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Does the interaction of soluble amyloid-β-dimers with the serotonin receptor 5-HT_{1B} have an impact on adult neurogenesis in the subventricular zone of mice?

Dissertation

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For my parents

Keziban and Ferhat Deliktaş

Thank you for your endless love and unwavering belief in me

Zusammenfassung

Die Alzheimer-Erkrankung (AD) ist die häufigste Form neurodegenerativer Demenz und betrifft weltweit etwa 50 Millionen Menschen. Hohe Konzentrationen fehlgefalteter Amyloid-Beta- (Aβ) und Tau-Proteine spielen eine zentrale Rolle in der Pathogenese der AD und führen zur Bildung unlöslicher Aggregate wie Plaques und Fibrillen, die mit verschiedenen neurotoxischen und neurodegenerativen Prozessen sowie mit Veränderungen mehrerer Gehirnfunktionen, einschließlich Gedächtnis und Lernen, verbunden sind. Vor Kurzem wurde gezeigt, dass insbesondere lösliche Aβ-Spezies die neuronale Funktion beeinträchtigen, vor allem in frühen Stadien der Erkrankung. Die transgenen Dimer-(tgD)-Mäuse (zur Verfügung gestellt von Prof. Dr. C. Korth, Institut für Neuropathologie, Heinrich-Heine-Universität Düsseldorf) produzieren stabile, neurotoxische Aβ-Dimere, ohne Plaquebildung oder Neuroinflammation zu entwickeln. Daher stellen sie ein geeignetes Modell dar, um die Auswirkungen löslicher Aβ-Spezies auf die strukturelle und funktionelle neuronale Plastizität zu untersuchen. Ähnlich wie in frühen Stadien der AD zeigen tgD-Mäuse leichte kognitive Defizite, verändertes Verhalten sowie Veränderungen im Neurotransmitterhaushalt, insbesondere in der Serotonin-Umsatzrate.

Im adulten Gehirn findet eine lebenslange kontinuierliche Neubildung von Neuronen statt, die als adulte Neurogenese bezeichnet wird. Diese tritt nicht nur in der subgranulären Zone (SGZ) des Hippocampus auf, sondern auch in der subventrikulären Zone (SVZ) der Seitenventrikel sowie im dazugehörigen rostralen Migrationsstrom (RMS). In der SVZ und im RMS gebildeten Neuronen wandern zum Bulbus olfactorius (OB), wo sie in bestehende neuronale Netzwerke integrieren und zur neuronalen Plastizität im olfaktorischen System beitragen. Kürzlich konnte unsere Arbeitsgruppe eine beeinträchtigte adulte Neurogenese in der SGZ des Hippocampus der tgD-Mäuse nachweisen, die durch Modulation des Serotoninrezeptors 5-HT_{1B} wiederhergestellt werden konnte. Unklar ist jedoch, ob die adulte Neurogenese in der SVZ/ im RMS bei tgD-Mäusen ebenfalls beeinträchtigt ist und ob die Modulation des 5-HT_{1B}-Rezeptors darauf einen Effekt hat.

Zur Untersuchung dieser Fragestellung wurden männliche Wildtyp- (WT) und tgD-Mäuse (n = 24 pro Gruppe) im Alter von 4–6 Monaten eingesetzt. Zur chronischen Verabreichung der Wirkstoffe wurde eine osmotische Minipumpe subkutan implantiert. Die Kontrollgruppen von WT- und tgD-Mäusen erhielten ein Vehikel, während eine WT-Behandlungsgruppe einen 5-HT_{1B}-Rezeptor-Agonisten erhielt und eine tgD-Behandlungsgruppe einen 5-HT_{1B}-Rezeptor-Antagonisten. Nach einer Erholungsphase wurden den Mäusen der Proliferationsmarker Bromdesoxyuridin (BrdU) injiziert. BrdU-positive Zellen (BrdU+)

wurden mittels Immunhistochemie nachgewiesen. Zusätzlich wurden Genexpressionsanalysen durchgeführt.

In den Kontrollgruppen zeigten tgD-Mäuse im Vergleich zu WT-Mäusen signifikant weniger BrdU+-Zellen im RMS sowie in der glomerulären Schicht (GL) des OB, was auf eine reduzierte Proliferation neuronaler Vorläuferzellen hinweist. Die Behandlung von tgD-Mäusen mit einem 5-HT_{1B}-Rezeptor-Antagonisten führte zu einer signifikanten Erhöhung der Anzahl BrdU-positiver Zellen in der SVZ, im RMS und in der Körnerzellschicht (GCL) des OB. Im Gegensatz dazu führte die Verabreichung eines 5-HT_{1B}-Rezeptor-Agonisten bei WT-Mäusen zu keiner Veränderung der Anzahl von BrdU+-Zellen im Vergleich zu WT-Kontrollen.

Zusammenfassend unterstützen diese Ergebnisse die Hypothese, dass lösliche Aβ-Dimere die adulte Neurogenese in SVZ/RMS beeinträchtigen und dass dieser Effekt möglicherweise mit einer erhöhten Aktivität des 5-HT_{1B}-Rezeptors bei tgD-Mäusen zusammenhängt. Die pharmakologische Hemmung des 5-HT_{1B}-Rezeptors könnte daher eine potenzielle Strategie darstellen, um die adulte Neurogenese in frühen Stadien der AD zu fördern.

Abstract

Alzheimer's disease (AD) is the most common form of neurodegenerative diseases affecting about 50 million patients globally. High levels of misfolded amyloid beta (Aβ) and tau proteins play a central role in AD pathogenesis, leading to insoluble aggregates such as plaques and fibrils that are associated with several neurotoxic und neurodegenerative processes as well as alterations in several brain function including memory and learning. Recently, soluble Aβ species are increasingly considered to impair neuronal function, particularly during the early stages of the disease. The transgenic dimer (tgD) mice (kindly provided by Prof. Dr. C. Korth, Institute for Neuropathology, Heinrich-Heine Universität, Düsseldorf) produce stable, neurotoxic Aβ dimers without developing plaque formation or neuroinflammation. Therefore, they represent a suitable model to study the effects of soluble Aβ species on structural and functional neuronal plasticity. Similar to early stages of AD, tgD mice exhibit mild cognitive deficits, altered behavior, changes in neurotransmitter balance, in particular, serotonin turnover rate.

In the adult brain, life-long continuous production of new neurons, termed as adult neurogenesis, occurs not only in the hippocampal subgranular zone (SGZ) but also in the subventricular zone (SVZ) of the lateral ventricle and the associated rostral migratory stream (RMS). Neurons generated in the SVZ migrate to the olfactory bulb (OB), where they integrate into existing neuronal circuits and contribute to neuronal plasticity in the olfactory system. Recently, our group showed an impaired adult neurogenesis in the SGZ of the hippocampus (SGZ), that could be rescued with serotonin receptor 5-HT_{1B} modulation. However, it remains unclear whether adult neurogenesis in the SVZ is also impaired in tgD mice and its regulation via 5-HT_{1B} receptor.

To adress this question, male wild-type (WT) and tgD mice (n=24) 4-6 months old were used. An osmotic mini pump was implanted subcutaneously for chronic drug delivery. Control groups of WT and tgD mice received vehicle while additional group of WT mice received a 5-HT_{1B} receptor agonist, and additional group of tgD mice received a 5-HT_{1B} antagonist. After a recovery period, mice were injected with the proliferation marker bromodeoxyuridine (BrdU). BrdU-positive cells ($Brdu^+$) were detected using immunohistochemistry. In addition, gene expression analyses were performed.

In the control groups, tgD mice showed significantly fewer $Brdu^+$ cells in the RMS and in the glomerular layer (GL) of the OB compared to WT mice, indicating reduced proliferation of neuronal progenitor cells. Treatment of tgD mice with a 5-HT_{1B} receptor antagonist significantly increased the number of $Brdu^+$ positive cells in the SVZ, RMS and the granular

cell layer (GCL) of the OB. In contrast, administration of 5-HT_{1B} receptor agonist to WT mice did not alter the number of $Brdu^+$ cells compared to WT controls.

Taken together, these findings support the hypothesis that soluble Aβ dimers impair adult neurogenesis in SVZ/RMS and that this effect may be associated with increased 5-HT_{1B} receptor activity in tgD mice. Pharmocological inhibition of the 5-HT_{1B} receptor may therefore represent a potential strategy to enhance adult neurogenesis in early AD stages.

Abbreviations

Aβ	amyloid-beta
Aβ-S8C	amyloid-beta with Swedish and cysteine mutation
ACh	acetylcholine
AD	Alzheimer's disease
ApoE	apolipoprotein E
APP	amyloid precursor protein
BrdU	bromodeoxyuridine
BPSD	behavioural and psychological signs and symptoms of dementia
cAMP	cyclic of adenosine monophosphate
CNS	central nervous system
DA	dopamine
DAB	3,3'-Diaminobenzidine
DCX	doublecourtin
DG	dentate gyrus
DMT	disease modifying therapeutics
DNA	deoxyribonucleic acid
dRMS	distal Rostral Migratory Stream
Fo	Formula
GABA	gamma-aminobutyric acid
GCL	granular Cell Layer
GL	glomerular Layer
HCl	hydrochloric acid

H_2O_2	hydrogen peroxide
HHU	Heinrich-Heine-Universität
i.p.	intraperitoneally
KO	knockout
LV	lateral ventricle
MAPT	microtubule associated protein tau
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
NeuN	neuronal nuclear protein
NMDA	N-methyl-D-aspartate
NPC	neuronal stem/progenitor cell
NS	normal serum
OB	olfactory Bulb
PBS	phosphate-buffered saline
PBST	phosphate-buffered saline with Triton
pRMS	proximal Rostral Migratory Stream
PSA-NCAM	polysialylated neuronal cell adhesion molecule
PS-1	presenilin-1
P2Y	a family of purinergic G-protein coupled receptor
P2Y2-/-	P2Y2 knockout mouse
RMS	rostral Migratory Stream
ROS	Reactive oxygen species
rpm	revolutions per minute
SCN	suprachiasmatic nucleus

SERT	serotonin reuptake transporter
SGZ	subgranular zone
SSRIs	selective serotonin reuptake inhibitors
SVZ	subventricular Zone
3xTg-AD	triple transgenic mouse
tgD	TgDimer mice
WT	wildtype
ZETT	Zentrale Einrichtung für Tierforschung und wissenschaftliche Tierschutzaufgaben

International System of Units

g	gram
x g	gravitational acceleration
mg	milligram
l	liter
ml	milliliter
mm	millimeter
M	molar mass
µm	micrometer
pg	picogram
pmol	picomol

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1. Introduction

1.1 Alzheimer's Disease

Alzheimer's Disease (AD) is the most prevalent, neurodegenerative disease affecting over 45 million people globally is expected to triplicate by 2050 (Crous-Bou, Minguillón et al. 2017). AD is an age-related disease that mainly affects people over 65 years and has the highest account for dementia cases in individuals over 85 years (Sindi, Mangialasche et al. 2015, Dos Santos Picanco, Ozela et al. 2018).

Besides, other risk factors like lack of physical and mental activity, depression, metabolic disturbances such as diabetes mellitus, midlife obesity, and hypertension increase the risk of developing AD (Chu 2012, Hüttenrauch, Brauß et al. 2016). Thus, AD is considered a multifactorial disorder where heritable and vascular factors also play an important role for its development (Sindi, Mangialasche et al. 2015).

To-date, the underlying pathomechanism of AD is not sufficiently understood. However, there are two hypotheses for the development of AD: the amyloid cascade hypothesis and the cholinergic theory (Breijyeh and Karaman 2020). The amyloid cascade hypothesis displays an imbalance between Aβ production and clearance (Haass and Selkoe 2007). Aβ is produced through the proteolytic cleavage of the amyloid precursor protein (Chen, Xu et al. 2017). An increased Aβ production might be missense mutations in the APP itself or mutations in proteins that enhances the procession of APP like presenlin proteins PS1 or PS2 (Hardy and Selkoe 2002). When Aβ production exceeds its elimination, oligomerisation of Aβ followed by Aβ deposits may occur (Haass and Selkoe 2007). The neurotoxic Aβ oligomers can form diffuse, insoluble plaques inducing direct and indirect cytotoxic effects on neurotransmission, axonal transport and signaling cascades (Reddy 2017) as well as increased microglial and astrocytic activity and neuroinflammation which leads to neuronal loss and neurotransmitter deficits (Selkoe and Hardy 2016).

The emphasis of previous AD studies lied on insoluble Aβ forms, but recent studies show that soluble Aβ oligomers are also neurotoxic, lead to cognitive decline and are strongly correlated to AD manifestations (Rosenblum 2014). Interestingly, soluble forms of the Aβ oligomers inhibit long term potentiation and enhance long term depression (Shankar, Li et al. 2008). Moreover, soluble Aβ oligomers reversibly degrade neuronal activity in rodents (Holscher, Gengler et al. 2007, Scopa, Marrocco et al. 2020). It also has been described that soluble Aβ oligomers have an impact on spine density and synaptic plasticity in rats (Jack, Bennett et al. 2018). Recently, our group showed an impaired adult neurogenesis in

the SGZ of the hippocampus (SGZ), that could be rescued with serotonin receptor 5-HT1B modulation (Faramarzi, 2024). Hence, soluble Aβ oligomers/dimers could be an essential target of to modify early AD manifestations.

In the cholinergic theory, experimental studies in humans and primates have shown that acetylcholine (ACh)-producing neurons, which are essential for learning and memory (Craig, Hong et al. 2011), undergo degeneration, which leads to increased deposition of amyloid and tau pathology in the hippocampus and cortex (Hampel, Mesulam et al. 2018). resulting in cognitive impairment (Ramos-Rodriguez, Pacheco-Herrero et al. 2013). The progress of dementia in AD correlates with the severity of the degeneration of cholinergic neurons (Hampel, Mesulam et al. 2018). Moreover, Aβ is a potential reducer of choline uptake and ACh release (Breijyeh and Karaman 2020), which may further deteriorate the neuropathology.

Besides, other neurotransmitters as dopamine, glutamate, and serotonin play a huge role in brain function and their dysregulation contributes to AD pathogenesis (Reddy 2017). The modulation of neurotransmitter household through drugs can improve AD symptoms. For instance, inhibitors of the enzyme cholinesterase can improve memory and language by increasing ACh levels (Marucci, Buccioni et al. 2021). Neurotransmitter abnormalities also seem to correlate with behavioral and psychological dysfunction in AD patients (Rajmohan and Reddy 2017). Hence, better understanding of the complex interplay of Aβ accumulations and changes in neurotransmitter signalling are essential for perspective treatments in AD.

