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An Enduring Controversial Story in the Human Brain: Adult Hippocampal Neurogenesis in the Dentate Gyrus

Abstract

Adult hippocampal neurogenesis (AHN) is a well-studied phenomenon that involves the derivation of new neurons from neural progenitor cells in the dentate gyrus region of the hippocampus, an area responsible for cognitive functions such as learning and memory storage. Moreover, the hippocampus is known to be implicated in neurological conditions such as Alzheimer's disease. Although AHN has been extensively observed in animal models for twenty years, its existence and persistence in humans have been widely debated in academia, heavily based on post-mortem immunohistochemical markers. Using the search engines PubMed and Google Scholar for "Adult Human Neurogenesis," 143 articles that were most relevant to the history of AHN discovery, detection in rodents, immunohistochemical studies on post-mortem human sections, and therapeutic development targeting AHN were reviewed. This review article highlights the current understanding of AHN in rodents and humans, its implications in neurodegenerative diseases and therapeutics, and the inconsistencies and methodological variabilities encountered in studying AHN in humans. Furthermore, the correlation between AHN and diseases such as mood disorders and Alzheimer's disease is still not well established, with conflicting findings reported. Standardization of transcriptomic methodologies and increased availability of post-mortem human brain samples are crucial in advancing AHN research. This review article attempts to discover the fascinating and controversial world of adult human neurogenesis and its potential implications in treating neurological disorders. Apart from the discussion on AHN existence, tackling devastating diseases with this supplemental knowledge can lead to therapeutic advancements which greatly rely on understanding not only the presence of AHN but the mechanisms mediating its availability.

Introduction

In the 1960s, Joseph Altman was the first biologist who discovered a generation of new neurons within the hippocampal region of rodents, via autoradiographic investigation[1,2]. The traditional view held by researchers was that neurogenesis did not occur in adult mammalian brains[3-6]. However, these findings were groundbreaking as they challenged this belief and demonstrated that newly born neurons could be incorporated into adult brains. Neuroscientists within academia did not entirely accept this development until the late 1990s[3-6]. Novel technologies such as bromodeoxyuridine (BrdU) labelling[7] and immunohistochemistry targeting polysialylated neural cell adhesion molecule (PSA-NCAM), a plasma membrane glycoprotein expressed by neuronal progenitors[8-10], allowed for the labelling of newly generated granule cells and neural progenitor cells. Utilizing this labeling technique, scientists observed AHN in the dentate gyrus (DG) of rodents' hippocampal region, which supported Altman's proposal of hippocampal neurogenesis within rodent brains.

In 1994, an immunohistochemistry study was performed on the hippocampi of children with extrahippocampal seizures, to expand the study of hippocampal neurogenesis into human subjects[11]. In structurally non-atrophic brains of children under two years of age with epilepsy, PSA-NCAM-positive immature neurons were observed in the granule cell layer (GCL) and subgranular zone (SGZ) of their hippocampi[11]. Furthermore, early studies by Eriksson and colleagues used the BrdU-labeling technique to mark newly formed neurons within post-mortem hippocampi from adult cancer patients that colocalized with the neuronal marker NeuN[12]. This provided evidence for the presence of neurogenesis within the human hippocampus. Alternatively, through immunohistochemistry with stem cell markers and immature neuronal markers (INMs), different research groups demonstrated hippocampal neurogenesis in mammals[13,14].

After several years of research, the term "adult hippocampal neurogenesis (AHN)" was coined and refers to the constant generation of dentate granule cells from neural stem cells (NSCs) in the SGZ of the hippocampal dentate gyrus[15,16], a narrow band between the hilus and GCL with a highly distinct molecular profile containing doublecortin positive (DCX+) and PSA-NCAM+ cells[17]. These newborn NSCs are described as type 1 radial glia-like cells (RGLs), which go through several consecutive stages of development[18]. Proliferating intermediate progenitor cells (IPCs, type 2 cells) can form from RGLs, further differentiating into neuroblasts (type 3)[19,20]. Once they fully integrate into the GCL, they mature and become dentate granule neurons[21,22]. Each developmental stage corresponds to different neuronal markers such as GFAP, Sox2, and Nestin for RGLs; Ki67, MCM2, and PAX6 for IPCs; DCX, PSA-NCAM, and NeuroD for neuroblasts; and NeuN/Calretinin for young immature neurons[22-25].

