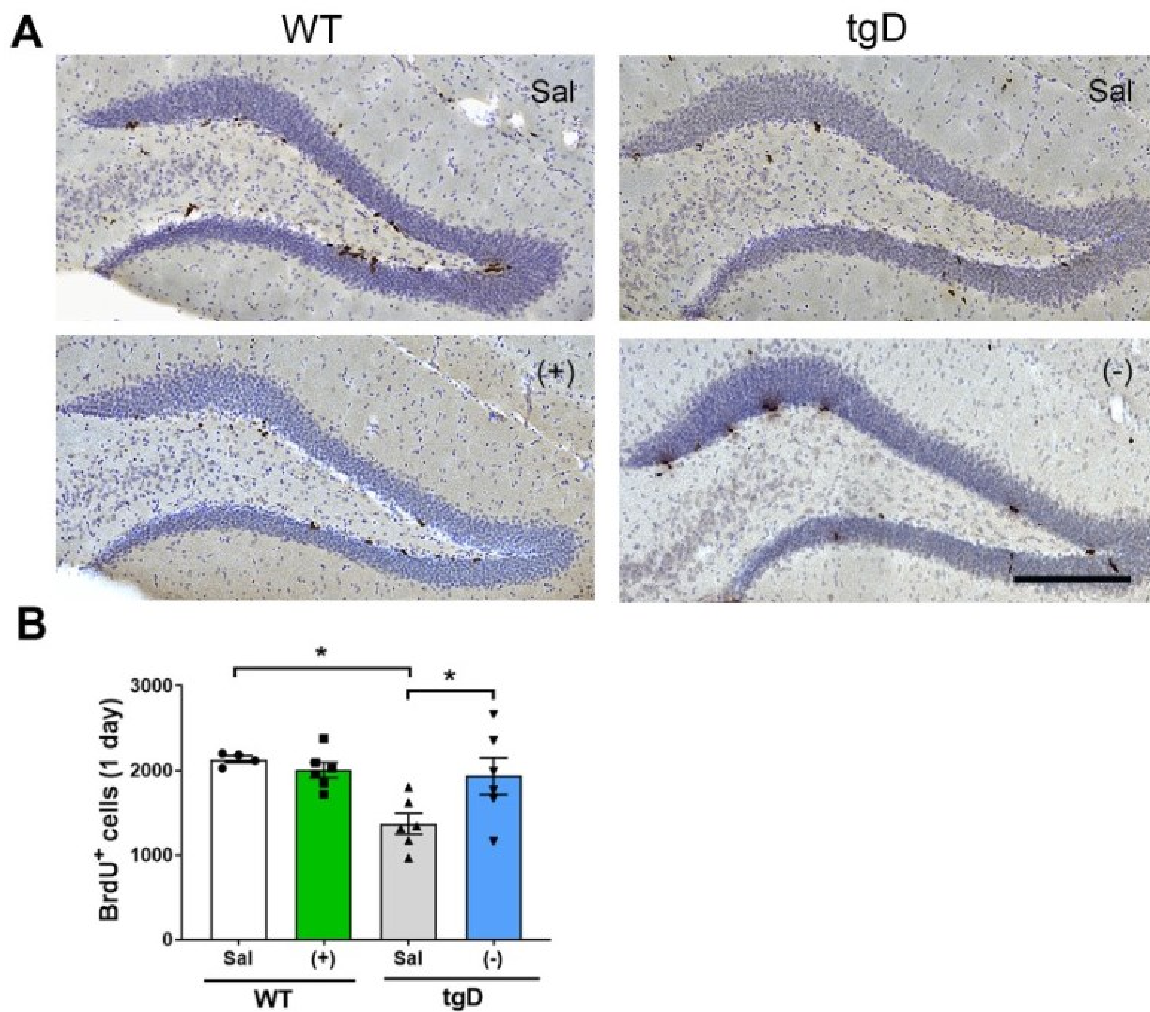


Fig 7.1: Effect of 5-HT1β signalling on hippocampal cell proliferation

*(A) Representative photomicrographs of bromdesoxyuridine positive (BrdU+) cells (brown) and cresyl violet staining (blue) in the dentate gyrus (DG) of WT and tgD mice treated with saline (Sal), 5-HT1β agonist (+) or 5-HT1β antagonist (-). Scale bar = 200 μm. (B) Quantification of the absolute number of BrdU+ cells within the DG. n = 6 per group. * : P < 0.05, **: P < 0.01 by ANOVA and Tukey post hoc test. Abbreviations: BrdU – bromdesoxyuridine; DG – dentate gyrus; WT – wild-type.*