AD is presently an incurable, disabling disease that decreases the life quality of affected individuals as their independence diminishes due to functional impairment (Viña and Sanz-Ros 2018).

The symptoms caused by AD can be various and are categorized into cognitive and non-cognitive symptoms. The cognitive symptoms of AD include impaired memory, language and motor control (Selles, Oliveira et al. 2018) leading to disturbed orientation of time and space (Breijyeh and Karaman 2020), verbal expression and reception (Verma and Howard 2012) in addition to balance, coordination and precision of movements (Yan, Rountree et al. 2008).

Non-cognitive symptoms depict functional impairments and are mainly responsible for the loss of life quality, high costs and early institutionalization of individuals (Petrovic, Hurt et al. 2007, Lyketsos, Carrillo et al. 2011). Behavioral and psychological signs and symptoms of dementia (BPSD) include a heterogeneous group of symptoms and there are four syndromes described: hyperactivity (agitation, aggression, euphoria, disinhibition, irritability,

aberrant motor activity), mood liability (depression and anxiety), and instinctual (appetite disturbance, sleep disturbance, and apathy) clusters (Mathys, Fang et al. 2018) that affect more than 90% of the patients and tend to occur in early stages of AD (Schwertner, Pereira et al. 2022). Moreover, the relationship between sleep disturbance and AD progression is reciprocal (Van Erum, Van Dam et al. 2018, Irwin and Vitiello 2019).

There are three general stages of AD: preclinical, mild cognitive impairment and dementia whereas mild cognitive impairment can transform into dementia, which is a steady progression of cognitive decline (Rasmussen and Langerman 2019).

An early recognition is essential before the onset of irreversible loss of neurons (Ausó, Gómez-Vicente et al. 2020). Thus, it is crucial to define early biomarkers and behavioral profiles to predict the progression from preclinical to clinical stages of mild cognitive impairment and AD dementia (Sperling, Aisen et al. 2011, Dubois, Hampel et al. 2016). Early detection of AD is challenging, however cerebrospinal fluid analysis of biomarkers like Aβ42 and total tau (Blennow, Dubois et al. 2015), MRI neuroimaging to detect brain atrophy (Frisoni, Fox et al. 2010) and neuropsychological evaluations are clinical standards for diagnosing AD in patients in advanced stages (Laske, Sohrabi et al. 2015).

Currently, available AD treatments cannot cure the disease nor impede the progression of it as they only show symptomatic relief (Holtzman, Morris et al. 2011). Thus, AD progression represents a tremendous burden for individuals, caregivers, and healthcare systems (Meijer, Casanova et al. 2022). To enhance cognition cholinesterase inhibitors and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine are used (Scheltens, De Strooper et al. 2021). For BPSD symptoms atypical antipsychotics, antidepressants, and anticonvulsant mood stabilizers can be integrated into the therapy (Cerejeira, Lagarto et al. 2012). Sleep disturbances are usually treated with melatonin or benzodiazepines (Ferini-Strambi, Galbiati et al. 2020) . The treatment of advanced AD stages with disease-modifying therapies is still in clinical trials as an efficacy could not be proven yet (Breijyeh and Karaman 2020).

1.2. Adult Neurogenesis

Adult neurogenesis (AN) is the lifelong generation of neuronal progenitor cells (NPC) capable of self-recruitment in the mammalian brain. AN is limited to two brain regions: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus (Ming and Song 2011, Cocks, Carta et al. 2016, Nieto-Estévez, Defterali et al. 2016). These brain regions are known as 'neurogenic niches' and provide the suitable milieu for the multi-step progression of adult neurogenesis including the proliferation of neuronal stem/progenitor cells (NPC), migration of neuroblasts, differentiation into mature neurons and their functional integration into pre-existing neuronal circuits (Ali and von Gall 2022). Not only the location of adult neurogenic niches is restricted, but also the timespan of adult neurogenesis as it diminishes with age (Lee, Clemenson et al. 2012) (Fig.1).

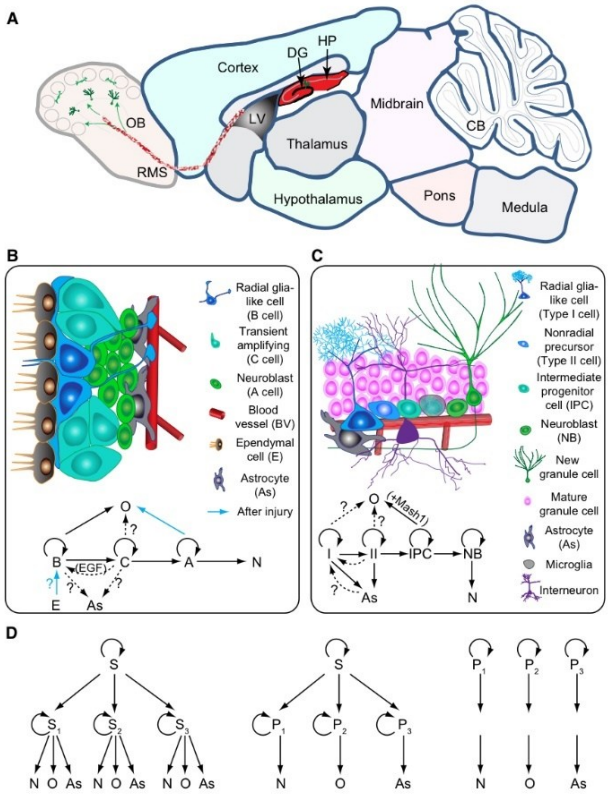

Fig.1: Schematic illustration showing models of neural stem cells and lineage relationship in the adult dentate gyrus (DG) and subventricular zone (SVZ). A) Demonstration of the localization of the two neurogenic niches: the DG of the hippocampus (HP) and the SVZ in the lateral ventricle (LV). B) Categorizing different stages of neural stem cells. C) Different maturation phases of neural stem cells (adapted from *(Ming and Song 2011)* with permission)

The SVZ is one of the two main neurogenic niches in the adult mammalian brain where adult neurogenesis takes place. It is located in the lateral and septal wall of the lateral ventricle, consists of four different layers and represents the largest neurogenic area in the adult mammalian brain (Alfonso, Le Magueresse et al. 2012, Mizrak, Levitin et al. 2019, Lombard, Digregorio et al. 2020). In the SVZ, three types of neural stem cells exist: quiescent type B cells, rapidly proliferating type C cells and migrating type A neuroblasts (Obernier and Alvarez-Buylla 2019). Thus, type C and type A of the precursor cells can be well identified with proliferating marker Bromodeoxyuridine (BrdU) (Zhao, Deng et al. 2008). The glial-like GFAP positive type B stem/progenitor cells are precursors of the MASH1 positive transit-amplifying C cells which in turn generate neuroblasts, migrating doublecourtin (DCX positive type A cells (Fig.2) (Alvarez-Buylla A 2008, Gonzalez-Perez, Romero-Rodriguez et al. 2009, Kriegstein and Alvarez-Buylla 2009).

These migrating type A neuroblasts build elongated cellular aggregates guided by GFAP-positive astrocytes forming a neuroblast chain and migrate tangentially for a long distance along the RMS to reach the olfactory bulb (Kriegstein and Noctor 2004, Lim and Alvarez-Buylla 2016). The flow of cerebrospinal fluid and the path of the RMS guide the new-born cells on their journey from the SVZ to the OB (Nait-Oumesmar, Picard-Riéra et al. 2008). Then, type A cells change their direction migrating radially as they reach the olfactory bulb (Zarco, Norton et al. 2019). The radial migration requires a disjunction of the neuroblasts from the neuroblast chain induced by mediators like reelin (Ali, Tundo-Lavalle et al. 2020) . After the disjunction, the neuroblasts become integrated into preexisting neuronal circuits of the olfactory bulb whereas 97% differentiate into granule cells and the remaining develop into periglomerular cells (Malvaut and Saghatelyan 2016). The newly generated neurons in the OB are essential for experience-dependent plasticity of neural networks (Dejou, Mandairon et al. 2024).

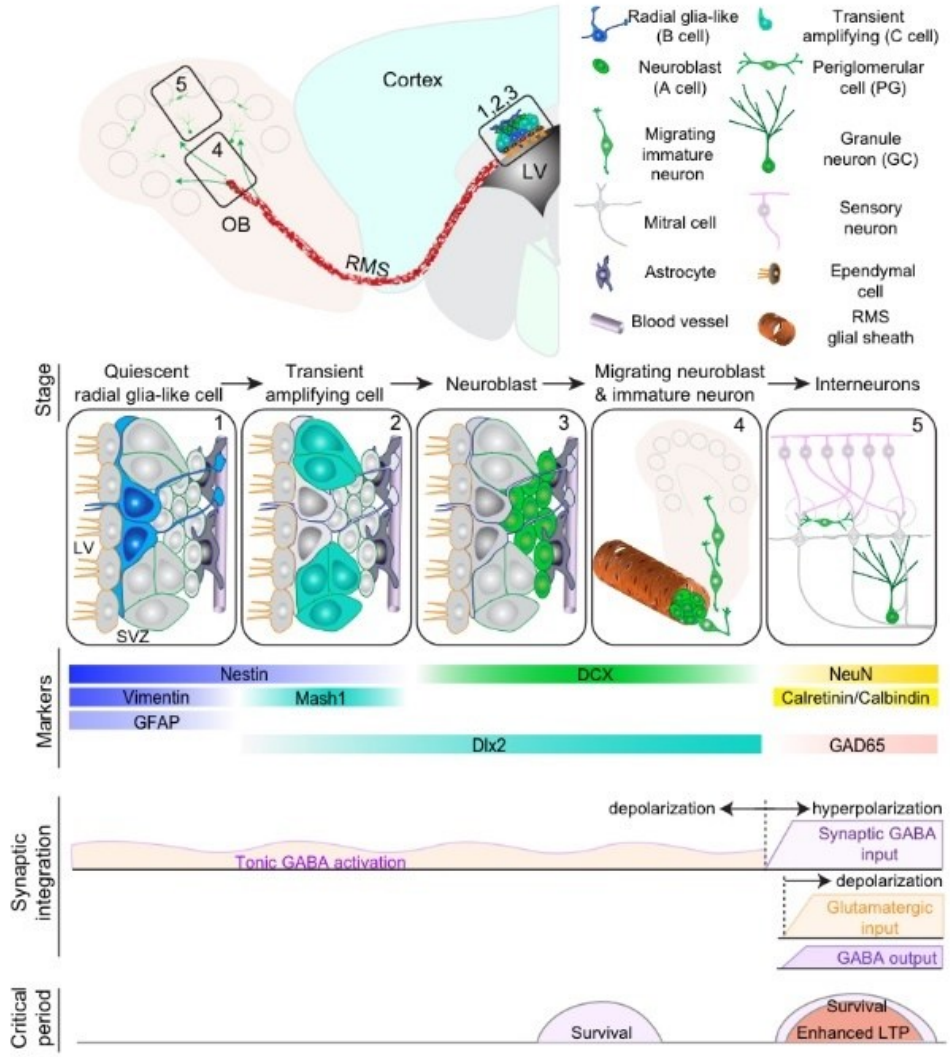

Fig.2: Schematic illustration showing the rostral migratory stream in sagittal mammalian brain with different stages of neuroblasts and their markers (adapted from *(Ming and Song 2011)* with permission)

The mammalian olfactory system is a complex network of neurons that begins in the olfactory epithelium of the nasal cavity and projects to the olfactory bulb (OB) in the brain (Belluscio, Lodovichi et al. 2002, Buschhüter, Smitka et al. 2008, Imamura, Ito et al. 2020). It is a critical structure in the mammalian brain responsible for processing odor information. Sensory information from the olfactory epithelium is transmitted by olfactory sensory neurons, which synapse with two main types of projection neurons in the olfactory bulb: mitral cells in the mitral cell layer (MCL) and tufted cells in the glomerular layer (GL) (Adam, Livneh et al. 2014) . Moreover, the OB is organized into further distinct layers, each containing specific neuron types that contribute to olfaction.

1.Olfactory Nerve Layer (ONL):
This outermost layer contains the axons of olfactory sensory neurons (OSNs) from the nasal epithelium. These axons project into the OB and synapse within the glomerular layer (Tufo).

2. Glomerular layer (GL):
The GL houses spherical structures called glomeruli, where OSN axons synapse with the dendrites of mitral and tufted cells. Each glomerulus represents a single odorant receptor, facilitating the initial coding of odor identity. Surrounding the glomeruli are juxtaglomerular cells, including periglomerular (PG) cells, external tufted (ET) cells, and superficial short-axon (sSA) cells, which modulate the input from OSNs (Nagayama, Homma et al. 2014). ETs most likely send their axons only to anterior areas of the olfactory cortex (OC) which include anterior olfactory nucleus (AON), anterior piriform cortex (APC), and olfactory tubercle (Igarashi, Ieki et al. 2012).

3. External Plexiform Layer (EPL):
Located beneath the GL, the EPL contains the secondary dendrites of mitral and tufted cells, which interact with the dendrites of granule cells. This layer is crucial for lateral inhibition and contrast enhancement in odor discrimination (Lodovichi 2021).

4. Mitral Cell Layer (MCL):
The MCL consists of the cell bodies of mitral cells, the principal projection neurons of the OB. Mitral cells receive input from the glomeruli and send processed odor information to various regions of the OC including the anterior and posterior OC (Igarashi, Ieki et al. 2012) .

5. Internal Plexiform Layer (IPL):
Situated below the MCL, the IPL contains axons from mitral cells and axon collaterals of ET cells. This layer plays a role in the integration and modulation of olfactory signals before they are relayed to higher brain centers.

6. Granule Cell Layer (GCL):
The GCL is composed mainly of granule cells, which are axon-less interneurons extending dendrites into the EPL. Granule cells form reciprocal synapses with mitral and tufted cells, providing inhibitory feedback that sharpens odor representation and contributes to the plasticity of olfactory processing.

In the main olfactory bulb, each axon of a projection neuron drains one or few glomeruli (Mori, Nagao et al. 1999). The glomeruli are organizational integrities modeling signals before transposing them to upper instances reaching consciousness (Zou, Chesler et al. 2009).

