Activity- and pharmacology-dependent modulation of adult neurogenesis in relation to Alzheimer's disease

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Preface

Alzheimer's disease (AD) is an incurable and fatal disease that threatens the lives of aging individuals. 100 years after the disease was first described, solutions for effective prevention and treatment still remain elusive[1]. Significant research efforts have so far led to insights concerning the cellular and molecular basis of AD that have illuminated potential causes of AD in the human brain. Increasing lines of evidence indicate that multiple factors contribute to the clinical manifestation of AD.

In this respect, the discovery of endogenous neural stem cells in the adult brain, and their capacity to functionally integrate into existing brain circuits has received considerable attention in the neuroscience field[2]. Our improved understanding of regulation and induction of adult neurogenesis has garnered interest in the therapeutic capacity of adult neurogenesis. Understanding the basic mechanisms of adult neurogenesis may allow us to further understand the relationship between neurogenesis and AD. This is relevant since a) adult neurogenesis occurs in only few areas of the nervous system, including the hippocampus[3], b) neurogenesis is positively correlated with learning and memory function[4], and c) the hippocampus is heavily affected in AD, both structurally and functionally; neuropathology and cell loss are extensive in this brain region, where reduced volume reflects disease progression[5,6]. Moreover, AD patients suffer from prominent cognitive decline early in the course of disease. Since neurogenesis in rodents can be modified by pharmacological and environmental interventions, positive induction of neurogenesis could form a relevant target when evaluated within a multifactorial disease context[7].

In this thesis I will explore the relationship between adult neurogenesis and AD in both human brain tissue and mouse models using the latest findings and tools. This will be done by starting broadly with details about these topics and later focusing on experiments that bring these areas together.

1. ALZHEIMER'S DISEASE
1.1 Epidemiology: aging and AD incidence

An impetus for doing AD research starts with projected costs, both human and financial, associated with the expected burden of care in the

near future. In the 21st century, changing demographics will place a huge burden on healthcare systems around the world. In fact, the world population is currently undergoing a demographic shift in age, and this shift in age has been reported as, "no other force is likely to shape the future of national economic health, public finances, and policy making as the irreversible rate at which the world's population is aging" [8].

Aging is the primary risk factor for developing AD; both the prevalence and incidence of AD increases dramatically with age [9]. Early AD onset cases, synonymous with familial inheritance of rare mutations, make up less than 5% of all reported AD cases, while a number of gene polymorphisms are associated with increased risk to develop the disease. For the majority of sporadic occurring cases of AD, no major genetic risk factor is known. Hence, aging is a primary cause for AD development. However, understanding aging in general and the aging process of the brain in particular, has been a long-standing problem for mankind. As average life expectancy increases throughout the world so does the number of elderly men and women who become increasingly susceptible to AD.

AD is the most prevalent neurodegenerative disease worldwide and has become a national health problem in the US and the Western world. A report issued in 2009 estimated that 7 million people in Western Europe and over 4 million in North America are currently afflicted with the disease[10]. It is estimated that by 2050, these numbers will increase to 13 and 11 million respectively if preventative treatments do not become available [9,10]. Worldwide prevalence is estimated to quadruple by 2050 at which time 1 in 85 persons will be living with the disease[11].

Because AD is such a debilitating disease and patients can suffer from AD for several years or even longer during which they require intensive care and nursing, the cost of AD for society is substantial. National direct and indirect cost of caring for AD patients have been estimated to be upwards of 100 billion USD per year, and this figure will undoubtedly rise with the projected growing number of AD cases[12]. In The Netherlands, the projected 10-year costs of care are close to 100,000 EUR per individual (calculated by1996 values)[13]. According to the World Alzheimer Report, the worldwide costs of dementia in 2010 were roughly 600 billion USD, with roughly 70% of the costs occurring in Western Europe and North America [14]. The total costs

associated with care are projected to reach 1.1 trillion USD by 2050 within the United States, a 500% increase in current expenditure[15].

1.2 Neuropathology

Alois Alzheimer, a German psychiatrist and neuropathologist, first described Alzheimer's disease in 1907. He used classical Silver staining techniques to identify neuropathological aberrations that are still used to this day for diagnosis. We now know they are based on accumulations of two aberrant proteins that are likely implicated in AD etiology. Despite an early identification of these protein accumulations, it wasn't until 1984 and 1986 when major findings were published that identified amyloid-β peptide[16] and hyperphosphorylated tau protein[17] as the main components of the classic AD neuropathological alterations.

AD has two characteristic neuropathological hallmarks, i.e. senile plaques and neurofibrillary tangles, that are mainly constituted of amyloid-β (Aβ) and hyperphosphorylated tau protein respectively. Accumulation of these proteins is contributes to neuronal dysfunction, atrophy, and degeneration of the neurons in the hippocampus, cerebral cortex, and other select brain subregions. These distinguishing neuropathological features, however, may not account for all clinical outcomes. While the diagnosis of AD can only be definitely ascertained through postmortem histological examination of the brain for these lesions[18], the further molecular, biochemical and genetic characterizations of the Aβ and tau proteins have advanced our understanding of how these lesions relate to, and explain at least part of AD etiology, progression, and the clinical manifestation of the disease.