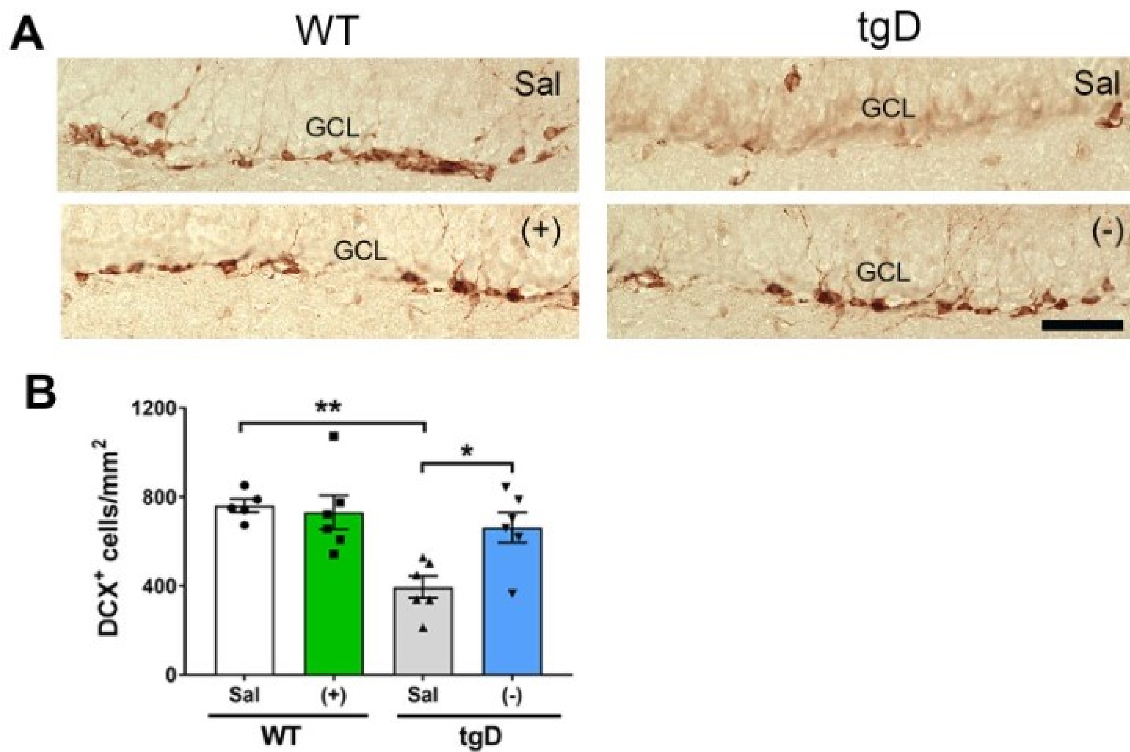


Fig. 7.2: Effect of 5-HT1β signalling on neuronal progenitors

*(A) Representative micrographs of doublecortin-positive (DCX+) cells (brown) in the DG of WT and tgD mice. Granular cell layer (GCL). Scale bar = 50 μm. (B) Quantification of density of DCX+ cell density within DG. n = 6 per group. * : P < 0.05, **: P < 0.01 using ANOVA and Tukey Post hoc test. Abbreviations: DCX – doublecortin; DG – dentate gyrus; GCL– granular cell layer; WT – wild-type.*

3.3 Survival of newborn progenitors is not affected by 5-HT1β receptor antagonist

After 28 days, there was also a significant decrease in the number of surviving NPCs in tgD mice (189±10.59) compared to WT mice (436.5±53.97) (P=0.001), consistent with the initial reduction in neural progenitor proliferation (Fig.7.1 A, B). Using linear regression, we observed that the survival rates of newborn progenitors in tgD mice did not change compared to WT mice (Fig.8 C).

Twenty-eight days after the last BrdU injection, application of the 5-HT1β receptor antagonist to tgD mice significantly increased the number of BrdU+ cells in the DG (345±34.67) by approximately 54.78% compared to saline-treated tgD mice (P=0.0234). Consistent with the lack of an initial effect of 5-HT1β receptor agonist on proliferation in WT mice, administration of 5-HT1β receptor agonist to WT mice did not affect the number of BrdU+ cells 28 days after the last BrdU injection (369±42.42) (P=0.6116). Furthermore, there were no statistically significant differences between the survival of newborn cells in WT and 5-HT1β agonist treated WT (Fig.8 E). To investigate whether the decrease in BrdU+ cells and therefore neurogenesis affects the total volume of the DG, we performed a total volume measurement. There was no significant difference between WT and tgD mice (Fig.8 D). Application of 5-HT1β agonist had no effect on DG volume in WT mice. However, we observed a significant increase in the total volume of the DG of tgD mice treated with 5-HT1β antagonist (0.01793mm3±0.002510) compared to tgD mice treated with saline (0.01992mm3±0.0008256) (P=0.0192).