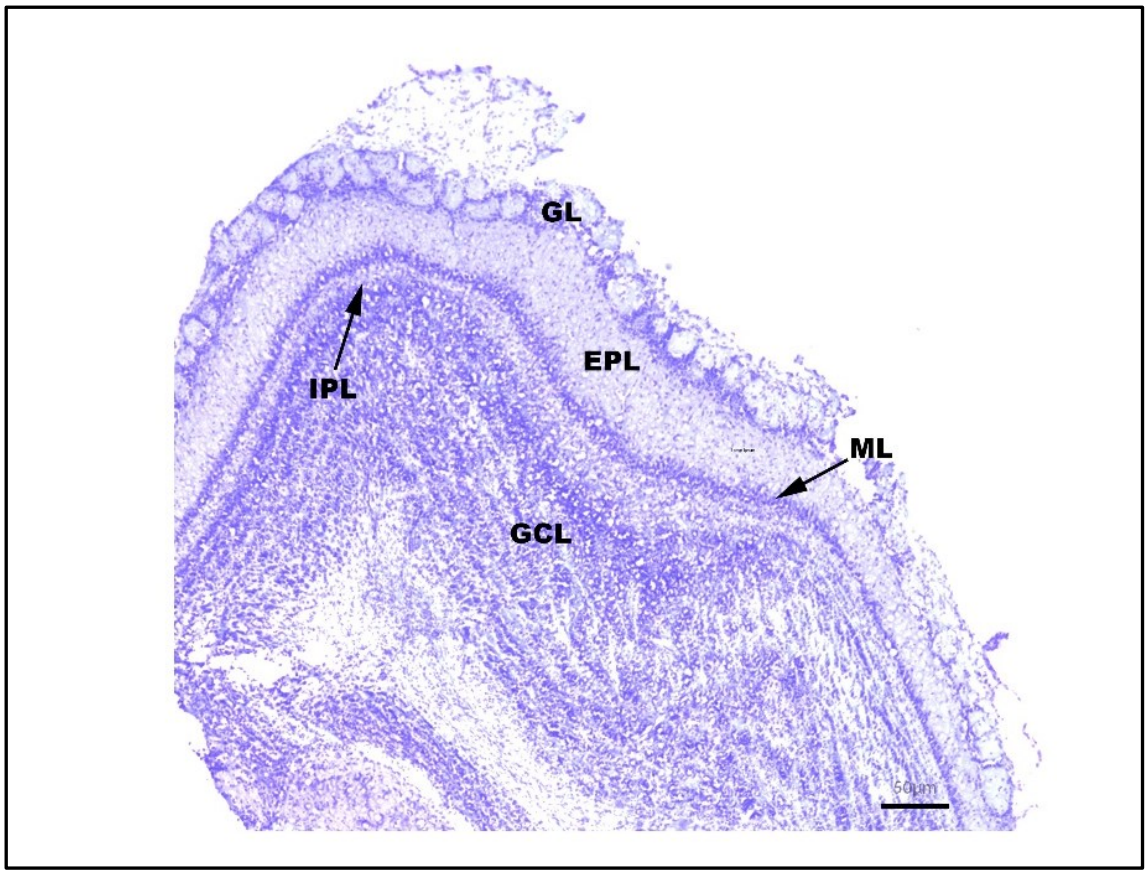

Fig.3: Representative photomicrograph of the mouse olfactory bulb (OB) and its individual layers stained with Nissel (cresyl-violet) staining. Glomerular layer (GL), mitral cell layer (ML), external plexiform layer (EPL), internal plexiform layer (IPL), granular cell layer (GCL). Scale bar = 50µm

Adult neurogenesis in the OB contributes to pattern separation and plays an essential role in adapting to the environment (Sahay, Wilson et al. 2011). Neural progenitor cells migrate along the RMS to reach the OB developing into granule neurons and periglomerular interneurons where they generate functional GABA receptors (Alonso, Ortega-Pérez et al. 2008). Most of the adult-born neurons are GABAergic granule cells which are bidirectional dendro-dendritic synapses lacking an axon and communicating with the lateral dendrites of mitral and tufted cells (Panzanelli, Bardy et al. 2009, Kelsch, Sim et al. 2010). Thus, granule cells prevent an interference between mitral and tufted cell activity establishing precise odor distinction (Petreanu and Alvarez-Buylla 2002). Periglomerular neurons are mainly

GABAergic in addition to some dopaminergic tyrosine hydroxylase-expressing neurons (Maher and Westbrook 2008). Interestingly, loss of olfactory function is an early remark of impaired adult neurogenesis due to neurodegenerative diseases like Alzheimer or Parkinson (Attems, Walker et al. 2014).

In addition to the SVZ, adult neurogenesis also takes place in the hippocampus. In the SGZ of the hippocampus, radial glia-like cells, also known as type 1 cells, generate intermediate progenitor cells, or type 2 cells, which own transient amplifying characteristics (Gonçalves, Schafer et al. 2016). Type 2 cells form neuroblasts that express doublecortin (DCX), which are also commonly referred to as type 3 cells (Jurkowski, Bettio et al. 2020). The neuroblasts migrate towards their destination within the DG, where the surviving neuroblasts then develop into granule cells (Ehninger and Kempermann 2008). These cells express NeuN, which is a marker of mature neurons, and integrate into the hippocampal neuronal network as they extend dendrites into the molecular layer and axons toward the CA3 area (Kempermann 2022) (Fig.4).

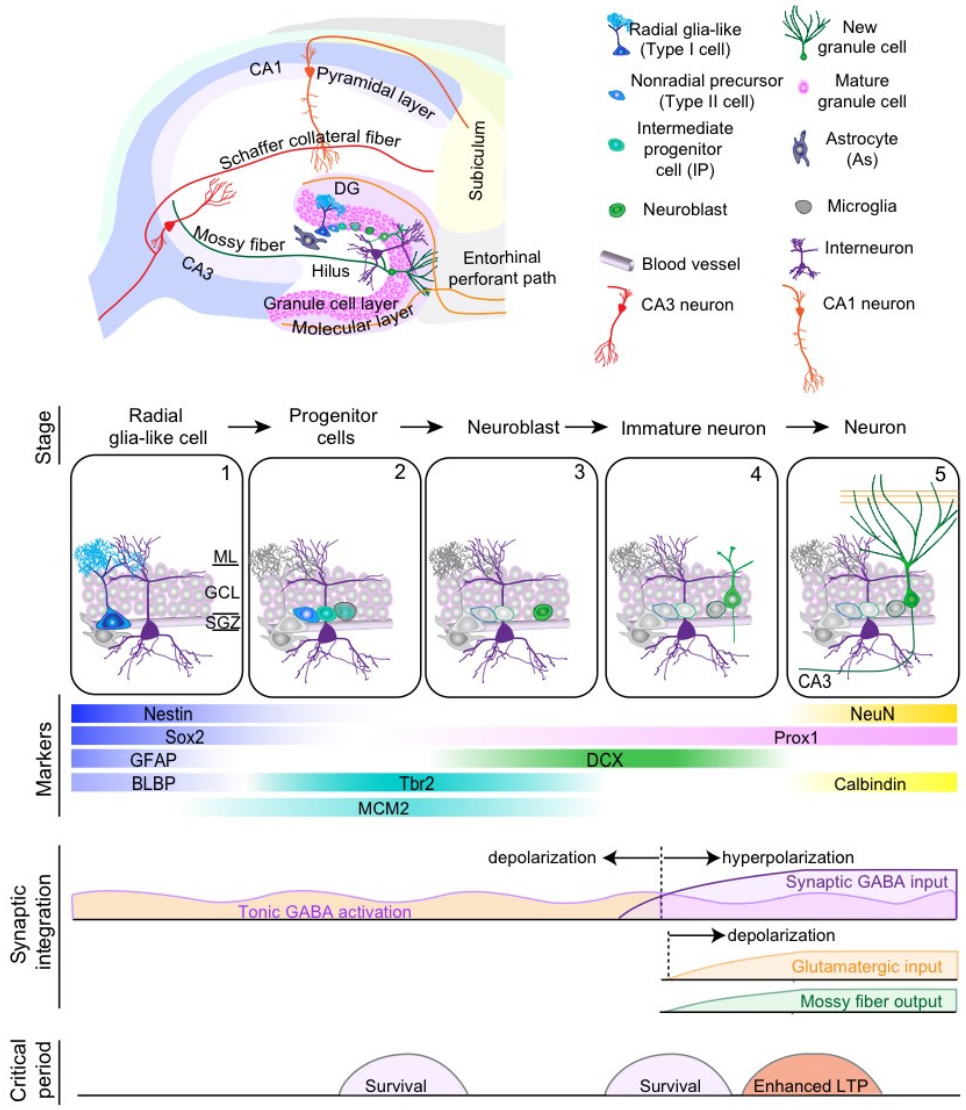

Fig.4: Schematic illustration showing the dentate gyrus of the hippocampus and the different stages of neuroblasts and their corresponding markers (adapted from (Ming and Song 2011) with permission)

In the hippocampus, adult born neurons play a key role in memory processes and spatial and object recognition (Jessberger, Clark et al. 2009). Moreover, hippocampal neurogenesis contributes to stress response and emotional control (Snyder, Soumier et al. 2011, Cameron and Glover 2015). In both regions, the hippocampus and the OB, newly generated neurons play a huge role in pattern separation which is crucial for environmental adaptation and non-overlapping memories from similar experiences (Sahay, Wilson et al. 2011, Nakashiba, Cushman et al. 2012).

Adult neurogenesis is a complex process that is regulated by multiple intrinsic and extrinsic factors in both neurogenic niches including growth factors, hormones, neurotransmitters

and transcriptional factors (Toda and Gage 2018). Moreover, circadian clock genes (Ali, Schwarz-Herzke et al. 2015, Ali, Schwarz-Herzke et al. 2019), stress (Du Preez, Onorato et al. 2021), ageing (Apple, Solano-Fonseca et al. 2017), diet (Poulos, Bayer et al. 2017), social interaction (Holmes 2016) and enriched environment (Leal-Galicia, Saldívar-González et al. 2007) also influence adult neurogenesis. Besides, physical excersises (Kleemeyer, Kühn et al. 2016) leads to increased neurogenesis in the hippocampal DG (Maass, Düzel et al. 2015) resulting in an enlarged hippocampal volume and improved memory formation (Nauer, Dunne et al. 2020).

Adult neurogenesis significantly contributes to neural plasticity (Kempermann, Gage et al. 2018) and even neurons that are generated in the SGZ/SVZ of rodents are also fundamental for brain plasticity and serve for a better comprehension of the interrelation of neurogenesis and learning (Braun and Jessberger 2014, Toda, Parylak et al. 2019). On the other hand, impaired adult neurogenesis is associated with neurodegenerative diseases which are a prime reason for human disabilities (Müller-Schiffmann, Herring et al. 2016). The development of neurodegenerative illnesses usually rely on the accumulation of pathologically misfolded proteins that becomes more evident with aging (McAlary, Chew et al. 2020, Donnelly, Coleman et al. 2022). In AD, the age-dependent accumulation of Aβ correlates with impaired neurogenesis (Salta, Lazarov et al. 2023) and is associated with cognitive deficits (Wirths 2017). Furthermore, adult neurogenesis is also reduced in other neurodegenerative diseases such as Parkinson’s disease and Huntington’s disease (Terreros-Roncal, Moreno-Jiménez et al. 2022). Thus modulation of adult neurogenesis may provide a therapeutic approach to modify the progression of neurodegenerative diseases and improve the associated symptoms.

1.3. Serotonergic neurotransmission

Serotonin (5-HT) is a critical neurotransmitter with diverse effects on behaviour and biological functions (Nichols and Nichols 2008).

5-HT is principally synthesized in the raphe nuclei of the brain stem but it can also be produced in the enterochromaffin cells of the intestinal mucosa (Hornung 2003, Wu, Denna et al. 2019). However, peripherally produced serotonin cannot pass the blood brain barrier and, thus, does not function as neurotransmitter for the central nervous system (CNS) (El-Merahbi, Löffler et al. 2015). Within the brain, there are two major functions of serotonin: the neurotransmitter function affecting cognition, attention, emotion, pain and sleep and the trophic function regulating neural cell division and synaptogenesis (Brummelte, Mc Glanaghy et al. 2017).

Serotonin binds to seven receptor families which are known as 5-HT_{1-7} receptors (Leibowitz and Shor-Posner 1986). In the hippocampus, all the serotonin receptor families are remarkably expressed and contribute to the 5-HT functions as cognition, mood regulation and appetite (Berumen, Rodríguez et al. 2012). Interestingly, most of the 5-HT receptors in the hippocampal circuit are expressed on neurons that do not receive direct serotonergic innervation, so 5-HT in the CNS is rather a neuromodulator that is distributed via volume transmission (Dale, Pehrson et al. 2016). All serotonin receptors are G-protein coupled receptors except for 5-HT_3 which is a ligand-gated ion channel (Nichols and Nichols 2008). Moreover, the 5-HT_1 receptor is divided into four subgroups 5-$HT_{1A, B, D, E}$ which all are Gi/Go protein coupled and exert inhibitory presynaptic and postsynaptic effects via decreasing cAMP levels through adenylyl cyclase inhibition (Granados-Soto, Argüelles et al. 2010). In neurons, the 5-HT_1 receptor is either located presynaptically on axon terminals as autoreceptors or they are located postsynaptically as heteroreceptors (Barnes and Sharp 1999, Nichols and Nichols 2008). The 5-HT_{1B} receptor is primarily a presynaptic autoreceptor inhibiting the release of 5-HT (Tiger, Varnäs et al. 2018). The function of 5-HT_{1B} heteroreceptors remains unclear, but they probably inhibit the release of neurotransmitters in nonserotonergic neurons such as dopamine, GABA, glutamate and ACh (Ruf and Bhagwagar 2009). Meanwhile, the activation of 5-HT_{1B} – autoreceptors results in a decline of 5-HT levels (Moret and Briley 2000, Rutz, Riegert et al. 2006, Lesch and Waider 2012). 5-HT_{1B} receptors are mainly expressed in the basal ganglia (Threlfell, Greenfield et al. 2010) and are involved in regulation of learning, vigilance, mood, sexual behaviour, sleep and appetite (Moret and Briley 2000).

Both neurogenic niches, the hippocampus and the SVZ, show serotonergic innervation (Berumen, Rodríguez et al. 2012, Fomin-Thunemann and Garaschuk 2022). For instance, the hippocampus shows a dense serotonergic input and expression of 5-HT receptor subtypes (Dale, Pehrson et al. 2016). Interestingly, the density of serotonergic innervation depends on the hippocampal region: it is highest in the CA3 and lowest in the CA1 region (Berumen, Rodríguez et al. 2012). In addition, serotonergic fibers reach the glomerular layer of the RMS and OB (García-González, Khodosevich et al. 2017, Fomin-Thunemann and Garaschuk 2022).