The genetic factors known to cause early-onset AD have provided unique insights as potential mechanisms for disease pathogenesis. To date, three genes have been identified as containing fully penetrant, causal mutations that result in early-onset AD, also referred to as familial AD. They have been described as both genetically complex (indicating that there is no simple mode of inheritance that accounts for their heritability) and heterogeneous in AD [19]. These mutations can be found on the genes encoding the amyloid β protein precursor (APP), presenilin-1 protein (PSEN1) and the presenilin-2 protein (PSEN2). Their identification was fundamental to clarify the mechanisms behind the familial forms and have improved our understanding of the sporadic forms of AD. These heterogeneous mutations interact with

each other and with non-genetic risk factors exerting only small or modest effects with weak genotype-phenotype correlations [20]. All three mutations involve the differential processing of APP and result in various lengths of amyloid-β (Aβ) peptides. The discovery that Aβ can act as an initiator of disease pathogenesis in early-onset AD has led to the formulation of the 'amyloid cascade hypothesis' and the general expectation that Aβ may be critical in sporadic cases as well [21].

Box 1.2.1: Proteolytic processing of Amyloid Precursor Protein (APP)

Aβ peptide is a proteolytic cleavage product of APP, a type-1 transmembrane protein of unknown function. The two aspartyl proteases responsible for cleavage and conversion of APP to Aβ are referred to as β- and γ-secretases [22]. However, most APP molecules undergo cleavage by another enzyme called α-secretase, that cleaves this protein near the middle of the Aβ domain [23], resulting in a cleavage product with a large soluble ectodomain (APP$_s$-α) that is released into the extracellular space. The remaining C-terminal fragment can then be cleaved by γ-secretase to create a smaller fragment known as the p3 fragment. The exact function of such proteolytic processing in normal, healthy neurons has yet to be defined, although research has indicated that cleavage of APP by γ-secretase allows the release of the APP intracellular domain (AICD) to the nucleus where it is thought to participate in transcriptional signaling[24]. Cleavage of APP by β-secretase leaves a longer C-terminal fragment that is retained in the cellular membrane and is subjected to further cleavage by γ-secretase, which finally results in the Aβ peptide cleavage product. Given the involvement of Aβ in the diagnosis of AD, the biochemistry and mechanisms for production of Aβ peptides have been rigorously investigated both in *in vivo* and *in vitro* experiments. While Aβ is constitutively secreted by mammalian cells and normally occurs in plasma and cerebrospinal fluid (CSF), Aβ and APP misprocessing are still targeted as primary causes of AD[25,26]. *See next page.*
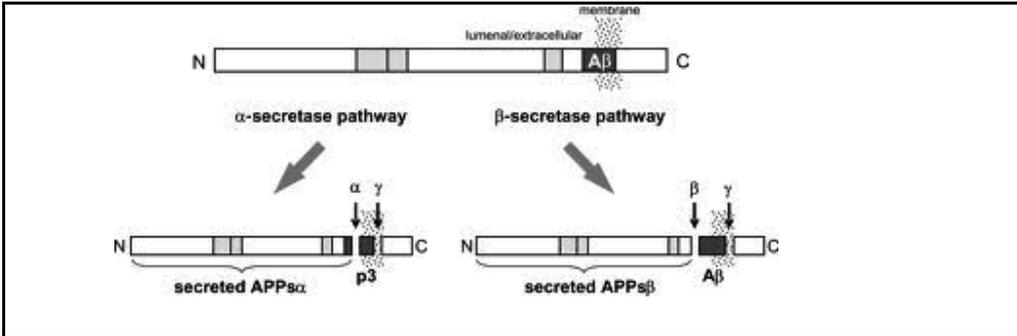
The second major pathological feature of AD is hyperphosporylated tau protein. Microtubule-associated protein (MAP) tau promotes assembly of tubulin and helps to maintain intracellular transport and structural stability in most cell types including neurons. Tau is abnormally phosphorylated in AD brain along numerous serine and threonine epitopes on the protein. Such hyperphosphorylation causes disengagement of tau from microtubules and aggregation of filamentous proteins. Hyperphosphorylated tau is considered to be the major subunit in both the paired helical filaments (PHF) as well as the neurofibrillary tangles (NFTs) [17,27].

Box 1.2.2: Mechanisms of tau hyper-phosphorylation and cytotoxicity

Tau is the substrate for a number of kinases, such as glycogen synthase kinase 3 (GSK3), cyclin dependent protein kinase 5, and protein kinase A [28]. Dephosphorylation of phospho-tau protein has been shown in vivo by alkaline phosphatase, protein phosphatase 2A (PP-2A), PP-2B, and PP-1, all of which convert it into a normal state lacking toxic properties [29,30]. PP-2A and PP-1 are responsible for 90% of the serine/threonine protein phosphatase activity in mammalian cells [31]. Activities of PP-2A and PP-1 have been shown to be compromised in AD [32,33], indicating that insufficient dephosphorylation could also be implicated in the appearance of phospho-tau.