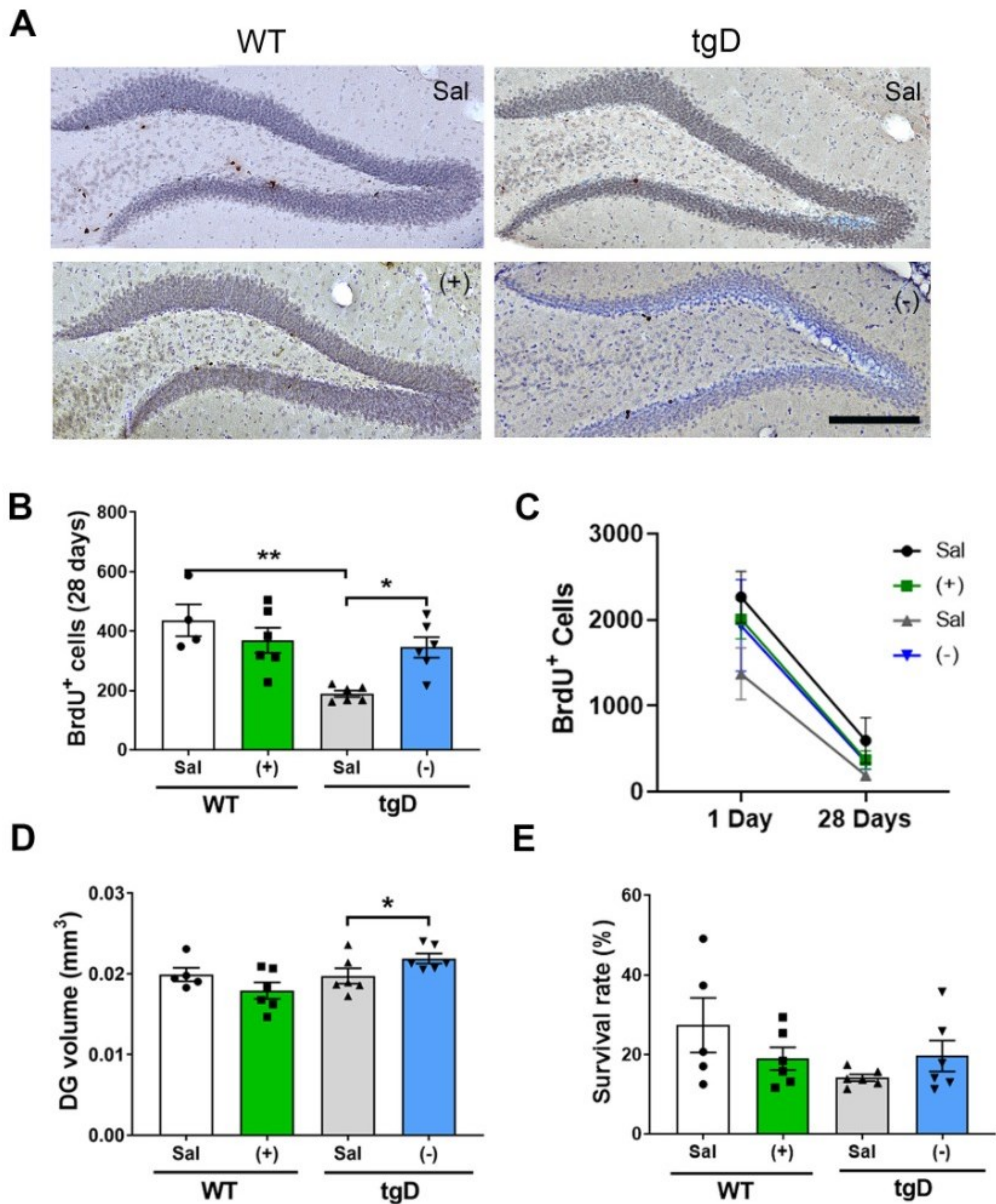


Fig. 8: Long-term effects of 5-HT1β signalling on cell survival

(A) Representative photomicrographs of BrdU+ cells (brown) and cresyl violet staining (blue) in the DG of WT and tgD mice treated with saline (Sal), 5-HT1β agonist (+) or 5-HT1β antagonist (-) 28 days after the last BrdU administration. Scale bar = 200 µm.

*(B) Quantification of the absolute number of BrdU+ cells within the DG. n = 6 per group. **: P < 0.01 by ANOVA and Tukey post hoc test. (C) Linear regression of BrdU+ cell survival. (D) Quantification of total DG volume. n = 6 per group. *: P < 0.05 by ANOVA followed by Tukey post hoc test. (E) Quantification of survival rate. n = 6 per group. **: P < 0.01 by ANOVA and Tukey post hoc test. Abbreviations: BrdU – bromdesoxyuridine; DG – dentate gyrus; WT – wildtype.*

3.4 Spatial distribution and migration of neuronal progenitors are not affected in tgD mouse or by 5-HT1β receptor modulation

The spatial distribution and migration to the final position of BrdU+ cells 1 day and 28 days after the last BrdU administration were analysed by estimating the percentage of BrdU+ cells located in the SGZ, inner third, middle third or outer third of the GCL out of the total number of BrdU+ cells. Our results indicate that the spatial distribution and migration to the final location was similar in both WT and tgD mice (P > 0.05). Furthermore, treatment of WT and tgD mice with 5-HT1β agonist and antagonist, respectively, had no significant effect on the spatial distribution and migration of neuronal progenitors (Fig.9).