Importantly, 5-HT modulates the proliferation and differentiation of neural stem and progenitor cells (Brezun and Daszuta 1999). For instance, 5-HT increases the level of neurotrophic factors such as BDNF and facilitates the generation of new neurons (Leschik, Gentile et al. 2022). Moreover, neuronal stem cells culture expressed 5-HT_{1A} receptor mRNA and also produced serotonin as well as tryptophan hydroxylase, an enzyme that is essential for serotonin synthesis (Benninghoff, Gritti et al. 2010). Modulation of 5-HT receptors affects NPCs proliferation and survival (Banasr, Hery et al. 2004, Lesch and Waider 2012). Consequently, modulation of serotonin signaling appears to be a promising therapeutic strategy for regulation of adult neurogenesis (Narboux-Nême, Pavone et al. 2008, Alenina and Klempin 2015).

1.4 Tg-Dimer mouse as a model for early AD

The transgenic Tg-Dimer (tgD) mice represents an AD mouse model that exclusively express soluble Aβ dimers. tgD mice carry a transgene for the full-length human APP751 including the Swedish mutation (APP-K670N/M671L) which leads to seven-fold increase of Aβ production (Müller-Schiffmann, Herring et al. 2016, van Gerresheim, Müller-Schiffmann et al. 2023). Additionally, the expressed APP includes the Aβ-S8C mutation where a serine at position 8 of Aβ is replaced by a cysteine leading to the formation of soluble Aβ-S8C dimers consisting of two Aβ monomeres connected through a disulfide bridge. Thus, during the life time of tgD mice, there is no insoluble amyloid species, plaques formation, neurofibrillary tangles, nor neuroinflammation (Abdel-Hafiz, Müller-Schiffmann et al. 2018, van Gerresheim, Müller-Schiffmann et al. 2023).

Despite the absence of Aβ plaque formation, tau pathology and neuroinflammation, the tgD mice show mild cognitive deficits and also anxiety phenotype with deviant serotonin turnover rate and acetylcholin levels, which is comparable to early characteristics of AD (van Gerresheim, Herring et al. 2021). Neurotoxicity, altered synaptic signaling and an impairment of cognitive function are associated with increased Aβ dimer levels (Abdel-Hafiz, Müller-Schiffmann et al. 2018) in tgD mice. Thus, tgD mice are mimicking early stages of AD (Müller-Schiffmann, Herring et al. 2016) and could provide a valuable model for testing treatment strategies during early stage AD. Importantly, serotonin turnover and the release of other neurotransmitters such as acetycholine is regulated by presynaptic auto- and hetero $5HT_{1B}$ receptors (Stenfors and Ross 2002). Based on her findings that tgD mice showed an altered serotonin turnover and changes in neurotransmitter relase (Abdel-Hafiz, Müller-Schiffmann et al. 2018), Abdel-Hafiz came up with the hypothesis that the activity of the $5HT_{1B}$ receptors is upregulated by soluble Aβ dimers which is an important basis for this study.

1.5 Aim of work

The irreversible progression of AD is a tremendous burden for individuals, caretakers and the health system. The rising number of AD patients, due to increased life expectancy, represents an ethical and financial challenge for society. Likewise, AD remains only symptomatically treatable and progresses continuously indicating an increasing relevance for the future including more people affected. The pathological changes in early AD before the onset of plagues and neuroinflammation are not yet fully understood.

In our study, we used tgD mice, that solely express soluble Aβ dimers without plaque formation, tau pathology or neuroinflammation and mimick early stages of AD, to test if chronic exposure to soluble Aβ dimers affects adult neurogenesis in the SVZ. Furthermore, we aimed to test if soluble Aβ dimers impacts the adult neurogenesis via an interaction with the 5-HT_{1B} receptor.

Our results may contribute to better understanding of structural changes in the early AD stages and may support the development of new pharmacotherapeutic strategies that are more benificial in early stages before the onset of plaque formation and neurodegeneration.

2. Material and Methods

Table 1: Products used for cutting cryostat sections

Products	Brand	Type	Lot/Ref/ID
Anti-roll plate	Leica	70mm	Ref: 1407742497
Blades	Fisher Scientific	Feather Microtome, C35 Type, 20 Blades	Ref: 20750012
Cryostat	Leica	CM3050	/
OCT Mounting	VWR	/	Lot: 03823083
Single edge blades	Personna	Width: 40mm	/
Slides	Star Frost	adhesive	ASIN: B077D9R3WN
Warming plate	Medite	OTS 40	/

Table 2: List of products required for staining of the sections

Products	Brands	Type/Formula(Fo)	Lot/Ref/ID/CAS/Cat
ABC Kit	Vector Labs	Reagent A + B	Lot: ZG 0817
Acetic Acid(99 - 100%)	Merck	Fo: CH3COOH	Cas: 64197
Normal goat serum	Vector Labs	/	Cat: S-1000, Lot: ZE0122
Boric Acid	Merck	Fo: H3BO3	Lot: A0152865
Cresylviolet	Merck	Fo: C19H18ClN3O	5235
Diaminobenzidine	Sigma	Fo:C12H14N4C12H18Cl4N4(4HCl)	/
Entellan	Sigma	500ml packaging	Lot: HX15893461
Hydrochloric acid	Merck	Fo: HCl, fuming	Lot: K42316717
Hydrogen peroxide	Merck	H_2O_2, 30%	Lot: K5282291040
Monopotassium-phosphate	Merck	Fo: KH2PO4	Lot: A0185473130
Potassium chloride	Merck	KCl	Lot: K41789236
2-Propanol	VWR	Fo: Ch3H80	Lot: 19K294015
Shandon Coverplates	Thermo Scientific	Shandon Coverplates	Lot: 322310, Ref: 72110017
Shandon Sequenza	Thermo Scientific	Shandon Sequenza	/
Sodium acetate	Merck	Fo: CH3COONA	Lot: AO346768
Sodium chloride	Merck	Fo: NaCl	Lot: K51809104946, Cas:7647145
Sodium dihydrogen phosphate	Merck	Fo: NaH2PO4	Lot: 2723C234, Cas: 7558807
Triton X-100	VWR	Fo: C14H22O (C2H4O)	Lot: 18J0356257

Table 3. List of primary and secondary antibodies

Products	Brand	Type	Lot/Ref/ID
Monoclonal Rat Anti BrdU	Bio-Rad	1:2000	Cat #MCA6144 Lot: 100004195
Biotinylated Goat Anti-Rat IgG	Cell Signaling	1:500	Cat # 877-616-Cell Lot: 6

Table 4: List of the composition of the solutions used

Solution	Composition
1. ABC Kit (1:100)	Reagent A 110µl +Reagent B 110µl + 11.000µl PBS
2. 0.1M Boric Acid	6.18g boric acid + 1000 ml H2O
3. 0.1% Cresyl violett	1g cresyl violet + 1L sodium acetate buffer at 60°C for 15 minutes and then filtered
4. DAB+H2O3	90ml + 1xPBS + 0.4ml DAB 50mg/ml +20µl H2O2
5. 0.3% H2O2	30% H2O2 + 200ml H2O
6. 2N HCl	170ml of 37% HCl (=12N) to 830ml H2O
7. 5% Normal Goat Serum	38ml PBST + 2ml Normal Goat Serum
8. 10x PBS	28.8g disodium phosphate + 4g monopotassium phosphate + 4g potassium chloride + 164g sodium chloride + 2l MilliQ
9. 1x PBS	200ml of 10xPBS + 1800ml Aqua bidest
10. 1x PBST	1l 1xPBS + 0.25ml Triton x-100
11. Sodium acetate buffer	0.205g sodium acetate + 3.1ml 99% acetic acid + 1l H2O

Tab.5: Gene sequences used for the qPCR

gene	forward	reversed
bdnf	TACCTGGATGCCGCAAACAT	GCTGTGACCCACTCGCTAAT
fgf	CGAGAAGAGCGACCCACAC	TGTAACACACTTAGAAGCCAGCA
prdx	TGGCGCTTCTGTGGATTCTC	CTGAGCAATGGTGCGCTTG
aldh2	GCTGGGCTGACAAGTACCAT	CGGGAAGTTCCACGGAATGA
nqo1	CATTGCAGTGGTTTGGGGTG	TCTGGAAAGGACCGTTGTCG
hgf	TGATCCCCCATGAACACAGC	CCCCTCGAGGATTTCGACAG
mt	TCCGATGGATCCTGCTCCT	AGCAGCAGCTTTTCTTGCAG
cat	GCCAATGGCAATTACCCGTC	GAGGCCAAACCTTGGTCAGA

2.1 Experimental animals

Adult 4-5 months old male homozygous tgD mice on a C57BL/6N background and wild-type C57Bl/6N mice (WT) (n=24 per genotype), weighing 30 to 35 g, were used for this project. The mouse line was generously provided by Prof. Dr. Carsten Korth, Institute of Neuropathology at Heinrich-Heine-University, Germany and was bred at the Central Animal Facility (Zentrale Einrichtung für Tierforschung und wissenschaftliche Tierschutzaufgaben (ZETT)) at the University hospital Düsseldorf.

During the animal experiments, the mice were kept individually in sound- and light-proof chambers (Bioscape, Germany) in standard APEC polycarbonate cages. The mice were housed under a standard photoperiod of 12 hours light / 12 hours dark with light on at 6 am and off at 6 pm (light intensity was 300 lux during light phase). Water and food were ad libitum for all animal groups.

Animal experiments were performed 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 were conducted in accordance with European Directive 2010/63/EU. All effort was made to minimize the number of animals used and to minimize their pain and distress.

2.4. Experimental groups

The WT and tgD mice were divided into four groups (n=6 mice per group) randomly.

The first group was WT mice implanted with an osmotic minipump that released NaCl, injected with BrdU for three consecutive days, and perfused either one day (n=6 mice per group) or 28 days (n=6 mice per group) after the last BrdU injection.

The second group was tgD mice implanted with an osmotic minipump that released NaCl, injected with BrdU for three consecutive days, and perfused either one day (n=6 mice per group) or 28 days (n=6 mice per group) after the last BrdU injection.

The third group was wild-type implanted with an osmotic minipump that released a selective 5-HT_{1B} agonist, then injected with BrdU for three consecutive days, and perfused either one day (n=6 mice per group) or 28 days (n=6 mice per group) after the last BrdU injection.

The fourth group was tgD mice implanted with an osmotic minipump that released a selective 5-HT_{1B} antagonist for tgD mice, then injected with BrdU for three consecutive days, and perfused either one day (n=6 mice per group) or 28 days (n=6 mice per group) after the last BrdU injection.

2.2 Surgery and drug administration

Groups of WT and tgD mice were subcutaneously implanted with a mini-osmotic pump (ALZET® model 2006, Campbell, California,USA) reliably releases continuous amounts of its charge for 42 days) that were filled with saline 0.9%. In addition, other cohorts of wild-type mice were implanted with the mini-osmotic pump filled with 5-HT_{1B} agonist (CP 94253 hydrochloride, Tocris BioScience, Ellisville, MO, USA) and tgD mice with mini-osmotic pump filled with 5-HT_{1B} antagonist (SB 216641 hydrochloride, Tocris BioScience, Ellisville, MO, USA). The mini-osmotic pump was implanted into the back of each animal.

The 5-HT_{1B} agonist was given at a dose of 4mg/kg/day that has been chosen based on antidepressant-like effect (Tatarczyńska, Kłodzińska et al. 2004) and 5-HT synthesis (Kovačević, Skelin et al. 2012).

The 5-HT_{1B} antagonist was given at a dose of 3mg/kg/day that has been modified from Stenfors & Ross (Stenfors and Ross 2002), who described 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 the observed skin irritation at this concentration in our study.

The selective 5-HT agonist (CP 94253 hydrochloride) used in this study is centrally active after systemic administration in vivo, while the selective 5-HT_{1B} antagonist (SB 216641 hydrochloride) has approximately 25-fold selectivity over 5-HT_{1D} and little or no affinity for

other receptor types. ALZET® model 2006 pump was built of a main body and an associated flow moderator. A sterile infusion needle and cannula were used to fill the substance into the pump with no air bubble formation. The pumps had an approximate fill volume of 200µl and released a constant rate of 3.6 µl for over 42 days. Then, the flow moderator was inserted into the pump's main body, and we ensured that no liquid escaped from the pump. More information is reported by Theeuwes and Yun (F Theeuwes, 1976). We placed the pump in a 5ml tube filled with 0.9% NaCL with the flow moderator facing up and incubated at 37°C for 72 hours before use (Fig. 5).

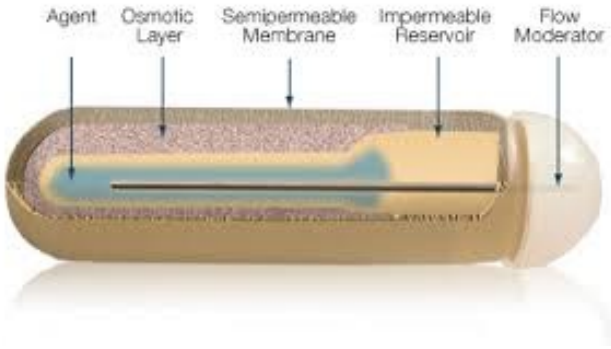

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Fig.5 Schematic illustration of the ALZET® osmotic mini pump. This minipump utilizes the osmotic pressure difference between the osmotic layers inside of the pump and the tissue that it is surrounded by in the place of implantation. The higher osmolality of the osmotic layer causes water to enter inside the pump through its outer surface which is simultaeneously a semi-permeable membrane. The water reaching and passing through the osmotic layer generates a pressure that compresses the flexible reservoir so that the solution of the pump is released at a predetermined rate (adapted with permission by ALZET® from: https://www.alzet.com/products/alzet_pumps/how-does-it-work/).

Animals were anesthetized using a closed Mini-Tag chamber (TEM SEGA, Poznari, Poland) with isoflurane inhalation at a flow rate of 1.5-2%. Primarily, the pain reflexes were checked. If the mouse didn't react to pain, a 20 mm^2 area on the mouse's back was shaved and disinfected. With a surgical scalpel a sagittal mid-scapular incision of 20 mm was cut and with an artery clamp the skin was separated from the subcutaneous fat to create a pocket. Following this, the pump with the flow moderator was placed in this pocket in the left cranial thoracic region. Then, the wound margins were adapted with 4-0 VICRYLTM absorbable sutures (Ethicon ®, Johnson&Johnson, USA) in single button sutures and covered with silver spray (Selectavet, Dr Otto Fischer GmbH, Germany).