Hyperphosphorylated tau (phospho-tau) accumulates into PHF/NFT but is not directly toxic within neurons and does not induce an apoptotic cascade. The manner in which tau is toxic is not related to formation of NFT but appears specific to the hyperphosphorylation of tau. Indeed, it has been shown that as much as 40% of phospho-tau is

not aggregated into NFTs [34]. Pathologically active phospho-tau does not bind tubulin but instead sequesters normal tau in addition to other MAPs, which subsequently interrupts the assembly and disassembly of normal microtubules [35,36].
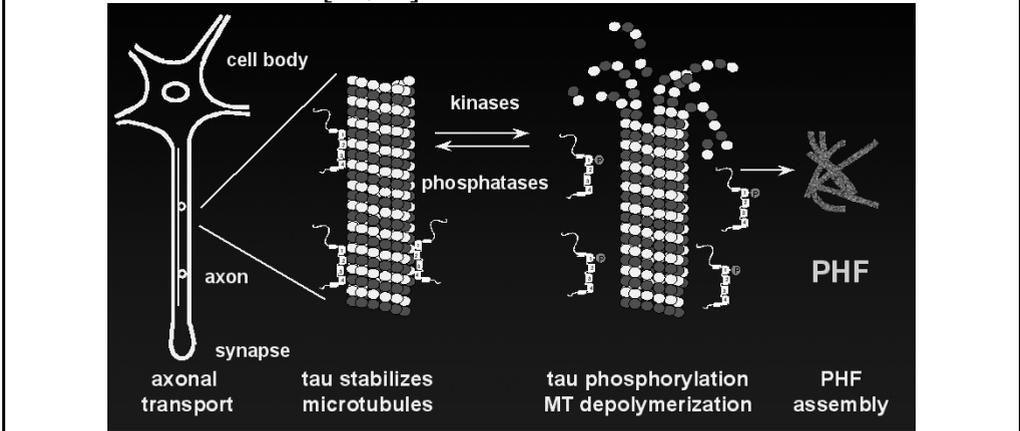
1.3 Memory and behavioral deficits associated with AD

Alzheimer's disease is most evident during the daily activities where memory and eventually executive functions are impaired. Manifestations of the disease evolve from mild memory impairments to severe cognitive dysfunction. Many times changes in mood accompany the decline in memory[37,38]. It is important to understand that at this time no single test or behavioral measurement can confirm AD. Nonetheless, neuropsychological studies combined with imaging studies have established that the cognitive deficits associated with AD are distinct from age-associated cognitive decline [39]. Despite much progress in identifying peripheral biomarkers and changes in brain through imaging, measuring functional memory deficits still remain a sensitive way to measure Alzheimer disease over time[40].

Initially, impairments may manifest as an inability to retain recently acquired information. This is described as episodic memory, as opposed to semantic memory. Episodic memory is associated with a time and place while semantic memory is not[41]. Deficits in working spatial memory have been documented in AD[42] with interventional studies showing an ability to improve specific spatial tasks [43]. The hippocampus plays a critical role in episodic memory (reviewed by Pennartz)[44].

Since mice can be tested for performance in memory tasks, this gives us the ability to test pharmacological and activity-based interventions in AD related behaviors of mouse models. Indeed, identifying and measuring behavioral changes in recently developed AD mouse models is considered critical to proving the efficacy of a candidate drug or treatment paradigm.   We will further review hippocampus-dependent behaviors later in this introduction.

1.4 Alzheimer's disease: Overlap between neurobiology of aging and disease

While AD is primarily classified as a proteinopathy, the implications of Aβ and tau protein accumulations are very complex. It is clear that human aging causes a number of physiological changes; untangling processes associated with normal aging from Alzheimer's disease process is not an easy task. In addition to amyloid and tau pathology, inflammation plays and unknown role in the progression of the disease. Adding to the complex general pathological picture, post-mitotic neurons in the hippocampus have been observed to re-enter the cell cycle as part of an apoptotic cascade, often in close association with the tangle pathology. These features of AD pathology further demonstrate the uniqueness of the disease.   As we review adult neurogenesis, some special consideration will be discussed in light of these particular disease features.

2   ADULT NEUROGENESIS
2.1 Adult Neurogenesis: conserved, functional and modifiable

One of the most exciting findings in recent neuroscience research has been the discovery that new neurons are produced in the adult brain.   The field has advanced rapidly since the introduction of Bromodeoxyuridine (BrdU) to trace cell lineage and life-long neurogenesis has been demonstrated in almost all mammals, including humans[45]. Neurogenesis in mammals decreases dramatically with age and studies of humans indicate similar reductions. Although there is no direct link between changes in adult neurogenesis per se and the risk for, or severity of, Alzheimer's disease, both are strongly correlated with aging. Given their restricted occurrence in the hippocampus, a brain structure critical to higher cognitive functions, and the fact that neurogenesis is modifiable, the relation between Alzheimer's disease and adult neurogenesis is of considerable interest.