Currently, AHN has drawn much attention and is widely studied in the field of neuroscience due to its role in hippocampal neural circuits involved in learning and memory[5,26]; regeneration of brain tissues[27,28]; and various diseases such as epilepsy[29], ischemia[30,31], Alzheimer's disease[32,33], and several psychiatric conditions[34]. Progress has also been made in terms of improving experimental techniques, such as using nuclear magnetic resonance spectroscopy to find neural progenitor cells and NSCs in the living human brain via their respective markers[35,36]. Integration of research on AHN suggests that the topic should be studied at the transcriptomic level using single-cell RNA sequencing and other transcriptomic methods[37-41].

Controversial evidence has been brought forward by Sorrells and colleagues that has put into question the existence of human AHN[42]. According to Sorrells et al., a sharp decline in hippocampal neurogenesis is observed during the infancy period, suggesting that neurogenesis in the dentate gyrus does not continue in adult humans[42]. Soon after this study, Boldrini and colleagues showed stable immature neuron pools and proliferating progenitor cells within human hippocampi throughout aging, whereas only quiescent stem cells decrease in number in aged human hippocampal dentate gyri[43]. Comparably, Moreno-Jiménez and colleagues applied improved immunohistochemical techniques to illustrate many DCX+ immature neurons in the human DG, which serves as evidence for the persistence of AHN across development in humans[44].

The exact reasons underlying the variating presence of AHN remains unclear since results can vary due to differences in techniques and specimens studied[44]. The processing of post-mortem brains may vary from study to

studied[44]. The processing of post-mortem brains may vary from study to study, as well as immunohistochemical aspects in terms of tissue preparation and procedure, and variations in antibody and probe utilization[45]. In this review, we will examine the history of and recent progress in understanding AHN in different experimental models. Moreover, we will discuss how AHN is implicated in major depressive disorder and especially in Alzheimer's disease, and the therapies targeting AHN, concluding with future goals for this topic of research.

Methods and Search Criteria

To conduct a thorough research of rodent and post-mortem brain tissue studies, we entered "Adult Human Hippocampal Neurogenesis" in Pubmed and Google Scholar and narrowed down the results to only highly cited research articles that employed immunohistochemistry (IHC) procedure. To ensure comprehensiveness, it should be noted that the search criteria for both Pubmed and Google Scholar includes all articles found on those search engines, regardless of their publication year. We also used AND to connect adult hippocampal neurogenesis with terms such as "dentate gyrus", "subgranular zone", and "neural stem cells" in our keyword search. We do not include niches such as AHN signaling pathways or AHN included as a subsection for research focusing predominantly on other topics. We limit our focus to studies mainly dissecting the existence or absence of AHN in rodents and humans but not non-human primates with complementary approaches besides IHC. For later sections on disease-induced changes in AHN and therapeutics, we conducted a more specialized systematic search with targeted terms involving "neurodegeneration", "Alzheimer's disease", "major depressive disorder", "treatment", and "therapeutics" to further discuss AHN and summarize findings for different disciplinary perspectives that suggest the presence or absence of neurogenesis rather than conclusive proof on the topics.

Adult Neurogenesis in Rodents

Most of the scholarly understanding of AHN comes from mouse studies performed in the past three decades, specifically in mice[22,38,46,47]. Besides the techniques using thymidine-H3 (2) and thymidine analogs[10] to confirm division and differentiation of NSCs inside the SGZ of DG, at least one other study applied similar methods to find the subventricular zone (SVZ) of lateral ventricles to constitute a specialized source of neuronal progenitor cells with lifelong neurogenesis[48]. Until now, many studies using a modern genetic manipulation technique with rodents consistently demonstrate that granule cell generation occurs within the SGZ of the adult DG in the hippocampus[46]. The adult NSCs harboured in the SGZ express glial fibrillary acidic protein as an astrocyte marker and have the characteristics of astrocytes[49] while possessing a radial glial cell morphology[50]. However, these markers do not distinguish NSCs from astrocytes and non-NSCs, which also express molecular markers like the glutamate-aspartate transporter[51]. Therefore, the field of AHN research continues to develop new techniques such as colocalized cellular markers to better distinguish NSCs from other cell lineages and to improve our appreciation of the mechanisms behind AHN.