As pain therapy, the mice received the nonsteroidal anti-inflammatory pain killer Caprofen subcutaneously with a dosage of 5 mg/kg body weight (Rimadyl ®, Zoetis, Berlin, Germany). This analgesia started 30 minutes before surgery and continued for 3 days. The

animals' conditions were controlled daily after the surgery. After the operation, the mice were kept in separate cages and allowed to recover for 2 weeks.

2.3. 5'-bromo-2'-deoxyuridine (BrdU) administration

BrdU is a nucleotide analog integrating to the nuclei of mitotic cells, and thus used as proliferation marker, and then can be detected through an immunohistochemical staining with matching antibodies (Condezo and San Martín 2021).

After two weeks of post-operative recovery, the wild-type and tgD mice received intraperitoneal (i.p.) BrdU (Roche Diagnostics GmbH, Germany) (BrdU) injections with a dosage of 100 mg BrdU/kg twice daily for three consecutive days at 7 am and 6 pm (Ali AAH 2015, Ali AAH 2019). For this, 10 mg of BrdU was dissolved in 1 ml 0.9% NaCl and was given to the animals in a weight-adjusted manner, for instance, for a 30 g mouse, a volume of 0.3 ml was applied at each injection.

2.5. Tissue processing

The mice were deeply anaesthetized with an intraperitoneal injection with a lethal dose of ketamine/xylazaline mixture (100mg/ 10mg/kg body weight). When the paw reflex was lost, the mice were intracardially perfused with 0.9% NaCl for 3 minutes to wash the blood followed by 4% paraformalin for additional 7 minutes using a Ministar peristaltic pump (World Precision Instrument, Sarasota, FL, USA). Before the brain extraction, the mice's head was sprayed with 70% ethanol to avoid fur distribution. Through a mid-sagittal incision of the scalp the skull was exposed. With a lateral cut around the foramen magnum one midline and two lateral lines were cut to dissect the skull cap over the cerebellum, then the brains were removed from the skull. Brains, were postfixed in 4% paraformalin for maximally additional 24 hours.

Thereafter, the brains were cryoprotected by storing them in 20% and then 30% sucrose until they sunk. Then, each brain was separated into hemispheres, one hemisphere (right) was snap frozen in isopentane, while the other hemisphere (left) was placed in OCT medium-containing molds and kept 10 minutes on dry ice, then, brain were stored at -80°C until cut into cryostat sections.

The left hemispheres were used in this study, as they were sliced into 6 series of 20 µm sagittal sections using a cryostat (LEICA CM3050 S, Germany) and then mounted on slides (Starfrost, adhesive slides, Radnor, Pennsylvania, U.S.A.). Sectioning started from the medial side of the hemisphere and continued laterally until the whole OB was sectioned at

temperatures between -13 and -16 °C. On a heating plate (Medite, Burgdorf, Germany) the sections were dried for two hours at a temperature of 37°C. The sections were stored in a plastic box and vacuumed then frozen at -20°C till further processing.

2.6. Histological staining

Every sixth section from each brain was stained with cresyl-violet. Slices were incubated with the sodium acetate buffer for 5 minutes and then with cresyl-violet solution in cuvettes for 2-3 minutes at room temperature (RT). Sections were incubated with Isopropanol 100% in three steps for 20 seconds, 2 minutes, and then, 5 minutes, respectively, at RT. Then the sections saturated two times in Rothistol each for 5 minutes in separate cuvettes. The sections were finally covered with Entellan, a cover-sliped and left to dry. These sections were used to dentify the location of the RMS and for morphometric analysis of OB

2.7. BrdU Immunohistochemical staining

For immunohistochemical staining, series of sections containing the RMS, previously identified in parallel series using cresyl-violet staining, was selected for immunohistochemical staining.

First, the sections were washed in phosphate buffered saline (PBS). The endogenous peroxidase activity was blocked by incubating the sections in 0.3% hydrogen peroxide for 30 min at RT. Then the sections were washed in PBS again. For DNA denaturation, the sections were incubated in 2N hydrochloric acid (HCl) for 30 minutes at 37°C.

This was followed by neutralization, as the sections were immersed in 0.1M boric acid for 10 minutes and then were washed with PBS containing 0.25% Triton-X 100 (PBS-T 0.25%) for permeabilization. Then, slides were mounted on Shandon Cover Plates and placed in Shandon Sequenza.

To block nonspecific antibody reaction, we incubated the sections in 5% normal goat serum (Vector Laboratories, United States) in PBS-T 0.25% for one hour at RT followed by incubation with the primary antibody rat monoclonal anti-BrdU antibody overnight at 4°C.

The sections were then washed by adding 2 ml of 1x PBS are applied twice to every cover each for 5 minutes. Sections were then incubated with the second antibody for 1 hour at RT. Another 3 washing steps with 2ml of 1x PBS for 5 minutes each followed. The sections were incubated for 1 hour in the ABC kit by Vectastain followed by washing with 1x PBS for 5 minutes at RT. The Shandon Cover Plates were taken out of the Shandon Sequenza and

slides were dismantled from the Shandon Cover plates and further washed in 1x PBS for 3 times at RT.

An incubation in DAB for 8 minutes followed. After DAB incubation the slides were rinsed with H_2O for 5 minutes to stop the chromogenic reaction. A counter staining with cresyl violet followed as described previously.

2.8. Image acquisition and analysis

The stained brain sections were examined using a KEYENCE BZ-X800 microscope (Keyence, Osaka, Japan). As an orientation, microphotograph of cresyl-violet stained brain sections was taken using bright field mode with a 2x objective lens to identify the regions of interest (ROI). For visualization of BrdU immunoreactive cells, the bright field mode and 40x objective were used. Images of the ROI including 1-12 images of the SVZ, 9-18 images of the proximal rostral migratory stream (pRMS), 5-15 images of the distal rostral migratory stream (dRMS), and 15 images for the GCL and GL (in sections where the RMS was found) from each mouse brain were acquired. The ROI was delineated, and surface area was measured and converted to mm^2. The BrdU positive cells within the ROI were counted manually using ImageJ https://imagej.net/ij/download.html (National Institute of health (NIH), Bethesda, Maryland, USA) and the cell density/mm^2 was calculated.

For morphometric analysis, the olfactory bulb was delineated in every sixth section using freehand tool in ImageJ software. In each animal, the measured area was then multiplied by 6 and by the thickness of each section (20µm) to obtain the volume of the OB in µm3 according to Cavlieri's principle (Karaca, Buyukmert et al. 2020). Moreover, the thickness of the individual layers in the olfactory bulb were also measured in µm and averaged in each animal.

2.9. Gene expression analysis in Olfactory bulb

2.9.1. Tissue preparation:

Adult male 5-6 months old mice that received minipump delivering saline (both genotypes), 5-HT_{1B} agonist (WT) or 5-HT_{1B} antagonist (tgD) (n = 6 per group) were killed by lethal dose of isoflurane. Brains were quickly removed from the skull, and the olfactory bulb was carefully dissected, collected in 2mL Precellys tubes (Eppendorf SE, Hamburg, Germany) and snap frozen.

2.9.2. RNA Extraction

Total RNA was extracted from tissue samples using the RNeasy Plus Universal Mini Kit (Cat#73404, Qiagen, Hilden, Germany). To each Precellys tube, 900 µL of QIAzol lysis reagent (Qiagen, Hilden, Germany) was added. Samples were homogenized using a Precellys Evolution tissue homogenizer (Bertin Technologies SAS, Montigny-le-Bretennoux, Paris, France) for 20 s at 5000 rpm, followed by incubation for 5 minutes at RT to facilitate the dissociation of nucleoprotein complexes.

Next, 100 µL of genomic (g)DNA eliminator solution was added, and samples were gently mixed for 15 seconds. Subsequently, 180 µL of chloroform was added and mixed for 15 seconds and left for 2–3 minutes at RT, then were centrifuged at 12,000 × g for 15 minutes at 4°C. The upper aqueous phase (~600 µL) was transferred to a new microcentrifuge tube, mixed with an equal volume of 70% ethanol, and loaded onto a RNeasy spin column.

RNA was bound to the column via two centrifugation steps at 8000 × g for 15 seconds. The column was washed with 700 µL RWT buffer and twice with 500 µL RPE buffer, with the final wash spun for 2 minutes. A final dry spin at full speed for 1 minute ensured complete ethanol removal. RNA was eluted with 30 µL RNase-free water by applying the volume directly to the membrane, allowing it to stand for 1 minute at RT, and centrifuging at 8000 × g for 1 minute. Then, the centrifugation was repeated at full speed for 1 minute at RT. Eluted RNA was stored on ice or at −80°C until use after measuring RNA concentration using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts USA).

2.9.3. cDNA Synthesis

Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the RevertAid First Strand cDNA Synthesis Kit (Cat:#K1622,Thermo Fisher Scientific, Waltham, Massachusetts, USA). RNA was diluted to a final concentration of 100 ng/µL. The reverse transcription reaction included 10 µL of RNA (1 µg), 1 µL Oligo(dT)$_{18}$ primer, and 1 µL Random Hexamer primer. This mixture was incubated at 65°C for 5 minutes in a PCR cycler and then stored on ice. Subsequently, the following reagents were added: 4 µL of 5× Reaction Buffer, 1 µL Ribolock RNase Inhibitor, 2 µL 10 mM dNTP mix, and 1 µL RevertAid M-MuLV Reverse Transcriptase, yielding a total reaction volume of 20 µL. The thermal cycling protocol was: 5 min at 25°C, 60 min at 42°C, 5 min at 70°C, and hold at 4°C. cDNA was stored at −20°C.

2.9.4. PCR Quality Control

To assess cDNA integrity, a control PCR targeting GAPDH was performed. The reaction included 0.1 µL cDNA, 1 µL each of forward and reverse GAPDH primers (10 pmol), 12.5 µL KAPA2G Fast PCR Master Mix, and 10.5 µL water (total 25 µL). The cycling conditions

were as follows: initial denaturation at 95°C for 3 min, followed by 34 cycles of 94°C for 15 s, 60°C for 15 s, and 72°C for 1 min, with a final extension at 72°C for 10 min and then kept at 4°C.

2.9.5. Quantitative PCR (qPCR)

For qPCR, 10 ng of cDNA was used in a 20 µL reaction containing 10 µL SYBR Green Master Mix (KAPA SYBR Green ABI Prism, Merck KGaA, Darmstadt, Germany) 5 µL of Primer Mix (0.5 µM each primer), and 5 µL of diluted cDNA (1:25 from a 50 ng/µL stock). For negative controls, water was used instead of cDNA. The qPCR plate was prepared on ice, sealed, centrifuged briefly to remove air bubbles, and run in using the following settings: Quantification/ Comparative Ct method, with a melting curve analysis enabled and the FAST-cycling protocol.

e.) PCR Product Purification for Standard Curve Generation

To generate standard curves, PCR products were purified using the MinElute PCR Purification Kit (Qiagen, Cat #: 28004). PCR reactions (quadruple per gene) were pooled into a single 1.5 mL tube (~80 µL total), and 5 volumes (400 µL) of PB buffer were added. The mixture was applied to a MinElute spin column and centrifuged at 17,900 × g for 1 min. The column was washed with 750 µL PE buffer, centrifuged, and then subjected to an additional dry spin. DNA was eluted with 10 µL RNase-free water. DNA concentration was determined via NanoDrop and stored at −80°C. To generate standard curves, a stock solution was prepared from the purified PCR product. A final concentration of 100 pg was used as the highest standard, and a 1:10 dilution series was prepared. Each standard curve reaction consisted of 5 µL DNA standard, 10 µL SYBR Green Master Mix, and 5 µL Primer Mix. Standard curves were generated separately for each gene. Olfactory bulb samples were used to generate standards for GAPDH and Rn18s using gene-specific primers. A standard curve for each primer was used to calculate primer efficiency.

The expression levels of several genes encoding for neurotrophic factors, including brain-derives neurotrophic factors (*bdnf*), fibroblast growth factor (*fgf*) and hepatocyte growth factor (*hgf*), as well as the oxidative stress genes, aldehyde dehydrogenase 2 (*aldh2*), catalase (*cat*), mitochondrial (*mt*) and NAD(P)H dehydrogenase (*nqo1*) were analyzed. The sequences of the used primers are listed in Table 5. mRNA expression levels of genes encoding for neurotrophic factors, regulation of cell cycle and cell proliferation as well as oxidative stress genes were normalized to housekeeping genes using the Pfaffl method to

obtain relative expression levels. β-Actin, Gapdh and Rn18s were used as housekeeping genes for the relative expression levels of genes of interest.

2.10 Statistical analysis

GraphPad Prism software was used for statistical analysis. First, we identified possible outliers via the ROUT method, where the Q was set with 10%. Then we tested the normal distribution of values within one group using Shapiro-Wilk test. When values passed the normality test, the ordinary one-way ANOVA was used followed by Tukey's multiple comparison test. Kruskal-Wallis test was applied followed by Dunn's multiple comparisons test, when values were not normally distributed. Values are expressed as mean ± standard error of the mean (SEM). P value < 0.05 was considered as statistically significant.

3 Results

3.1 Proliferation of neural progenitors in SVZ of tgD mice

To demonstrate the proliferation of NPCs in the SVZ, dRMS, pRMS, GL 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 SVZ, dRMS, pRMS, GL and GCL of tgD and WT mice and had homogeneously stained triangular, longitudinal, or round nuclei. The BrdU+ cells in the SVZ and RMS were mainly arranged in clusters, whereas in the GCL and GL BrdU+ cells were more sporadically distributed.

In the SVZ, the number of BrdU+ proliferating neural progenitors/mm^2 was not significantly different between the WT (4542 ± 473) and tgD mice (3025±582.6) (p = 0.18) one day after the last BrdU injection.

The number of BrdU+ proliferating neural progenitors/mm^2 in WT mice (4542 ± 473) treated with saline one day after the last BrdU injection was comparable with WT mice treated with 5-HT$_{1B}$ agonist (4019 ± 555.2) (p = 0.8).