Specialized micro-environments in particular appear to support the production of new cells in the brain. These zones are unique as they contain neural stem cells (NSCs) with the capacity to proliferate, migrate, and differentiate into adult, functional cell-types. NSCs proliferate and produce identical multipotent NSCs with the capacity to produce neurons, astrocytes, or oliogodendrocytes[46]. Self-renewal and the ability to differentiate into specialized cell types are exceptional properties that distinguish stem cells from other dividing cells, properties conferred by the microenvironment surrounding stem cells. Interestingly, stem cells isolated from the same regions behave differently when transplanted to other brain regions, confirming the important role for the local micro-environment or the neurogenic niche that enables stem cell maturation into fully functional neurons in these zones only [47](reviewed by Morrison). It is apparent that regulation of neurogenesis occurs through cell-intrinsic and cell-extrinsic mechanisms.

It is important to understand that stem cells have the capacity to generate cell types other than neurons. This implies that NSC proliferation does not necessarily result exclusively in the generation of new neurons. Likewise the use of a protein marker that identifies cell proliferation may also identify non-stem cells such as mature glia in the brain that can also proliferate in the adult brain.

In the brains of adult mammals, new cell birth and neurogenesis has been best described in two locations; the subventricular (SVZ) and subgranualar zones (SGZ) located in the lateral ventricles and hippocampal dentate gyrus, respectively. Two types of NSCs have been identified by morphology and molecular markers (reviewed by Zhao)[3], namely radial NSCs (Type-1) and non-radial NSCs (Type-2). Within the DG, GFAP astrocytes are the NSCs of the brain that generate new granule neurons through a series of immature cells [48,49]. Wnt signaling has been previously demonstrated to be responsible for the neurogenic activity of these astrocytes [50].

In the hippocampus, immature neurons migrate from the SGZ into the granule cell layer (GCL) of the dentate gyrus (DG) where they mature into granule neurons. Similarly, neuroblasts born in the SVZ migrate tangentially towards the olfactory bulb along the rostral migratory stream (RMS) [51,52] an area that has also been identified in human brain [53]. Migratory, DCX-positive (+) neurons present in the primate SVZ were found to co-express polysialylated neural cell

adhesion molecule (PSA-NCAM), identifying a migratory pathway to the striatum [54].

Recent studies show further that neurogenesis may also occur outside the classical neurogenic niches of hippocampus and SVZ; indeed, rare neurogenesis has also been reported in the cortex, amygdala, hypothalamus and substantia nigra, notably often in response to insult or other challenges [55-58]. Ischemia/reperfusion in the striatum can e.g. recruit new neurons from glial precursors in closely related brain regions like the subventricular zone [59,60]. Moreover, neurogenesis has been reported after hippocampal or cortical damage from excitotoxic, ischaemic or epileptic events [59,60] [61-64]. Interestingly, factors like brain-derived neurotrophic factor, insulin-like growth factor 1, fibroblast growth factor 2 and vascular endothelial growth factor [65-67] that are expressed after hypoxia, are known stimulators of adult neurogenesis [68-70].

2.2 Neurogenesis in Primates

The fact that neurogenesis occurs throughout the lifespan of many different species including rodents, primates and even humans, indicates an important role for this form of structural plasticity that is conserved throughout evolution. Chapter 3 of this thesis highlights a novel population of plastic cells described in the brains of the adult common marmosets, a small new-world monkey studied in relation to psychosocial stress. Marmosets are small New World monkeys native to Brazil. They have been studied in laboratory settings since the 1960s because of their small size and primate brain. Their behavior and social order have been extensively studied in their native habitat. The study highlights the fact that areas of the brain with adult neurogenesis occurring under normal physiological conditions continue to be refined.

As discussed earlier, neurogenesis is conserved in the dentate gyrus and olfactory bulb.   While marmosets have neurogenesis in these two areas, a distinct, less well characterized migratory stream exists in primates and allows neuroblasts to migrate directly to the amygdala, an area associated with emotional memories[71]. The temporal stream (TS) has only been described in rhesus and squirrel monkeys[55]. However, due to structural homology it was suspected to also exist in other primates.

> *Open Research Question:*
> *If neuronal progenitors migrate to the amygdala, how extensive is this cell population in primates compared to a traditional zone of adult neurogenesis such as the dentate gyrus?*

3 THE HIPPOCAMPUS: A CRITICAL STRUCTURE FOR LEARNING AND MEMORY DURING HEALTH AND DISEASE

3.1 Hippocampus: Structure and Function

As argued in the previous section, the hippocampus is unique in that it contains stem cells that continue to generate new neurons in the adult brain of several mammalian and primate species, including humans. The hippocampus is also well known for its critical role in higher-level cognitive functions. In regard to AD, the hippocampus is severely affected and reduced in volume in this condition. Also the two main neuropathological lesions of AD, i.e. amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs), advance through the brain in a hierarchical manner, with the hippocampus being affected already early in the disease [72]. In the early stages of disease, these protein accumulations are not directly correlated with cognitive decline, and pathology has e.g. been found in the hippocampus of elderly individuals, irrespective of their cognitive status[73]. Given this central role of the hippocampus in my thesis, I will address the main structural and functional properties of this structure in more detail below. This is also of relevance for the behavioral tests that were applied in the second half of my thesis.