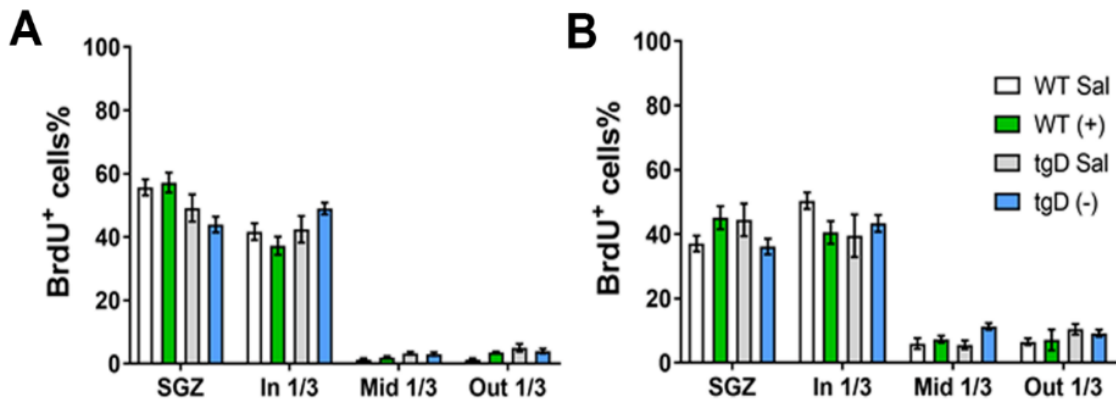


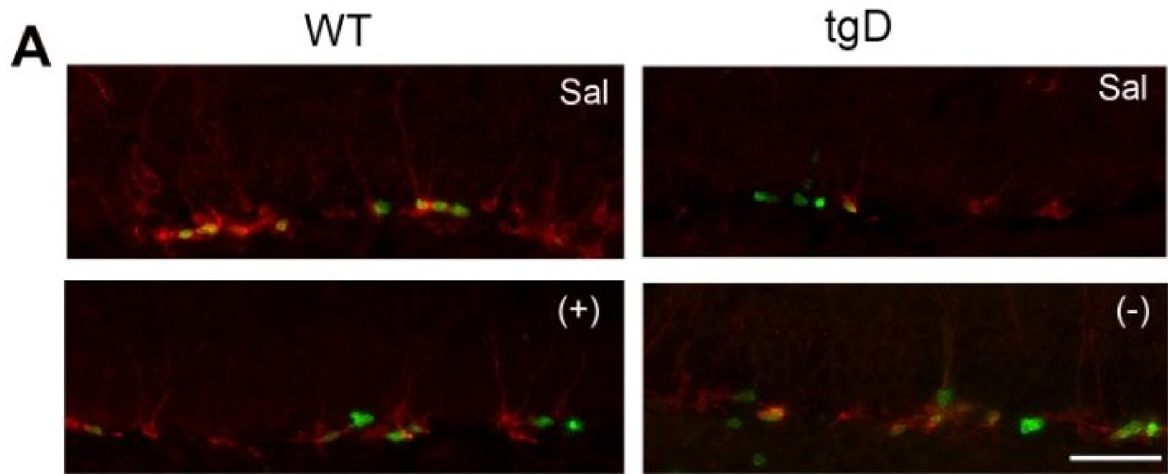
Fig 9.: Spatial distribution of BrdU-immunoreactive cells

(A) One day after BrdU injection. (B) 4 weeks after the last BrdU injection. SGZ: subgranular zone, In 1/3: inner third, Mid 1/3: middle third, Out 1/3: outer third of the granule cell layer. Abbreviations: BrdU – bromdesoxyuridine; SGZ – subgranular zone.

3.5 Neuronal differentiation was not affected in tgD mouse or by modulation of the5-HT1β receptor

The percentage of cells immunoreactive for DCX (DCX+), the marker for neuroblasts/immature neurons, among the BrdU+ cells was analysed one day after the last BrdU application. There was no significant difference in the percentage of BrdU/DCX+ co-labelled cells between WT and tgD mice (Fig.10.1). This suggests that despite the overall lower number of BrdU+ cells and thus fewer NPCs, these cells differentiate into mature cells at an equivalent rate. There was no difference in the percentage of BrdU/DCX+ co-labelled cells between WT and tgD mice after treatment of WT and tgD mice with 5-HT1β agonist and antagonist. This suggests that modulation of 5-HT1β receptor signalling has no effect on neuronal differentiation.

In addition, the differentiation of NPCs into mature neurons expressing NeuN was examined 28 days after the last BrdU injection. The percentage of cells co-expressing BrdU and the neuronal marker NeuN relative to total BrdU+ cells was not significantly different between experimental groups (Fig.10.2), suggesting that neuronal maturation in tgD mice is not disrupted and is not altered by modulation of 5-HT1β agonists and antagonists.