Through the proliferation of intermediate progenitors or self-renewing progenitor cells, neurons are generated from NSCs; however, a majority of NSCs do not undergo active proliferation but remain in a quiescent state[52]. The activated NSCs divide into daughter cells which enter quiescence, self-renewal, or differentiation into neurons or glia over approximately 7 weeks in mice[25]. There are four phases of neurogenesis: (1) precursor cell activation/proliferation, (2) early survival, (3) early postmitotic maturation, and (4) late maturation[53]. At the precursor stage, activated, multipotent astrocyte-like quiescent NSCs divide asymmetrically to form both progenitor cells and NSCs[54].

Comparatively, Ascl1 and Prox1 expressions are reported in early proliferating intermediate progenitor cells whereas PSA-NCAM, NeuroD, and DCX expressions are observed in late proliferating intermediate progenitor cells[51,55]. The cell morphologies of neural progenitor cells differ from mature dentate granule cells: they remain as round or ovoid cells smaller than mature dentate granule neurons with short processes, and form clusters[24].

Progenitor cells with neuronal fate specification become newly formed neuroblasts which develop into immature dentate granule neurons via excitation by GABAergic input to promote neuronal differentiation[56]. Many newly generated cells experience cell death following division, and these cells are eliminated by apoptosis with 50% of BrdU-labeled cells remaining, thereby reducing the quantity of newly generated granule cells/neurons[57]. Two weeks later, the subpopulation of surviving newly generated immature neurons migrates horizontally in the SGZ to establish fusiform cells with horizontally oriented extensions[58]. Finally, the neurons migrate to the GCL and are incorporated into the hippocampal network where they extend their apical dendrites and develop axons[59].

Newly formed neurons displaying INMs undergo axon elongation, branched dendritic spine formation, and synapse formation during the maturation process[22,60,61]. The development of granular cells is first marked by strong expression of PSA-NCAM and DCX with limited syntactic contact with CA3 pyramidal cells[62], axon terminals, radial glial processes, and nonpyramidal cells[63]. A potent negative regulator of cell interactions is PSA-NCAM[64], which diminishes from half of the immature neurons to allow synaptic contacts during dendrite formation of mature dentate granule cells[24]. The late maturational stage is also characterized by switching calretinin expression to calbindin expression[65], accounting for the decreased excitability in developed granule cells eight weeks after generation[66,67]. This eight-week period is critical for new neurons to create glutamatergic synapses with a diminished propensity for long-term potentiation (LTP)[68], which mediates the synaptic plasticity necessary for hippocampal memory formation[69].

Promising evidence that AHN declines with age is consistently reported in mouse models [9,70-72]. A small portion of proliferating cells (Ki67+), INM+ cells, and BrdU-labelled cells persists across development as seen through experiments in aged rodents– implicating a decreasing rate of AHN in older rodents[71-73]. Blockage or genetic ablation of new neuron formation interferes with cognitive abilities, such as conditioned learning, emotional processing, and memory, which involves hippocampal circuitry and neuronal generation from AHN[74,75]. It is noted that environmental and behavioral cues such as exercise could enhance neurogenesis in rodents to improve cognitive performance[76]; accordingly, AHN is negatively impacted in high-stress conditions, such as depression and neurodegeneration[10,77,78]. AHN is a specialized process within the neurogenic niche, where interruptions of such a niche can result in cognitive impairment of learning and memory processes[79]. Mechanistically, AHN is a simple process; however, due to its translational characteristic, it has an essential role in disease formation and maintenance, as well as researchers' understanding of cognitive processes. Rodent models of AHN have paved the way for future analysis and provoked translational studies aiming at humans, mainly through the use of post-mortem hippocampal tissues. It is worth mentioning that non-human primates greatly contribute to the discussion of AHN; however, for feasibility reasons, they will not be covered in this review.