3.2 Hippocampal anatomy and the trisynaptic circuit

The SGZ is part of the dentate gyrus (DG), an integral portion of the hippocampal formation. The DG largely contains granule neurons and has a trilaminar anatomy organized in a unique trisynaptic circuit involved in specific and largely unidirectional information processing (reviewed by Amaral)[74]. Anatomically, the trisynaptic circuit starts with projections from the entorhinal cortex to DG granule neurons. Mossy fibers from DG granule neurons then project to the large pyramidal neurons in the Cornu Ammonis 3 (CA3) subregion of the hippocampus and these CA3 pyramidal neurons then project to hippocampal CA1 neurons, that in their turn, project into the cortex. The CA1 and CA3 regions of the hippocampus are known to contain "place cells ", which exhibit high firing rates corresponding to a specific

location within a given environment. Very early work also established the important role of the hippocampus in forming new memories, whereas recent studies have implicated the DG in complex aspects of learning and memory, such as pattern separation and completion (see 3.3). As such, the hippocampus is considered to be involved in encoding spatiotemporal maps of the environment [75].

3.3 Hippocampal specific behaviors

The hippocampus is a brain structure of critical importance in cognition and executive functions like spatial and working memory tasks. In this thesis we will evaluate the role of adult neurogenesis for this structure and its functional relevance for health and disease. In order to measure hippocampal function in quantitative detail, the Morris water maze is commonly used for mice and rats.

The Morris Water Maze (MWM) was designed to test spatial learning and memory with high relevance for hippocampal function. Richard G. Morris first described the original maze requiring rodents to find a submerged platform in a pool of opaque water. Distal cues are placed on the walls to allow the animals to navigate to the platform. During learning and recall of placement of the submerged platform within the pool, a number of measurements are used to assess performance. A review of the testing criteria and the procedure provides an accurate description of the methodology used in this thesis[76].

Many studies have shown that modulation of adult neurogenesis either directly or indirectly, contributes to adaptations in hippocampal function [77,78]. Pattern separation and pattern completion are two related behaviors where adult neurogenesis is known to play an essential role (reviewed by Sahay et al., and Aimone et al) [79,80]. Pattern separation is the process of making similar inputs and representations less similar while pattern completion involves the reconstruction or remapping of stored representations from partial inputs [81].

We can subdivide structural plasticity in the adult brain into regulation of synapses connecting neurons and the generation of new neurons through adult neurogenesis. While synaptic plasticity is thought to be the main structural change corresponding to cognition, ongoing neurogenesis is a novel and unique form of structural plasticity that has the potential to modify structural arrangements in this brain

region on a longer time scale. While new neurons born in the subgranular zone (SGZ) progress through proliferation, migration and neuronal differentiation before becoming new DG neurons, stage-specific markers are available to identify the individual phases of the neurogenic process.

Although the total number of new neurons incorporated in the hippocampus per day may be quite low during aging, adult neurogenesis does generate new, functional neurons within an existing brain circuit and as such, represents a potential for adaptation. The extent of neurogenesis present from early age onwards has been described previously as the 'neurogenic reserve'; a special type of brain plasticity that could, when the hippocampus is actively engaged, allow for adaptation and resistance to accumulated deleterious insults, such as those developing during e.g. aging and/or Alzheimer pathology.[82].

4 HIPPOCAMPAL CELL PROLIFERATION IN ALZHEIMER'S DISEASE

4.1 Cell cycle abnormalities and adult neurogenesis during AD

When discussing adult neurogenesis and Alzheimer disease we must confront some unique evidence regarding proliferation in the hippocampus. Besides neurogenesis, there is evidence that mature neurons re-enter the cell cycle, driven by mechanisms that are not completely understood. Normally, proliferation in the brain, outside the neurogenic niches, only occurs during development; mature neurons in the brain are post-mitotic and do not divide to produce identical daughter cells. Only recently has proliferation of microglia and astrocytes started to be examined during aging and disease.

Cell-cycle abnormalities have been reported in mature neurons in hippocampus during AD, and additionally at stages considered prodromal to dementia such as mild cognitive impairment[83]. Understanding such proliferative changes in the aged and diseased brain are of considerable interest because expression of cell cycle proteins in the CA1 areas of the hippocampus is not seen without disease. Immunohistochemical studies identified cell cycle proteins in pyramidal neurons with significant AD pathology, namely tangle-bearing neurons of the hippocampus. These proteins included various cyclins and cyclin-dependent kinases [84-89]. Work in this area has identified that re-expression of cell-cycle proteins in mature neurons is part of an apoptotic cascade[90]. Whether such responses are

functionally significant or exclusively represents an abortive exit remains unclear.