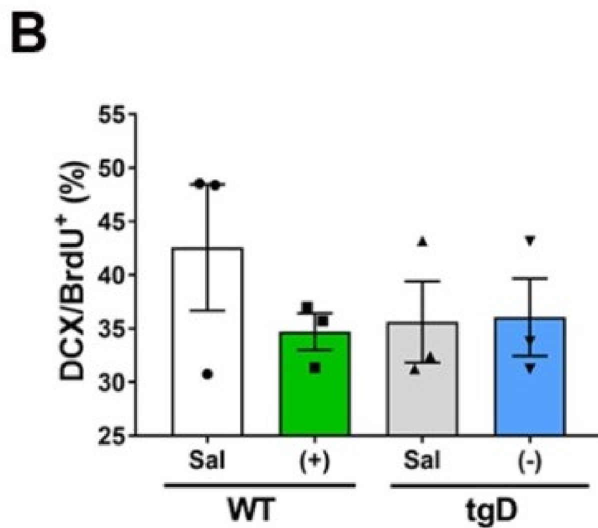
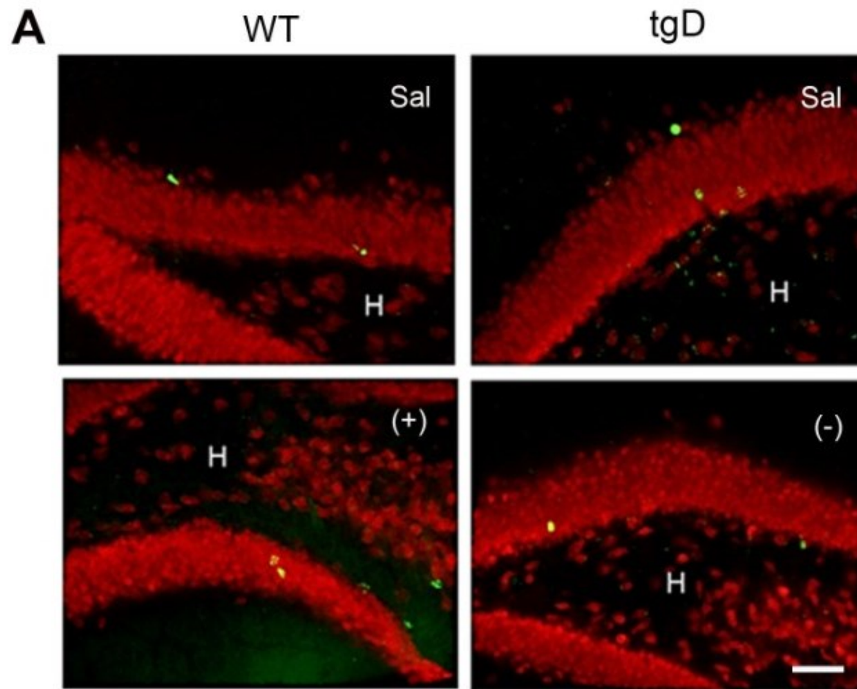


Fig. 10.1: Analysis of BrdU and DCX co-localisation

(A) Representative photomicrographs showing BrdU+ (green) and DCX+ (red) cells in the DG of the hippocampus. Scale bar = 50µm (B) Quantification of DCX/BrdU+ cells as a percentage of total BrdU+ cells 1 day after the last BrdU administration using the Shapiro-Wilk test followed by the Kruskal-Wallis test. Abbreviations: BrdU – bromdesoxyuridine; DCX – doublecortin; DG – dentate gyrus.



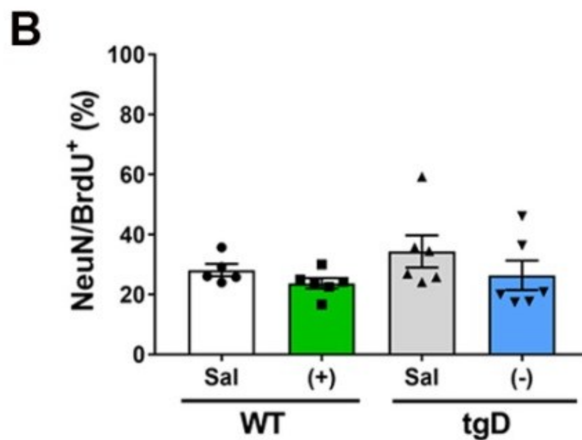


Fig. 10.2: Analysis of BrdU and NeuN co-localisation

(A) Representative photomicrographs showing immunofluoresce of BrdU+ (green) and NeuN+ (red) in the DG of the hippocampus. Scale bar = 50µm (B) Quantification of NeuN/BrdU+ cells as a percentage of total BrdU+ cells 28 days after the last BrdU administration using the Shapiro-Wilk test followed by the Kruskal-Wallis test. Abbreviations: BrdU – bromdesoxyuridine; NeuN – neuronal nuclear protein; DG – dentate gyrus.

4. Discussion

Considering our results, we can partially confirm our hypothesis. The proliferation of NPCs and the pool of neuroblasts show a significant reduction in tgD mice. This reduction was reversible by 5-HT1β regulation, suggesting that the observed change in tgD mice is due to the interaction of Aβ with the 5-HT1β receptor.
However, NPC survival rate, distribution and migration are unaffected. Differentiation is not affected either in tgD nor by modulation of the 5-HT1β receptor.
It remains a matter for future research to establish why proliferation of NPCs is impaired while other aspects of adult neurogenesis remain unchanged.