Adult Neurogenesis in Humans

The first endorsement of AHN stemmed from an immunohistochemical investigation in 1994, when Mathern and colleagues conducted PSA-NCAM IHC staining on non-atrophic brains of children with extrahippocampal seizures[11]. Neural storms manifest as massive surges in neural activity resulting in seizures and can cause seizure-induced neuronal damage or aberrant sprouting, which can impact the postnatal neurogenic development of the hippocampus[11,80,81]. Decreased quantities of immature neurons were detected in hippocampi of children with frequent seizures[11]. This further illustrated that severe epilepsy adversely affects processes involved with normal postnatal neurogenesis[11,80]. Mikkonen and colleagues obtained post-mortem hippocampi and the entorhinal cortex of patients with temporal lobe epilepsy to further scrutinize the immunoreactivity of

PSA-NCAM in comparison to specimens from autopsy controls without neurological diseases[81]. Likewise, a considerable number of PSA-NCAM+ cells in the hippocampal SGZ was reported in adult controls and patients with mild neuronal loss[81]. However, in epileptic patients with severe neuronal loss, PSA-NCAM+ cells in the SGZ are drastically undermined[81]. In summary, the initial investigations on post-mortem epileptic patients have yielded comparable results to Altman's research on rodents regarding existence of AHN in humans. These studies have uncovered that severe epilepsy can have a negative impact on normal neurogenic development of the hippocampus. Early studies on AHN using post-mortem epileptic patient brains have provided important guidance for future studies on AHN in the context of human tissues. These early studies have highlighted the potential impact of neurological diseases on the AHN process and have pioneeringly illustrated the intertwined relationship between AHN and disease. While much of the understanding of AHN comes from rodent studies, these findings suggest that continued investigation of AHN in human tissues may yield important insights into the mechanisms of neurogenesis and its role in the pathophysiology of neurological diseases.

Furthermore, Gu and colleagues offered evidence for postnatal neurogenesis by studying the distribution of Nestin immunoreactivity in human brain tissues[82]. They demonstrated that there are elevated concentrations of Nestin in SGZ cells, which have astrocyte-like morphology but are not double-labeled with astrocytic GFAP, suggesting the presence of neural stem cells or progenitors[82]. A methodologically unique study by Eriksson and colleagues utilized BrdU incorporation on post-mortem brains from adult cancer patients to estimate proliferating cells in the adult human hippocampal region[12]. Using immunofluorescent labeling for BrdU and colocalizing cells with neuronal markers including calbindin+ and NeuN+ cell bodies, new neurons in both the GCL of the DG and in the SVZ were illustrated[12]. Early IHC studies have provided evidence for postnatal neurogenesis in the adult human hippocampus through the distribution of a neuronal marker immunoreactivity, suggesting the presence of NSCs or progenitors and new neurons in the SGZ. However, there exist some inconsistencies in studies from the 1990s. One study that collected human brain tissues ranging from 7 months to 82 years old found that the maximal cell number of PSA-NCAM+ immature granule cells in GCL and SGZ exists during the first 3 years of life[83]. This level of PSA-NCAM+ immature granule cells is followed by a considerable decrease in PSA-NCAM+ cells from 3 years of age onwards, implying an age-dependent PSA-NCAM-mediated neuroplasticity with attenuation across the human lifespan[83]. This incongruent finding was not systematically examined in-depth, but certain factors such as individual differences in patients, sample conditions, and immunohistochemical methods could be contributing factors[84]. After a period of varying identification methods for neurogenesis in the SGZ, researchers investigating early adulthood agreed upon the existence of AHN in human hippocampi. Despite initial debates and inconsistencies, the consensus among early researchers was that AHN is present in the hippocampal SGZs, challenging long-held beliefs about the inability of the adult brains to generate new neurons.