Regarding neurogenesis during AD, a limited number of studies have attempted to examine adult proliferation and neurogenesis in the postmortem human brain using immunocytochemical markers. These studies reported equivocal results; one report described increased cell proliferation and expression of doublecortin (DCX), a marker of immature neurons [91] [92,93], in a cohort of senile AD cases, suggesting that neurogenesis is increased in AD [94]. Proliferation and transient neurogenesis have been observed in rodent models of brain injury [95]. The evidence indicates that neural stem cells are capable of proliferation during AD in a compensatory mechanism, but whether they receive the appropriate inputs from the hippocampus to mature naturally and integrate into the dentate gyrus is unknown.

Proliferation and markers of proliferation are not limited to neurons in the hippocampus. A study in a presenile patients found increases in proliferation but did not replicate the neurogenesis results; proliferating cells were significantly increased in the AD hippocampus but were morphologically identified as non-neuronal glia [96]. These cells were not observed exclusively in granule cell layer but additionally in subregions known to contain large numbers of glia cells, suggesting that glia proliferation occurred during AD.

4.2 Astrocytes and microglia in the AD brain

There has been a long-standing focus on neuronal changes during health and disease of the brain. This nearly exclusive focus on neurons in the brain has changed. The complex interactions between neurons and glia and the role of glia in neuronal function have become well accepted. This includes the use of protein markers such as the astrocyte marker GFAP, which is known to have multiple alternatively spliced isoforms with specific structural functions [97]. For instance, in the human SVZ, the isoform GFAP delta (GFAPδ) is expressed in quiescent neuronal progenitors[98].

Microglia are immune cells of the brain and serve an important role in surveillance, trophic support, and synaptic pruning. The extensive presence of microglia at sites of amyloid deposition in humans has been previously documented [99-101], strongly suggesting that these cells play an important role in metabolism, maintenance and/or morphology of the plaque pathology. While cell-cycle proteins in neurons have been

extensively documented, it remained unknown whether proliferation of microglia and/or astroglia occurs during disease progression and whether this can contribute to the proliferative changes seen in the AD hippocampus.

There is evidence that the innate immune system generates an active response to remove excess Aβ from the brain[102]. In light of this phenomenon, immunological approaches to clearing Aβ from the brain have been developed and active and passive immunization strategies have reached clinical testing. To briefly review this topic, Aβ deposition is thought to be reduced by Fc receptor mediated microglial phagocytosis[103]. Indeed, passive immunization with antibodies raised against Aβ have demonstrated that microglia are responsible for clearing Aβ plaques through Fc-receptor mediated phagocytosis and subsequent degradation[104].

The first immunotherapy study was stopped early due to serious side effects. AN-1792 (Elan Pharmaceuticals/Wyeth) reached Phase IIa clinical trials in the US and Europe when the program was suspended in January 2002 when approximately 5% of the patients in the active treatment group developed symptoms of aseptic meningo-encephalitis[105]. While AN-1792 suffered from considerable shortsightedness regarding drug safety, it became evident from this strategy, that understanding the role of microglia during each stage of AD was important to understand if immunotherapy was to be used successfully without causing deleterious inflammation.

Within months of publication of this thesis, phase III clinical data from the leading immunotherapy, a fully humanized monoclonal antibody will be expected. Anticipation of the results is extremely high within the Alzheimer's field; the results of the study will be intensely scrutinized. Active and passive immunotherapy will continue to be tested in clinical trials, although at this time we do not fully understand how microglia respond to plaques during disease progression.

➢ *Open Research Question:*
Do glia cells proliferate during AD in response to Aβ plaque accumulation?

5 MODULATING NEUROGENESIS DURING AGING AND AD

5.1 Known mechanisms of positive and negative regulation

This thesis is concerned with regulation under normal physiological conditions   i.e. non-acute/traumatic conditions.   We know that neurogenesis is highly susceptible to environmental/experience-dependent modulation such as voluntary exercise and environmental enrichment which stimulate survival and steer new cells to a neuronal phenotype [106,106]. Studies have further identified factors that can regulate production and survival of hippocampal neurons maturing during rodent adulthood. Some, like estrogen, environmental complexity[107-109], and NMDA-related excitatory input [110] positively regulate neurogenesis, while others like cholinergic denervation[111], stress, and aging [112] decrease levels of neurogenesis. Stress is well known to reduce different stages of neurogenesis [113] that have been implicated in depression and in antidepressant drug action[114-117]. Of interest, high circulating levels of stress hormones form a substantial risk factor for Alzheimer disease[118,119] Acute and chronic brain diseases generally elicit acute and chronic responses from the endogenous NSC population. Traumatic head injuries, epileptic seizures, and transient global and focal ischemia all increase hippocampal neurogenesis in rodents. The effects of transient neurogenesis occurring under these conditions have unknown effects on the hippocampal circuit (reviewed by Castellani et al.)[120].

In this thesis I explored the ability of pharmacologic agents and physical exercise to counteract the negative regulators of neurogenesis that occur during aging and stress conditions. The rationale behind these two approaches is explained in the next two sections.