The tgD mice treated with 5-HT$_{1B}$ receptor antagonist showed a significant increase in the number of BrdU+ cells (7836 ± 391.4 cells) one day after the last BrdU injection compared to tgD mice treated with saline and scarificed one day after the BrdU injection by approximately 38.6% (p = 0.0001) (Fig.6A, B).

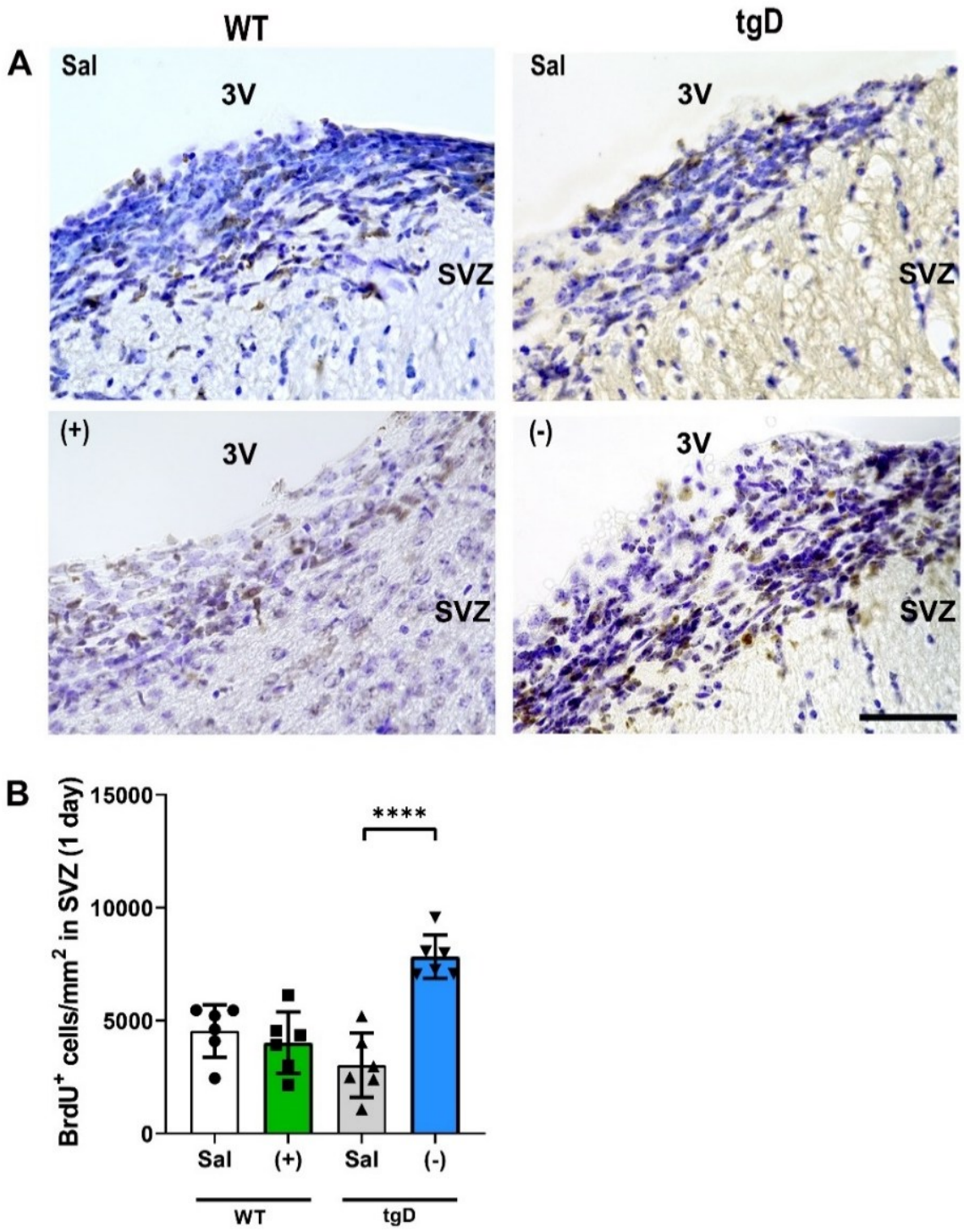

Fig.6 Proliferation of neural progenitors in subventricular zone (SVZ). A) Representative photomicrograph showing BrdU positive (+) proliferating neural progenitors (NPCs) (brown) within SVZ one day after the last BrdU injection in wild type (WT) and tgD mice treated with saline (Sal), 5-HT_{1B} agonist (+) or 5-HT_{1B} antagonist (-). Blue indicates cresyl violet-stained nuclei. B) Quantification of BrdU+ NPCs density per 1mm^2 area within SVZ 1day after last BrdU application. ****: $p < 0.0001$ using One-Way-Anova followed by Tukey's multiple comparison tests. Scale bar = 50µm.

3.2 Proliferation of neural progenitors in RMS

In the pRMS, the number of BrdU+ proliferating neural progenitors/mm^2 was significantly higher in WT (6372±281.3) compared with tgD mice (3942 ± 412.7) (p = 0.006) one day after the last BrdU injection by approximately 61.86%.

The number of BrdU+ proliferating neural progenitors/mm^2 in WT mice (4542 ± 473) treated with saline one day after the last BrdU injection was comparable with WT mice treated with 5-HT$_{1B}$ agonist (5123 ± 587.9) (p = 0.2).

The tgD mice treated with 5-HT$_{1B}$ antagonist showed a significant increase in the number of BrdU+ cells (9096 ± 366.7) one day after the last BrdU injection compared to tgD mice treated with saline by approximately 43.3% (p = 0.0001) (Fig.7A, B).

In the dRMS, the number of BrdU+ proliferating neural progenitors/mm^2 was significantly different between the WT (4485 ± 580.8) and tgD mice (2198 ± 234.9) (p = 0.0010) one day after the last BrdU injection.

The number of BrdU+ proliferating neural progenitors/mm^2 in WT mice (4485 ± 580.8) treated with saline one day after the last BrdU injection was comparable with WT mice treated with 5-HT$_{1B}$ agonist (4282 ± 414.4) (p = 0.6756).

The tgD mice treated with 5-HT$_{1B}$ receptor antagonist showed a significant increase in the number of BrdU+ cells (8418 ± 234.7) one day after the last BrdU injection compared to tgD mice treated with saline (p = 0.0001) (Fig.8A, B).

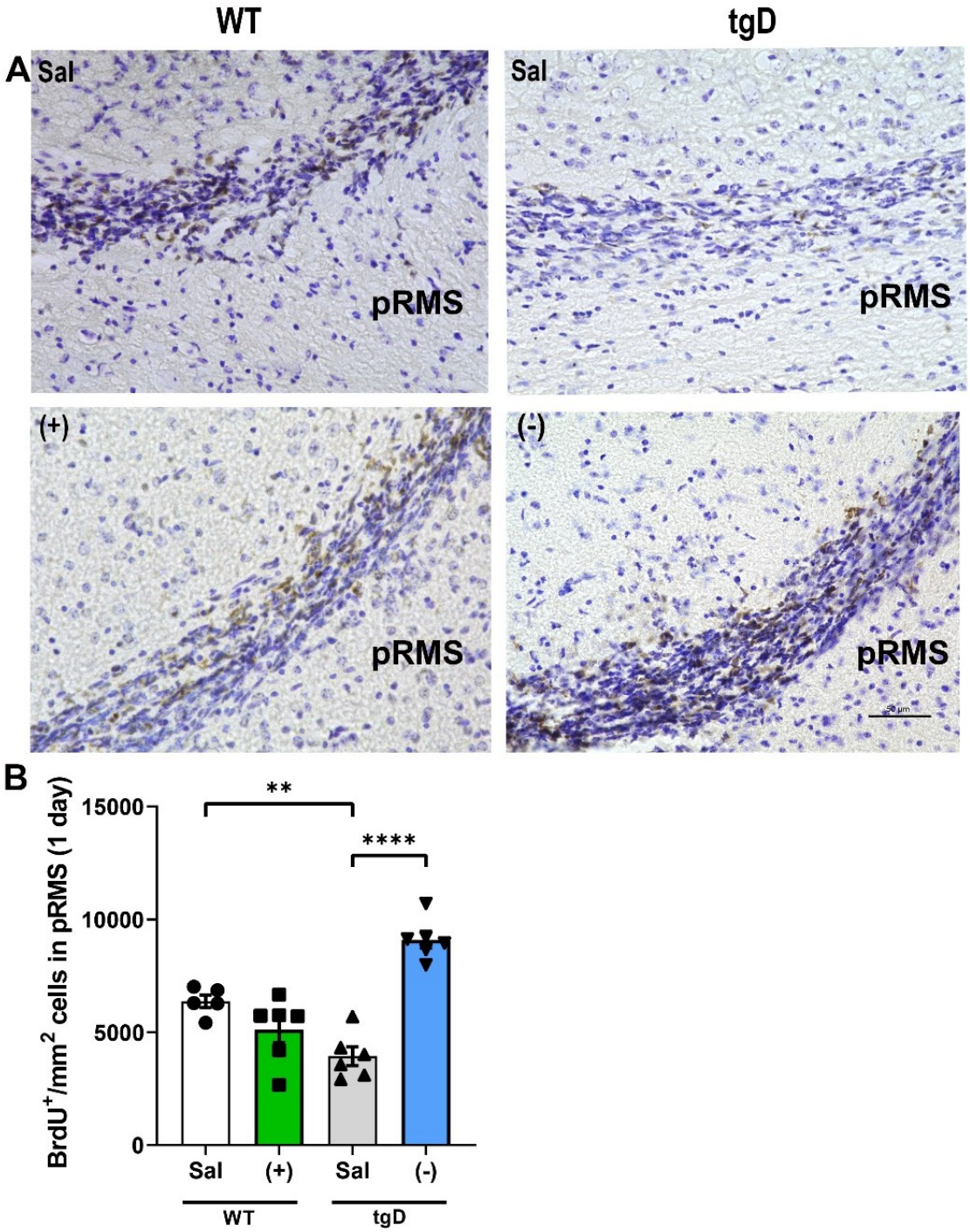

Fig.7 Proliferation of neural progenitors in proximal rostral migratory stream (pRMS). A) Representative photomicrograph showing BrdU+ NPCs (brown) within pRMS one day after the last BrdU injection in WT and tgD mice treated with saline (Sal), 5-HT_{1B} agonist (+) or 5-HT_{1B} antagonist (-). Blue indicates cresyl violet-stained nuclei. B) Quantification of BrdU+ proliferating NPCs density per 1mm^2 area within pRMS 1day after last BrdU application. **: $p < 0.0060$ ****: $p < 0.0001$ using One-Way-Anova followed by Tukey's multiple comparisons tests. Scale bar = 50µm.

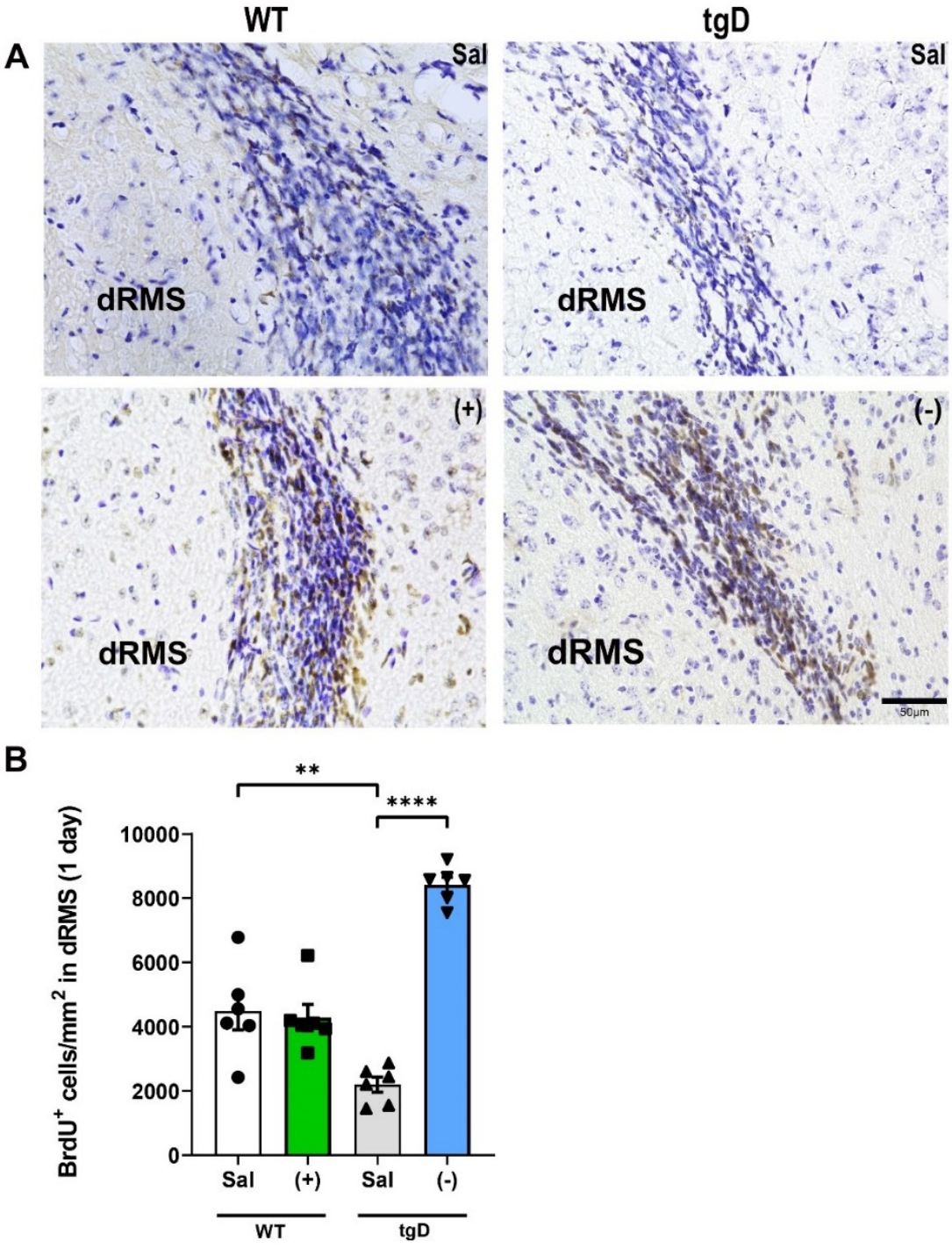

Fig.8 Proliferation of neural progenitors in distal rostral migratory stream (dRMS). A) representative photomicrograph showing BrdU+ proliferating NPCs (brown) within dRMS one day after the last BrdU injection in WT and tgD mice treated with saline (Sal), 5-HT_{1B} agonist (+) or 5-HT_{1B} antagonist (-). Blue indicates cresyl violet-stained nuclei. B) Quantification of BrdU+ proliferating NPCs density per 1mm^2 area within dRMS 1day after last BrdU application. **: $p < 0.0010$ ****: $p < 0.0001$ using One-Way-Anova followed by Tukey's multiple comparisons tests. Scale bar = 50µm.