In this thesis, we report that the proliferation of neuronal progenitors and the pool of neuroblasts in the hippocampal dentate gyrus are reduced in tgD mice. This suggests that soluble forms of Aβ affect adult neurogenesis and thus structural neuroplasticity. These findings are consistent with reduced proliferation of neuronal progenitors in several mouse models. For example, the 3xTg mouse, a transgenic AD model with mutations in the APP sw, PS-1 and MAPT genes (Oddo et al., 2003) with increased tau tangles, Aβ plaques and neuroinflammation (Hamilton et al., 2010), shows significantly reduced adult neurogenesis in both the SGZ in the hippocampus and the SVZ of the lateral ventricle. At 4 months of age, 3xTg mice exhibit a reduction of up to 63% in Ki67+, BrdU+ NPCs, and DCX+ neuroblasts. By 12 months of age, the ability to generate new neurons is completely abolished (Hamilton et al., 2010; Rodríguez et al., 2008; Rodríguez et al., 2009).

In addition, similar observations have been reported in 5xFAD mice, another AD mouse model that carries 5 mutations in AD-related genes, including 3 APP mutations and 2 PS-1 mutations. These mice show neuropathological changes such as extracellular and intracellular accumulation of amyloid plaques along with synapse loss, neuronal death, neuroinflammation and memory deficits.

At 2 months of age, these mice show reduced adult hippocampal neurogenesis in the DG, as evidenced by reduced DCX expression. By 7 months of age, the mice no longer generate new neurons (Moon et al., 2014). However, disruption of neuronal differentiation and, to a lesser extent, impaired proliferation are thought to be the primary causes of neurogenesis impairment in 5xFAD (Moon et al., 2014; Zaletel et al., 2018).

In contrast to these findings, Jin et al. (Jin, Galvan, et al., 2004) found that the number of BrdU-labelled cells in the DG is increased in another AD model, the PDGF-APP mice, which have two different mutations in the APP gene. These mice show signs of AD-like neuropathology such as amyloid plaques, dystrophic neurites, and gliosis. This reduction was mainly seen in older mice, aged 1 year, while the younger mice, aged 3 months, showed no significant differences compared to wild-type mice. However, another study showed no significant difference in neurogenesis in 5-month-old APP23 transgenic mice before amyloid deposition compared to wild-type controls, but 25-month-old amyloid-depositing APP23 mice showed significantly increased neurogenesis compared to controls (Crews et al., 2010; Ermini et al., 2008).

The varied results may be due to the complex nature of Alzheimer's disease. Numerous factors influence the disease, such as the degree of Aβ toxicity, the extent of plaques and neurofibrillary tangles, oxidative stress, neuroinflammation and neurodegeneration. The different genetic mutations in the transgenic models could also have an impact.

Interestingly, some post-mortem studies of the brains of AD patients support our findings. Lovell and colleagues found reduced survival and senescence of NPCs in the hippocampus of AD patients (Lovell et al., 2006). As AD progresses, the density of immature neurons in the hippocampus decreases, potentially leading to deterioration of the neuronal lineage (Moreno-Jiménez et al., 2019).

However, increased neuronal markers such as DCX, PSA-NCAM and TUC4 have been found in the hippocampus of AD patients (reviewed by Babcock et al., 2021). One explanation would be a compensatory mechanism triggered by neurodegeneration. However, both studies examined a relatively small number (7-14) of post-mortem brains from AD patients (Jin, Peel, et al., 2004; Ziabreva et al., 2006).

The survival of NPCs until the maturation and integration of newborn neurons in the SGZ is essential for hippocampal integrity and function. Many factors could influence the survival of NPCs, for example, exposure to an enriched environment promotes the survival of immature neurons (Kee et al., 2007; Tashiro et al., 2007). In contrast, ageing and stress can have negative effects (reviewed by Klempin & Kempermann, 2007). These findings shed light on the complex balance of factors that influence the survival of neural progenitor cells.

In our study, we found no difference in NPC survival rates between tgD and wild-type mice. In contrast, researchers using BrdU and NeuN found more NPCs surviving in the APP/PS1 transgenic mouse model of AD (Yu et al., 2009). Another study showed that early NPC survival in an AD mouse model using APP/Aβ transgenic mice was unaltered, while later there was a decrease in survival at 4-6 weeks of age by 12% decrease compared to normal mice. They also detected fewer maturation markers in only a third of the Alzheimer's mice, compared to about half of the normal mice (Verret et al., 2007).

In addition, Wen's group saw lower NPC survival rates in PS1 transgenic AD mice. However this may be because of failed neurogenesis during the embryonic growth, resulting in fewer mature and immature neurons overall, which eventually leads to AD (Wen et al., 2004).

In PS1/PS2 double knockout mice, there was no detectable change in the short-term survival of these mice. Yet, a significant decrease in the long-term survival of BrdU/NeuN positive cells was reported (Chen & Dong, 2009).

These inconsistent findings may be attributed to variations in the models employed, experimental protocols, animal age and markers utilised for analysis (Shruster et al., 2010).

Migration of neuronal progenitors within the neurogenic niche in the hippocampus and their final spatial distribution in the granular cell layer is an essential feature of memory and cognitive functional integrity of the hippocampus. Disruptions in the spatial distribution and migration can have significant implications for brain development and function (reviewed by Mu & Gage, 2011). Our results show that the spatial distribution and migration of neuronal progenitors are unaffected in the tgD mouse model, suggesting that these specific processes may not be directly affected by early amyloid pathology in the form of soluble Aβ in tgD mice.