5.2 Regulation of neurogenesis by antidepressants

Antidepressants, such as the SSRI Fluoxetine hydrochloride (fluoxetine) are prescribed to more than 40 million patients worldwide[121]. Yet, the exact mechanism of action for most antidepressants, as well as the pathophysiology of depression in general, is not well understood[122]. The altered HPA axis activity and observed hippocampal atrophy in a subset of depressed patients[123,124] as well as the time-to-effect of most antidepressants of more than a month, has raised the possibility that prolonged stress-induced reductions in adult hippocampal neurogenesis may at least in

part contribute to the structural and functional alterations in this condition [114-116,125]. In 2000, the first evidence was found that antidepressants increase neurogenesis in the brains of rats [126] suggesting that neurogenesis and anti-depressive action could be linked[127]. There is a wealth of research supporting a role for adult neurogenesis in the therapeutic effect of antidepressants, although deficiencies in neurogenesis have yet to be established as an etiological cause of depressive disorders.

Therapeutic benefits of antidepressants generally occur not until after a 4-week delay. Notably, this coincides with the maturation time-course of newly born neurons [112]. Supporting evidence has further been published showing that chronic treatment with antidepressants increases survival and rate of maturation for nascent neuroblasts [126,128]. Furthermore, experiments with different classes of antidepressants (tricyclics and SSRIs) showed reversal of depression-like phenotypes in tests such as novelty suppressed feeding (NSF)[129]. The link between neurogenesis and behavior was tested further by selectively deleting newly born cells; e.g. hippocampal progenitor selective knockouts and X-ray ablation of the neural stem cells, all blocked changes in NSF indicating that hippocampal neurogenesis is required for antidepressants to exert their effect on clinical improvements [129,130].

➢ *Open research question:*
Are other antidepressants, such as the duloxetine, a unique dual SSRI/SNRI, as effective as other antidepressants or physical exercise with regard to their ability to increase neurogenesis?

It is important to mention that many of the adult-generated cells die within the first few weeks [131],[132] due to selection mechanisms most likely determined by a balance between local neuronal activity and trophic support [133]. Significant proportions of the newborn cells (>50%), however, survive and eventually differentiate into fully functional neurons. Although neurogenesis on a short time scale is thought to have a rather limited direct input into the adult hippocampal circuit, modulation of neurogenesis for prolonged periods of time is thought to significantly influence hippocampal learning and pattern separation[134,135]. Moreover, neurogenesis appears to be required for the behavioral effects of antidepressants.[129], [77,136].

5.3 Regulation of neurogenesis by activity

Exercise similarly acts as an antidepressant [137] and induces neurogenesis, as further described in Box 4.

Box 5.3.1: Regulation of neurogenesis by voluntary wheel running

While a number of known physiological changes occur with physical exercise, we will primarily focus on effects in the brain, particularly the hippocampus. Most of the data we will review has been collected in mice allowed to freely exercise on a running wheel. As wheel running has been shown to potently stimulate neurogenesis in mice, this method will be used later in the thesis. Voluntary wheel running resulted in a robust enhancement in the survival of newly born cells in the DG of the hippocampus as well as an increase in synaptic plasticity[138,139]. This finding has been replicated in different mouse strains, ages and exercise paradigms, as reviewed by van Praag [140].

Running also enhances learning and memory in aged mice[141], which supported that age-related declines in neurogenesis could be reversed. However, details about benefits of running are still forthcoming. Many times, mice are allowed to exercise for 4 weeks, coinciding with the development time for new neurons. Whether long-term exercise paradigms has the same benefit in aged or genetically modified models of AD is unknown and addressed in this thesis.

Exercise elevates monoamine levels[142] including the precursor for serotonin synthesis, tryptophan hydroxylase [143], which may mediate the reported anti-depressant effect of exercise. Clinical data from humans shows that running and antidepressants have similar efficacy for treating major depressive disorder [144]. In the hippocampus, running is further known to increase the levels of different trophic factors [145,145,146,146], the extent of angiogenesis, dendritic spine density and synaptic plasticity [139]. Specific to the dentate gyrus (DG) subfield of the hippocampus is a robust increase in neurogenesis with exercise[140]. Both running and antidepressants increase BDNF levels [145,147], which is hypothesized to contribute significantly to neurogenesis and mood regulation [148].

➢ *Open Research Question:*
Does chronic exercise, started in middle age, prevent age-associated loss of spatial memory?

5.4 Alzheimer's disease mouse models

In the last part of the thesis we discuss and produce experiments in which we actively modified neurogenesis through activity and pharmacology in a transgenic AD mouse model. While identifying neurogenesis and pathology in the AD brain can be done through relatively straightforward methods, evaluating data from transgenic AD mice often requires a careful interpretation. Whereas AD has a long asymptomatic phase and generally a late onset with a complex, mixed neuropathology, most AD mouse models are in fact incomplete because they do not reproduce all aspects of AD. These mouse models allow key elements of AD to be tested instead of the full spectrum of the disease[149].