3.3 New-born neurons in OB

New-born neurons are integrated into GCL and GL of the OB (Dejou, Mandairon et al. 2024). In the GCL, the number of BrdU+ new-born neurons/mm^2 was not significantly different between the WT (414.7 ± 42.61) and tgD mice (384.7 ± 14.53) (p = >0.99) four weeks after the last BrdU injection. The number of BrdU+ new-born neurons/mm^2 in WT mice (414.7 ± 42.61) treated with saline four weeks after the last BrdU injection was comparable with WT mice treated with 5-HT$_{1B}$ agonist (395.9 ± 27) (p = >0.99). The tgD mice treated with 5-HT$_{1B}$ receptor antagonist showed a significant increase in the number of BrdU+ new-born neurons (732.2 ± 28.39) four weeks after the last BrdU injection compared to tgD mice treated with saline (p = 0.008) (Fig.9A, B).

In the GL, the number of BrdU+ new-born neurons /mm^2 was significantly different between the WT (365.6 ± 43.62) and tgD mice (163.7 ± 5.072) (p = 0.0165) four weeks after the last BrdU injection. The number of BrdU+ new-born neurons/mm^2 in WT mice (365.6 ± 43.62) treated with saline four weeks after the last BrdU injection was comparable with WT mice treated with 5-HT$_{1B}$ agonist (339 ± 16.59) (p = >0.99). The tgD mice treated with 5-HT$_{1B}$ antagonist showed a significant increase in the number of BrdU+ new-born neurons (292 ± 11.05) four weeks after the last BrdU injection compared to tgD mice treated with saline (p = > 0.99) (Fig.10A, B).

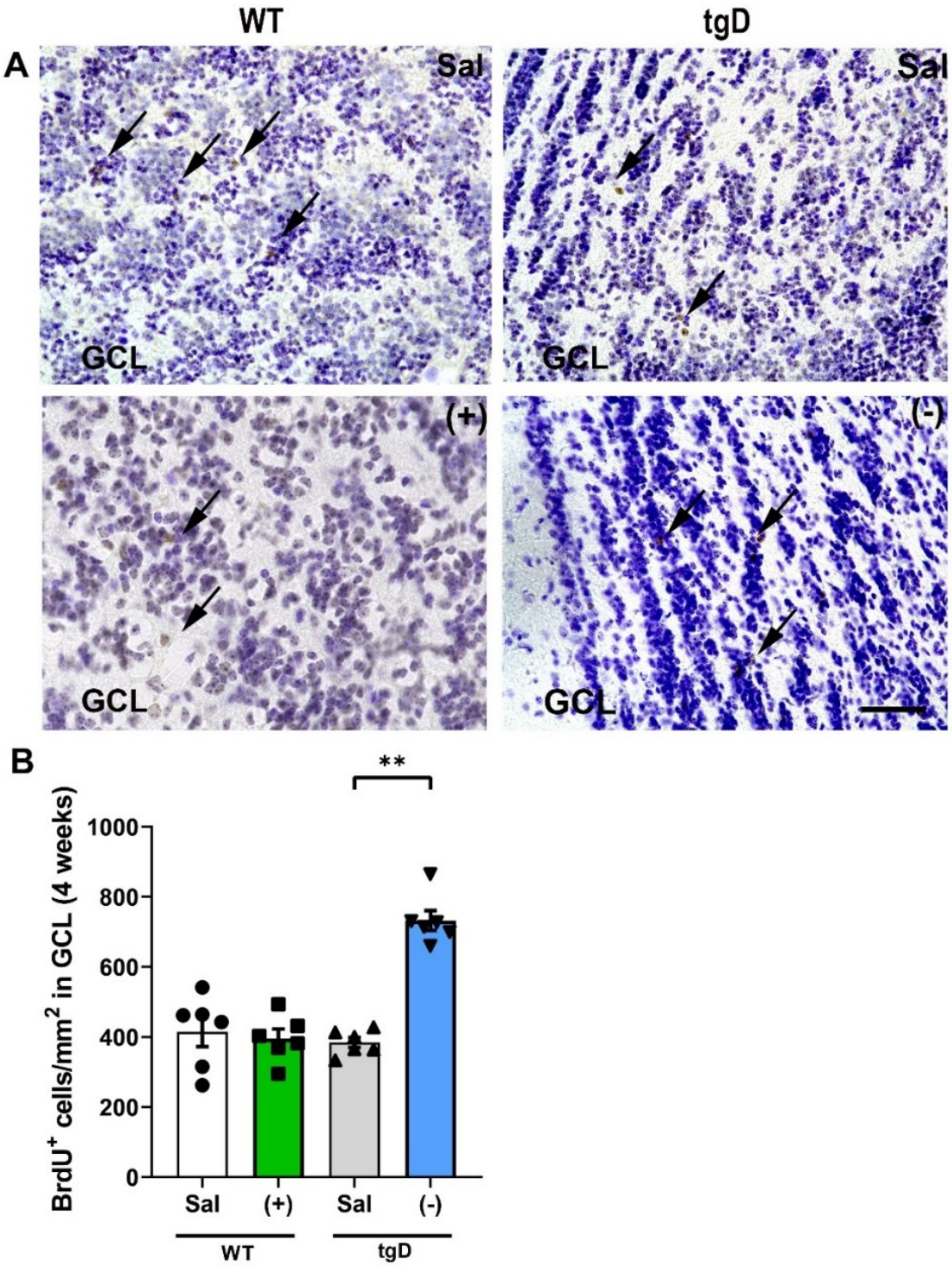

Fig.9 New-born neurons in granular cell layer (GCL) in olfactory bulb (OB). A) Representative photomicrograph showing the surviving BrdU+ new-born neurons (brown, black arrows) within GCL of OB four weeks after the last BrdU injection in WT and tgD mice treated with saline (Sal), 5-HT_{1B} agonist (+) or 5-HT_{1B} antagonist (-). Blue indicates cresyl violet-stained nuclei. B) Quantification of BrdU+ new-born neurons density per 1mm^2 area within GL of OB four weeks after last BrdU application. *: p = 0.0081 using Kruskal-Wallis followed by Dunn's multiple comparison tests. Scale bar = 50µm.

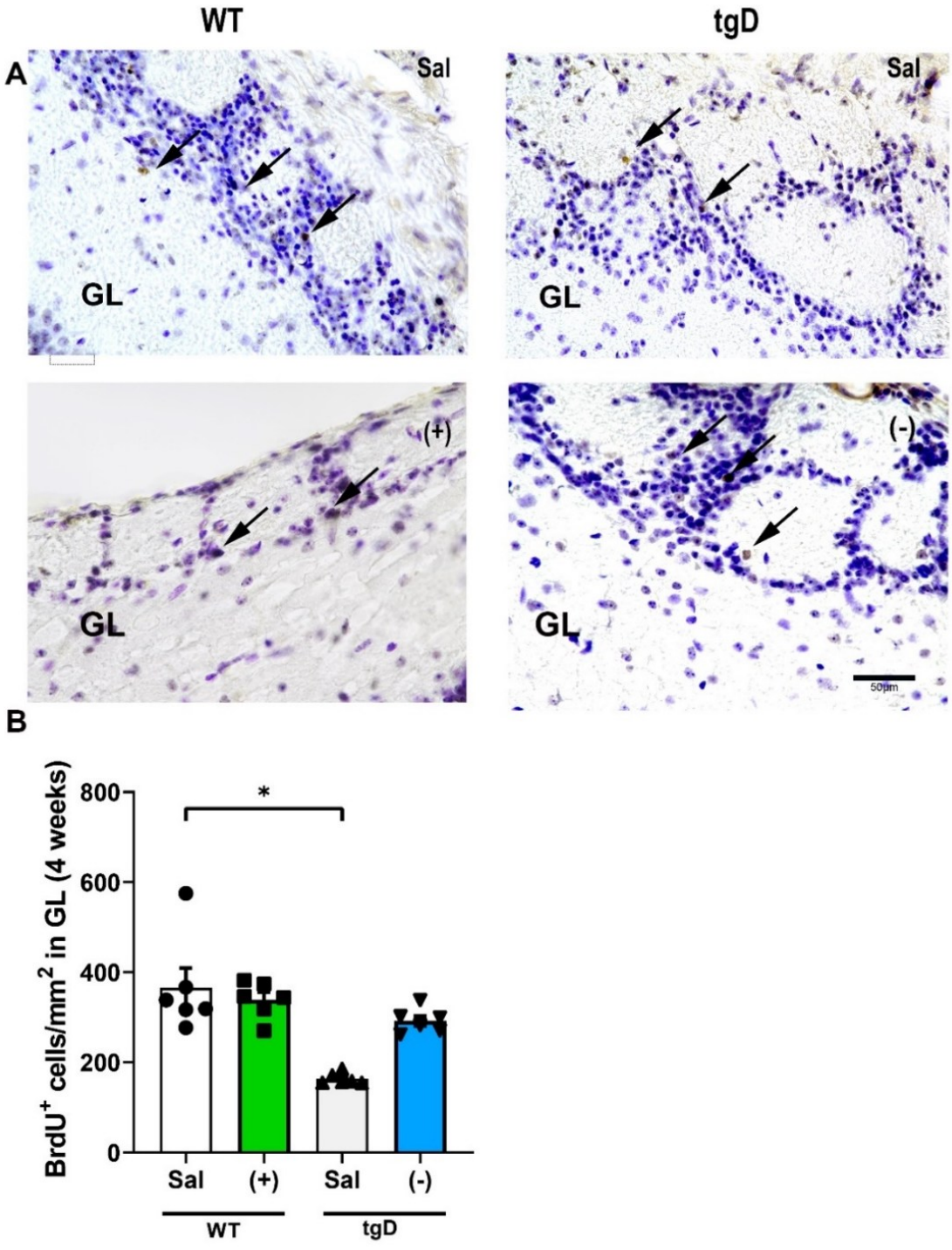

Fig.10 New-born neurons in glomerular layer (GL) in olfactory bulb (OB). A) Representative photomicrograph showing the surviving BrdU+ new-born neurons (brown, black arrows) within GL of OB four weeks after the last BrdU injection in WT and tgD mice treated with saline (Sal), 5-HT_{1B} agonist (+) or 5-HT_{1B} antagonist (-). Blue indicates cresyl violet-stained nuclei. B) Quantification of BrdU+ new-born neurons density per 1mm^2 area within GL of OB four weeks after last BrdU application. *: $p < 0.05$ using Kruskal-Wallis followed by Dunn's multiple comparison tests. Scale bar = 50μm.

3.4 Olfactory bulb volume and cytoarchitecture

The OB volume was measured for each brain of the WT and tgD mice treated with saline, 5-HT_{1B} agonist or 5-HT_{1B} antagonist. Statistical analysis revealed no significant differences in OB volume across the experimental groups (Fig. 11A). The GL, EPL, MCL, and IPL layer thickness were measured. There were no significant differences in OB layer thickness across the experimental groups (Fig.11B). We analyzed genes encoding neurotrophic factors and oxidative stress genes in OB. There were no significant differences between WT and tgD mice treated with saline, between WT treated with saline and WT mice treated with 5-HT_{1B} agonist or between in tgD mice received saline and those received 5-HT antagonist (Fig.11C, D).

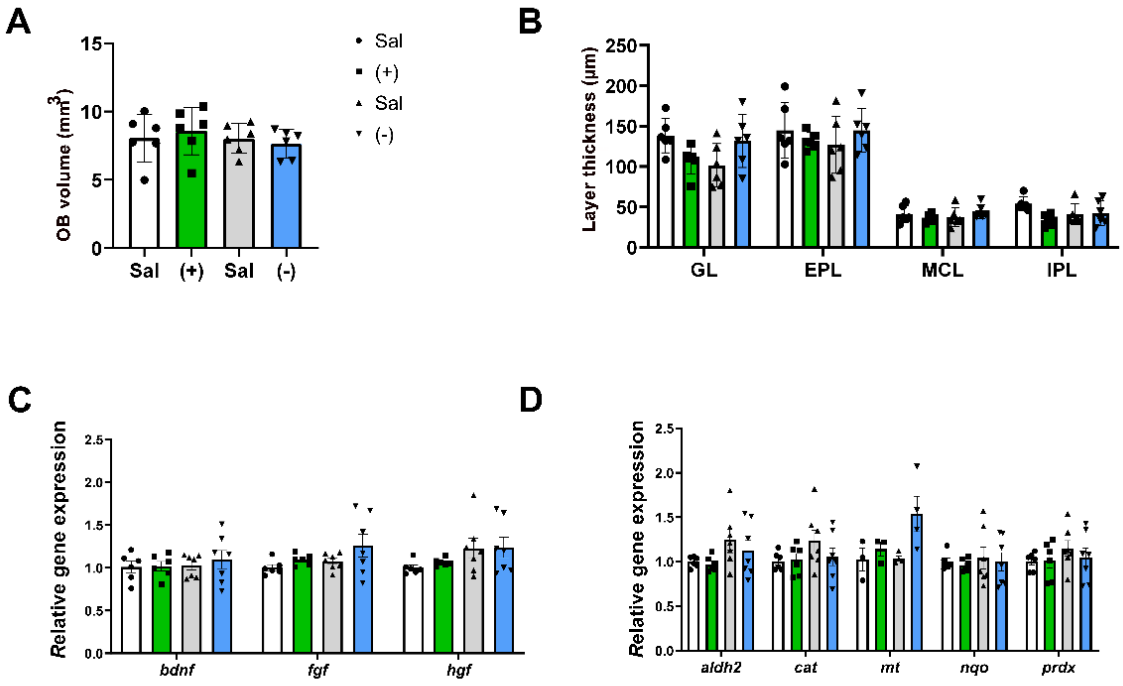

Fig.11 Olfactory bulb volume and cytoarchitecture. Quantification of the (A) OB volume and (B) OB layers: glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL) and internal plexiform layer (IPL). Quantification of the relative expression of C) genes regulating cell cycle and cellular proliferation. B) genes encoding neurotrophic factors and D) oxidative stress genes. *: $p < 0.05$ using One way ANOVA and multiple comparison.measured in WT and tgD mice treated with saline (Sal), 5-HT_{1B} agonist (+) or 5-HT_{1B} antagonist (-).