The final step in adult neurogenesis is the differentiation of newborn cells into mature neurons and their integration into the hippocampal circuitry.
In our study, Aβ dimers alone may not be associated with significant impairments in neuronal differentiation, as supported by other studies. Donovan and collegues found that the number of BrdU/ NeuN positive cells in PDAPP mice expressing human V717F mutant APP under the control of the platelet-derived growth factor-β promoter was not significantly different from WT mice. After 4 weeks, the same proportion of new cells became neurons, so there was no difference in neuronal fate (Donovan et al., 2006). Another study using double transgenic mice expressing the CaMKII-TAA and tetO-APPsxe/ind 102 genes supports this finding.
In a further study, immunohistochemistry using BrdU/ NeuN showed no difference between CaMKII-TTA/ APP mice and the control group in terms of differentiation into mature neurons in the SGZ (Yetman & Jankowsky, 2013).

The effect of AD on neuronal differentiation has been studied in several AD models (Ming & Song, 2011). For example, an AD mouse model with APPSw,Ind mutations showed increased neurogenic differentiation at an early stage (Jin, Galvan, et al., 2004).

However, this increase in neuronal differentiation was not maintained in older mice, suggesting a dynamic regulation of this process that changes with age and disease progression (Heo et al., 2007). Similarly, in Bi-Tg mice carrying pPDGF-APPSw,Ind and pNes-LacZ mutations, Aβ plaques promote the differentiation of NPCs (Gan et al., 2008).

Interestingly, specific Aβ isoforms were shown to have different effects, as oligomers and small aggregated forms of Aβ1-42 had positive effects on neuronal differentiation, whereas soluble forms of Aβ1-42 and all forms of Aβ1-40 had no effect (López-Toledano & Shelanski, 2004). In contrast, another study found that Aβ1-40 and Aβ1-42 induced astrocytic and neuronal differentiation (Chen & Dong, 2009). In addition, a third study showed that both Aβ1-42 monomers and oligomers induced neuronal differentiation, whereas the fibrillar form had no effect (Heo et al., 2007).

These divergent results highlight the complexity of the influence of Aβ on neuronal differentiation and suggest that different Aβ isoforms may have different effects. Overall, the effect of AD on neuronal differentiation is influenced by a combination of different factors such as Aβ isoforms, age and Aβ deposition (reviewed by Chuang, 2010).

In our study, the significant reduction in hippocampal NPC proliferation in tgD mice could be reversed by administration of a specific 5-HT1β receptor antagonist. These findings suggest a potential link between altered serotonin signalling through the 5-HT1β receptor and the proliferation of hippocampal neuronal progenitors in the tgD mice. Importantly, regulation of 5-HT1β receptors also appears to contribute to the induction of proliferation (Banasr et al., 2004). However, there is a limited amount of data on the effect of the 5-HT1β subtype alone on proliferation.

Serotonin plays an essential role in adult hippocampal neurogenesis (Jin, Galvan, et al., 2004; Song et al., 2016).

Studies showed that reducing central 5-HT levels in adult mice led to enhanced neurogenesis, characterised by increased proliferation of NPCs and improved survival of new born neurons in the hippocampus (Diaz et al., 2013). This effect was observed in mice with a significant reduction of serotonergic neurons, but not in those with a milder reduction (Song et al., 2016). Additionally, the dendritic length of adult neurons was increased in mice with reduced serotonin levels (Song et al., 2016).

Moreover, chronic administration of selective serotonin reuptake inhibitors (SSRIs), which increase extracellular 5-HT levels, promotes neurogenesis (reviewed by Song, Huang, et al., 2017). The findings suggest that both extremely low and high levels of central 5-HT can promote adult hippocampal neurogenesis, possibly through activation of different combinations of 5-HT receptors, and that central 5-HT plays multiple roles in regulating adult neurogenesis, as evidenced by findings from mouse models with dysfunctional 5-HT signalling at different developmental stages (reviewed by Song, Zhang, et al., 2017).

Pharmacological manipulation of 5-HT receptors has shown that different subtypes, such as 5-HT1A and 5-HT2, also regulate different stages of neurogenesis (Klempin et al., 2013). The 5-HT1A receptor subtype has been identified as a key mediator of the effects of serotonin. In vitro experiments have shown that isolated neural stem cells express 5-HT1A receptors and produce both serotonin and tryptophan hydroxylase (TPH), an enzyme involved in serotonin synthesis (Benninghoff et al., 2009; Klempin et al., 2013). Blocking 5-HT1A receptors or reducing serotonin levels reduces the self-renewal and proliferation of NPCs. On the other hand, stimulating 5-HT1A receptors increases the generation of neuronal cells. In contrast to 5-HT1A, the 5-HT2 receptor family exerts different effects on neurogenesis. Acute activation of the 5-HT2C receptor subtype can either decrease cell proliferation or have no effect (Banasr et al., 2004; Klempin et al., 2013). Conversely, blocking 5-HT2C receptors leads to an increase in the number of labelled cells. These findings suggest that the actions of 5-HT2 receptors and their impact on neurogenesis are distinct from those of 5-HT1A receptors.