To begin discussing the transgenic AD animals available, we need to revisit the proteins and enzymes implicated in AD, as genes coding human proteins are introduced into these animals. In a traditional sense, we expect that introducing a pathogenic protein from a human into a mouse would faithfully recapitulate the human condition. In practice however, the phenotype of Alzheimer disease mouse models must be carefully documented and experimentally tested as reviewed in Chapter 7.

The phenotype of each model is dependent on the promoters, transgenes, methods, and techniques used to experimentally study the animals.   Each model has its own pathological signature and this requires when designing experiments to evaluate neurogenesis.   In chapter 7 we take a more in-depth look at transgenic models of Alzheimer's disease including mice expressing mutant APP. Earlier we briefly described the biochemical basis for APP cleavage and the generation of Aβ peptides. Transgenic animals have been generated that express both mutant APP as well as the enzymes responsible for cleaving APP to Aβ. Given the interaction between these factors, many transgenic strains highly overexpress a mutant protein that is preferentially cleaved by a corresponding mutant enzyme, such as the bigenic mice that express mutant PS1 and human APP. As reviewed recently, most APP and APP/PS1 mouse models show reductions in cell

proliferation[150], but this depends on the age at which they are studied and the extent of neuropathology.

These APP or amyloid based animals lack however the pathological phosphorylation of tau protein and also tangles do not develop. This led to further development of mice with multiple mutant transgenes in which the development of these pathological features is either introduced separately and/or accelerated and aggravated. In the final chapter of this thesis I study modulation of neurogenesis in the well-known triple transgenic mouse line (3xTg mice). Much of the important details regarding the 3xTg mice will be discussed in Chapter 9 while in the general discussion I will also review and compare our findings with those produced in other laboratories.

The 3xTg mice were first described in 2003[151,152], and since that time have been widely distributed to investigators. They quickly became one of the most frequently used AD models because the 3 mutated transgenes are responsible for recapitulating several aspects of AD. An exciting report was also issued regarding neurogenesis in these mice. Pathogenic accumulations of Aβ occurred within neurons in the brain; these accumulations were responsible for behavioral deficits and correlated with the electrophysiological responses recorded from the brain. Interestingly, these mice were shown to have impaired neurogenesis compared to their non-transgenic littermates in a sex and age-specific manner[153]. Based on this evidence the following research questions were introduced:

➤ *Open Research Questions:*
Can antidepressant treatment or exercise increase neurogenesis and reduce AD neuropathology in these animals?
Do age and/or disease preclude the stimulation of adult neurogenesis?

In Chapter 8 these questions will be discussed in relation to experimental findings and ongoing work in this area[154].

6 OUTLINE OF THIS THESIS

This thesis includes 5 primary studies on aspects of adult neurogenesis in relation to Alzheimer's disease as well as 2 review chapters on the topic.

Chapter 2 provides a closer look at the therapeutic potential of neurogenesis to treat Alzheimer's disease. In this chapter we explore current thoughts about adult neurogenesis and the potential of neurogenesis as an effective therapy.

One goal for translational neuroscience is to conduct basic research in models with predictive value for humans. Indeed, our understanding of adult neurogenesis in primates is incomplete and expanding as new brain regions are studied. In chapter 3 we performed basic research on a adult new world primate and report on the occurrence of new neurons in the hippocampus as well as amygdala under stress conditions.

In chapter 4 we sought to identify the phenotype of the proliferating cells present in the hippocampus of aged (>70 years of age) humans and AD patients. This chapter provides results on the actual extent of proliferation in the affected human AD brain and relates this to glia cell responses and cognitive status. The wider question is determining how amyloid plaque pathology influences adult neurogenesis.

In chapter 5 we switch to activity and pharmacology dependent neurogenesis; two different antidepressant drugs, fluoxetine hydrochloride (marketed as Prozac) and duloxetine hydrochloride (marketed as Cymbalta), a novel anti-depressant with dual-pharmacology, are compared with exercise to evaluate their potential to stimulate neurogenesis in female C57Bl6J mice.

Chapter 6 uses the same non-transgenic inbred mouse strain as in chapter 5 but here, middle-aged animals are started on long-term exercise. Instead of measuring the acute effects of these interventions on neurogenesis and cognition, we chronically treated mice to investigate if long-term activity could preserve spatial memory during aging.

In chapter 7 we review mouse models of Alzheimer disease and experimental results already published. This includes changes in neurogenesis reported in many of the commonly used Alzheimer mouse models. This provides an overview of work already completed in this area and the questions that remain to be answered.

Chapter 8 utilizes a similar study design as described in Chapter 6 but here we used the 3xTg mouse model of Alzheimer disease, a widely studied model previously reported to have deficit in hippocampal neurogenesis. Middle-aged 3xTg mice were maintained on either 1) fluoxetine 2) open access wheel running or 3) combined fluoxetine and wheel running for 11 months. This study adds multiple components, namely the presence of AD pathology in the mouse model and the synergistic treatment paradigm.

In chapter 9 the results from all chapters are reviewed and discussed with indications for further areas of study and experimentation.

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