4 Discussion

The pathogenesis of AD is highly complex, with amyloid pathology playing a central role in progressive neurodegeneration and impaired brain regeneration (Holtzman, Morris et al. 2011). Although altered neurogenesis is likely associated with progressive amyloid pathology, the precise mechanisms underlying this dysregulation in early AD remain unclear (Sung, Lin et al. 2020, Terreros-Roncal, Moreno-Jiménez et al. 2022) (Sung, Lin et al. 2020).

The tgD mice used in this project exclusively express Aβ dimers without forming amyloid plaques, yet still exhibit mild cognitive decline and neurotransmitter dysbalance (Abdel-Hafiz, Müller-Schiffmann et al. 2018). As such, they serve as a valuable model for mimicking early, alternative stages of AD and for advancing our understanding of its initialization and dissect the effect of soluble amyloid species on the brain (van Gerresheim, Herring et al. 2021). Importantly, the serotonergic signalling regulates adult neurogenesis (Brummelte, Mc Glanaghy et al. 2017) and controls neuronal responses (Ledo, Azevedo et al. 2016), which may modify the AD progression. Our results indicate that soluble Aβ affects neuronal progenitor cell proliferation in RMS and survival in OB which could be reversed via modulation of 5-HT_{1B} receptor, indicating an interaction between soluble Aβ and 5-HT_{1B} which affects the adult neurogenesis in tgD mice and highlighting a novel possible therapeutic target an increased number surviving NPCs may enhance learning and memory processes as they contribute to neuroplasticitiy and neuroregeneration (Arzate and Covarrubias 2020).

In this study we observed a reduced proliferation of NPCs in the RMS in tgD mice.

In line with our findings, a decline in neurogenesis is correlated with neurodegenerative diseases and is accompanied by cognitive deficits (Apple, Fonseca et al. 2017). Several observations from other rodent AD models are in agreement with findings in our tgD mouse models. In the SGZ of tgD, reduced proliferation of NPCs has been reported, which may impair neuronal plasticity (Faramarzi, 2024). For instance, mice with APP mutations showed a reduced adult neurogenesis in the hippocampus without changes in neuronal fate determination (Donovan, Yazdani et al. 2006). Additionally, the 3xTg-AD mouse, which harbours mutations in the APP (sw), Presenilin - 1 (PS-1) and microtubule associated protein tau (MAPT) genes and develops extracellular Aβ deposits shows critically reduced adult neurogenesis in both neurogenic niches: the SGZ in the hippocampus and the SVZ of the lateral ventricle (Hamilton, Aumont et al. 2010). In addition to cognitive impairment, Aβ burden appears to contribute to olfactory dysfunction in AD mouse models (Wesson, Levy et al. 2010). Olfactory deficits in these animals often emerge before prominent cognitive

symptoms and are associated with early Aβ accumulation within olfactory structures such as the OB and epithelium (Wu, Rao et al. 2013). Studies in Tg2576 mice demonstrate that non-fibrillar Aβ deposits correlate with odor habituation and discrimination deficits at 3 months of age, preceding extensive cortical pathology (Wesson, Levy et al. 2010).

Furthermore, the 5xFAD transgenic mouse, that shows an overexpression of APP with three FAD mutations and two FAD mutations in human PS-1, amyloid deposits and gliosis mimicking human AD (Oblak, Lin et al. 2021) do not generate new neurons at the age of 7 months (Moon, Cha et al. 2014) and significant disturbances of neuronal differentiation (Zaletel, Schwirtlich et al. 2018). Conversely, certain studies have reported an increase in AN in APP/PS1 tg mouse models of AD, demonstrating that altered survival of newborn neurons and elevated neuroblast proliferation can precede amyloid plaque deposition (Unger, Marschallinger et al. 2016). Moreover, Jin et al. detected in the DG of PDGF-APP mice with two different mutations in the APP gene (Jin, Galvan et al. 2004) and, therefore, mimick AD neuropathology and show excessive amyloid plaques, gliosis and dystrophic neurites (Hsia, Masliah et al. 1999) an increasement in BrdU-labelled cells. Furthermore, in 5-month-old APP23 mice that express hAPP751(Swe) no significant difference in neurogenesis was described (Crews, Rockenstein et al. 2010), but the 25-month-old APP23 tg mice had an increased neurogenesis (Ermini, Grathwohl et al. 2008). Similar results were also seen in the TGCRND8 mice with soluble Aβ production and amyloid plaque accumulation mimicking AD which had an increased proliferation of newborn cells in the dentate gyruse compared to non-tg controls (Kanemoto, Griffin et al. 2014).

A possible explanation for these contrasting results can be the heterogeneity of AD which is a multifactorial disorder involving vascular, genetic, sexual and environmental factors, which may differently affect adult neurogenesis (Sindi, Mangialasche et al. 2015). Moreover, the different ages and genotypes of the transgenic mice cohorts, the analysed cell populations and the different neurogenic niches may promote divergent results (Unger, Marschallinger et al. 2016). This also indicates that the amyloid plaque load does not necesserarily correlate with decreased adult neurogenesis and, in turn, the cognitive impairment in alzheimer mouse models (Krezymon, Richetin et al. 2013). Nevertheless, soluble Aβ species in APP transgenic mice have been reported to induce aberrant brain network acitivity in mature mice, highlighting the potential role of early pathological changes in the development of AD (Ben-Nejma, Keliris et al. 2019). Thus, the apparent disconnect between amyloid plaque accumulation and neurogenic impairment underscores the importance of soluble Aβ species as early and functionally relevant mediators of neural dysfunction in AD (Zhang, Hao et al. 2011). Overall, soluble Aβ may be a critical factor affecting neurogenesis and network integritiy in AD (López-Toledano and Shelanski 2004).

Findings from post-mortem human brain showed less NPCs in the hippocampus of AD patients compared to age-matched controls (Lovell, Geiger et al. 2006). In addition, the progenitor cells are reduced and the maturation of neuroblasts is impeded in AD patients (Moreno-Jiménez, Terreros-Roncal et al. 2021). Although an increased early neuronal markers as DCX, polysialylated-neural cell adhesion molecule (PSA-NCAM) and neuron-glial related cell adhesion molecule (NrCAM/TUC-4) in AD patients' hippocampus was reported (Babcock, Page et al. 2021), this may be a compensatory transient upregulation in response to brain pathology in AD (Yu, Washington et al. 2016). Noteworthy, adult neurogenesis in the aging human brain continues but at dramatically lower rates making the quantification of adult-born neurons challenging (Jin, Peel et al. 2004, Kumar, Pareek et al. 2019, Moreno-Jiménez, Terreros-Roncal et al. 2021).

The survival and maturation of new born neurons is important for their integration into neuronal circuits that exist in the SGZ and SVZ (Turnley, Basrai et al. 2014) (Kee, Teixeira et al. 2007) which is supported by enriched environment in the neurogenic niches (Tashiro, Makino et al. 2007). Contrary to this, ageing and chronic stress decrease the amount of surviving and maturating NPCs (Klempin and Kempermann 2007, Schoenfeld and Gould 2012) and in turn their integration.

We demonstrated that the tgD mice had less surviving newborn-neurons in the GL of the OB, consistent with initial reduction of NPC proliferation in SVZ and RMS in tgD mice. An impaired migration of NPCs from SVZ and RMS to OB may play a role in reduced NPCs in OB (Turnley, Basrai et al. 2014), and can have critical consequences on brain plasticity and function (Mu and Gage 2011). However, the effect of soluble Aβ on NPCs migration remains to be tested in further studies.

Interestingly, several studies have reported impaired olfactory function in the early stages of AD, in both human and mouse studies. In APP/PS1 transgenic mice histological atrophy of the olfactory mucosa and deficits in olfactory behavior have been observed (Yao, Hua et al. 2017, Meyer, Niedermeier et al. 2025). In humans, olfactory dysfunction progresses with disease severity, with significant olfactory deficits detected in patients with manifest AD compared to individuals in the initial stages of mild cognitive decline (Jung, Shin et al. 2019). In our study, we observed no significant changes neither in the total OB volume nor in the cytoarchitecture of individual olfactory bulb layers. Similarly, MRI based measurments of OB volume in AD human patients also revealed no significant differences (Servello, Fioretti et al. 2015). However, an earlier MRI based study of OB volume in humans reported significantly reduced OB volume in AD patients (Thomann, Dos Santos et al. 2009). This discrepancy may be due to different stages of AD, subject populations. Our results are also

in contrast with previous research that has shown a shrinkage of the glomerular layer of post-mortem OB tissue in AD patients (Son, Steinbusch et al. 2022) and that reducing APP overexpression in mice leads to an enhancement of the glomerular layer observed through brain MRI (Saar, Cheng et al. 2015). As tgD mice mimiks early AD stages with mild cognitive decline (Abdel-Hafiz L 2018, van Gerresheim, Müller-Schiffmann et al. 2023), gross structural alterations in the OB may have not been manifested.

We also investigated the expression of several genes encoding neurotrophic factors (*bdnf, fgf, hgf*) and associated with oxidative stress (*aldh2, cat, mt, nqo1, prdx*).

Neurotrophic factors belong to a group of secreted proteins that enhance neuronal proliferation, differentiation, maturation and synaptic plasticity. Among these, *bdnf* is a key regulator for neuronal and synaptic development and plasticity (Agnihotri and Mohajeri 2022). *fgf* also plays a crucial role in neuronal development (Gantner, Hunt et al. 2021), while *hgf* is expressed in the nervous system and contributes to neuronal growth and survival (Desole, Gallo et al. 2021). The neurotrophic factors are affected in AD, for instance, *bdnf* and its expression is reduced by Aβ accumulation (Gao, Zhang et al. 2022). Interestingly, Aβ induced neuropathology in APP mouse models – including gliosis, synaptic deficits and other neuronal abnormalities – was effectively reversed following *fgf1* administration (Hung, Sun et al. 2025). Morevoer, *hgf*'s receptor kinasis c-Met was reduced in 5xFAD mice, leading to exacerbation of Aβ-associated neuronal impairments (Wei, Ma et al. 2022). In addition, the balance between generation and degradation of ROS is tightly regulated and its disruption also contributes to impaired neurogenesis and is linked to various neurodegenerative diseases (Walton, Shin et al. 2012). *Aldh2* is important for the catabolism of toxic aldehydes associated with oxidative stress, and *aldh2* knockout mice exhibit age-related cognitive deficits (D'Souza, Elharram et al. 2015). *Cat* is an enzyme, that decomposes ROS into water and oxygen, therefore reducing ROS levels (Chui, Zhang et al. 2020). Another enzymes that controls redox imbalances is *nqo1* (Drolet, Buchner-Duby et al. 2021) and prdx that metabolizes H_2O_2 (Yan, Rountree et al. 2008). ROS might contribute to the progression of AD by including neuronal damaging and mitochondrial dysfunction (Bhatt, Puli et al. 2021).

However, in tgD mice, we didn't observe a significant change in neurotrophic-factor nor in ROS homeostasis encoding genes. This is in agreement with preserved OB volume and cellular architecture in tgD mice.

In our study, the significant reduction of NPCs proliferation in the RMS could be rescued by administration of a specific 5-HT_{1B} receptor antagonist. We noticed also an increase in NPCs proliferation in SVZ and increased number of new born neurons in GCL of OB. Hence,

we suggest that there might be a positive influence of modulation of serotonin signalling through the blockage of the 5-HT_{1B} (autoreceptors and heteroreceptors-inhibitory) on the proliferation and survival of neuronal progenitors in the neurogenic niches in tgD mice, probably due to restoration of serotonin balance and indicating an interaction between 5-HT_{1B} receptor and soluble Aβ dimers.

In agreement with this hypothesis, increased serotonin level via long-term administration of SSRIs leads to enhanced proliferation and survival of neuronal progenitor cells (Malberg, Eisch et al. 2000, Santarelli, Saxe et al. 2003). Paralell to our finding it was described that 5-HT_{1B} receptors antagonist enhances the proliferation of NPCs (Banasr, Hery et al. 2004),

Importantly, the NPCs proliferation may be differently influenced depending on the modulated serotonin receptor subtypes (Klempin, Beis et al. 2013) and stages of life (Song, Huang et al. 2017). For instance, it has been observed that agonist of 5-HT_{1A} (autoreceptors-inhibitory) or 5-HT_4 (heteroreceptors-stimulatory) increase the proliferation of NPCs in the DG whereas antagonists of these receptors results in an opposite effect (Segi-Nishida 2017). Additionally, agonists of 5-HT_{2C} (heteroreceptors-stimulatory) decreases cell proliferation or has no effect (Banasr, Hery et al. 2004) and it blockage results in increased proliferating NPCs (Klempin, Babu et al. 2010). In contrast to our findings, 5-HT_{1B} knock out mice show normal NPCs proliferation (Xia, Deloménie et al. 2012). All in all, the effects of serotonin on NPC proliferation may not depend on 5-HT solely, but also on the interaction between serotonin and the different 5-HT receptor subtypes, the region and the pathological state (Soumier, Banasr et al. 2010). In particular, the influence of 5-HT_{1B} receptors on NPC proliferation may be affected by of AD pathology. In line with this hypothesis, modulation of 5-HT_{1B} receptors via agonist in WT mice has no effect on the proliferation of NPCs. This may suggest that in healthy WT mice, the influence of 5-HT_{1B} receptors on NPC survival may be absent or compensated. We also noticed that neither OB volume nor layer thickness was affected by modulation of 5-HT_{1B} receptors.

5 Summary, Conclusion and outlook

Soluble Aβ dimers significantly reduce the number of proliferating NPCs in RMS and surviving newborn neurons in OB of tgD mice. This implies that soluble Aβ impairs the structural neuronal plasticity, which is likely contributing to learning and memory deficits in early AD stages. Chronic modulation of 5-HT_{1B} via receptor antagonist rescued the reduction of NPCs, supporting an interaction of Aβ dimers interacting with 5-HT_{1B} receptors in the neurogenic niches.

As 5-HT_{1B} receptors are predominantly presynaptic and function as both an auto- and heteroreceptor, their interaction with Aβ dimers may disrupt not only serotonin release but also broader neurotransmitter signaling involved in neurogenesis, however further research is necessary to define the underlying mechanisms. Such findings may open new therapeutic avenues for early AD.

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