Importantly, 5-HT receptors are expressed in specific regions of the hippocampus and play a crucial role in adult hippocampal neurogenesis (reviewed by Alenina & Klempin, 2015). Increasing 5-HT levels by chronic administration of SSRIs increased the survival of adult neurons and promoted the maturation of immature neurons in the hippocampus (Malberg et al., 2000; Santarelli et al., 2003; Wang et al., 2014).
However, it has been shown that lifelong serotonin depletion in tph2 mice does not result in a measurable change in proliferation compared to WT mice (Klempin et al., 2013). These data suggest that there are permanent compensatory mechanisms in the lifelong absence of serotonin. Chronic activation of these receptors increases proliferation, whereas prolonged inhibition reduces net neurogenesis (reviewed by Song, Huang, et al., 2017).

We next investigated the effect of a 5-HT1β receptor antagonist on the survival of newborn progenitor cells in tgD mice. Our study showed that administration of a 5-HT1β receptor agonist to WT mice or antagonist to tgD mice did not affect the newborn progenitor survival rate, which may be due to the release of compensation caused by decreased proliferation in tgD mice. Another hypothesis is that the 5-HT1β receptor does not play a critical role in promoting the survival of NPCs.
The increased survival induced by chronic administration of fluoxetine, an SSRI, is blocked in 5-HT1β KO mice (Diaz et al., 2012). Similarly, increased survival and antidepressant-like behaviour induced by exercise are blocked in 5-HT3 KO mice (reviewed by Song, Huang, et al., 2017). This shows the complexity of the 5-HT signalling pathway in the survival of NPCs.

In our study, receptor modulation by 5-HT1β antagonists did not affect the spatial distribution and migration of NPCs in the tgD mouse model. In addition, administration of a 5-HT1β agonist to WT mice also had no effect on NPC distribution and migration. These findings suggest that manipulation of this receptor alone may not have a direct effect on the spatial distribution and migration of neuronal progenitors in the mouse hippocampus.

We found that neuronal maturation in tgD mice is not disrupted and is not altered by modulation of 5-HT1β agonists and antagonists. Most studies investigating the influence of serotonin on adult neurogenesis have focused primarily on the proliferation and survival of adult neurons rather than their maturation (reviewed by Song, Zhang, et al., 2017). But the loss of central serotonin levels, as observed in tryptophan hydroxylase 2/serotonin transporter enhancer protein (Tph2Pet1 CKO) and parvalbumin-expressing interneuron-specific diphtheria toxin receptor (PC/DTR) mouse models, can affect neuronal maturation and dendritic length of adult-born neurons (Jia et al., 2014; Song et al., 2016).

Another study examined the effects of extremely low and high levels of serotonin on neuronal cell development in rodents. Low serotonin levels, induced by the antagonist Mianserin, led to specific apoptosis of neuronal cells, while fibroblasts remained unaffected. In contrast, very high levels of serotonin resulted in significant changes, including a decrease in cell aggregation, impaired cell organisation and impaired differentiation of neuronal cells. This was reflected in altered expression of proteins such as glial cell line-derived neurotrophic factor (GDNF) and NeuN.

Furthermore, the formation of axons was significantly decreased in the studies described. While the organization of the cytoskeleton was unchanged with high serotonin levels, there were clear problems with the differentiation processes. This research underscores serotonin's critical role in neuron survival and development, and brings to light potential negative impacts of abnormal serotonin levels during development (Menegola et al., 2004).

Other neurotransmitters affecting adult hippocampal neurogenesis include GABA, the primary inhibitory neurotransmitter, which has a dual effect on adult hippocampal neurogenesis. Activation of the GABA system reduces cell proliferation but accelerates the synaptic integration of newly generated neurons. Conversely, the glutamate system, the main excitatory neurotransmitter, has a negative effect on neurogenesis.

Activation of the NMDA subtype of glutamate receptors inhibits cell proliferation, while blocking these receptors or lesioning the main glutamatergic input to the dentate gyrus increases cell proliferation (reviewed by Lieberwirth et al., 2016).

In our work here, modulating 5-HT1β did not affect neuronal differentiation capacity. It is worth noting that the effects of serotonin modulation on neuronal maturation in Alzheimer's disease may differ from the effects observed in these serotonin-related genetic models.

Summary, conclusion and outlook

In the hippocampus of the tgD mice, which produces soluble Aβ dimers, the number of proliferating cells and DCX cells is significantly reduced. This implies soluble forms of Aβ impair the structural plasticity of the hippocampus by reducing NPCs likely contributing to deficits in hippocampus-dependent learning and memory.

Treating tgD mice with a 5-HT1β antagonist partially reversed the reduction of NPCs, supporting the hypothesis of Aβ dimers interacting with 5-HT1β signalling. Since 5-HT1β is mostly presynaptic and functions as an auto- or heteroreceptor, Aβ dimers could conceivably interact with 5-HT1β signalling to affect not just serotonin release, which is known to regulate neurogenesis but also other neurotransmitters.

More research is required to determine if 5-HT1β antagonists modulate neurotransmitter release and improve cognitive and non-cognitive deficits in tgD mice. This approach may open up new therapeutic avenues to alleviate symptoms of Alzheimer's disease patients.

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