As it became known that BrdU is toxic to humans, researchers turned to immunohistochemistry for more molecular markers. For example, an extensive IHC study from the Kempermann group mapped fourteen neurogenic markers associated with rodent AHN and evaluated DCX (a microtubule-associated protein found in differentiating neurons) co-expression in samples from the human hippocampus across the entire lifespan, ranging in age from 1 day to 100 years of age[85]. Their efforts illustrated the existence of DCX immunoreactivity in the GCL and SGZ of every sample across this age range, but an exponential decline in DCX+ cell density due to aging[85]. Furthermore, all fourteen neurogenesis-associated markers were detected in DCX+ cells and double-labeling confirmed the neuronal lineage of these cells, but colocalization with DCX decreased with age[85], consistent with other reports of qualitative and quantitative reductions in the DCX expression patterns due to aging in the SGZ[86-88]. Additional innovative measures were also applied for neurogenic marker detection, such as nuclear magnetic resonance spectroscopy[35,36]. Scientists used this spectroscopy for non-invasive identification of augmented biomarkers in neural progenitor cells and NSCs from living human brains, with the potential of quantification at different neurogenic stages[35,36]. One creative alternative approach was to use carbon-14 incorporation from nuclear bomb test-derived 14C in the genomic DNAs of human hippocampal neurons for cell turnover dynamics[89]. Using 14C incorporation data, they found occurrence of continuous AHN with an additional 700 newborn neurons to the hippocampus every day[89]. Furthermore, a sizable subpopulation of newly generated hippocampal neurons is subject to annual turnover with a rate of 1.75% that persists across the lifespan[89]. The total level of additional neurons formed in humans is debated, historically due to the difficulty in quantification and the controversy over the extent of postnatal neurogenesis. Nevertheless, modern research utilizing advanced techniques has provided compelling evidence to support the presence of AHN, which is heavily and negatively affected by aging in adults.

Although it is necessary to investigate a vast demographic range of post-mortem brains via different methodologies and from different perspectives, the proposal of persistent neurogenesis in the human SGZ was opposed by a study from Dennis and colleagues[90]. This group used IHC and immunofluorescent biomarkers on post-mortem brain tissues from adults and juveniles that had been fixed for 2-3 weeks[90]. They discovered a reduction in proliferating cells in neurogenic niches of early infancy–marked by a dramatic decline of Ki67+ proliferating cells in the DG or SGZ shortly after the first years of life[90]. Dennis and colleagues also described a drastic decline in DCX+ clusters over the age of three, and localized a sparse amount of Ki67+/DCX+ cells in the SGZs of juvenile and adult individuals[90]. They marked a low density of proliferating cells and neuroblasts in neurogenic niches[90], in conflict with earlier discoveries of continued neurogenesis in the human SGZ. Similarly, a study by Sorrells and colleagues, which collected intraoperative and post-mortem specimens of human hippocampi from fetal and postnatal subjects to adult patients with epilepsy, demonstrated a rich neurogenic niche in infants[42]. After a post-mortem interval of approximately 48 hours, hippocampal samples were fixed for less than 1 hour before they were sliced and stained using immunohistochemistry for twenty-two neurogenic markers and in-situ hybridization against a single marker, DCX[42]. They observed that the number of Ki67+/Sox1+ or Ki67+/Sox2+ dividing neural progenitors and DCX+/PSA-NCAM+ immature neurons in the DG intensely diminished in the first year of life with nearly undetectable levels of DCX+ PSA-NCAM+ newborn neurons for neurogenesis cells in the adult SGZ aged between 18 and 77 years old[42]. These results, especially ones by Sorrell et al. published in Nature, stirred considerable debate in the field of neuroscience since they provided evidence that disputed drastically against previous findings on the existence of AHN across ages[47,91,92]. They demonstrated a lack of evidence for persistent neurogenesis in the adult hippocampus and called into question the validity of previous studies that reported long-lasting neurogenesis in the human SGZ, indicating the need for further investigation and scrutiny in the field.

Interestingly, a separate study performed in the same year of 2018 also characterized DCX+/PSA-NCAM+ cells but found contradicting results utilizing hippocampi collected during autopsy[43]. Boldrini and colleagues set a narrow post-mortem interval of only 26 hours to prevent brain protein degradation, and conducted immunocytochemistry and immunofluorescence experiments targeting seven widely-used neurogenic biomarkers[43].They illustrated persistent and stable numbers of DCX+ PSA-NCAM+ proliferating neuronal progenitors and Ki67+/Nestin+ immature neurons in the SGZ of DG across the ages of [14–79], disregarding a smaller pool of quiescent stem cells in aged DG indicated by Sox2 and GFAP expression[43]. The authors interpreted the results as firmly suggesting a reduction in the pool of dormant stem cells and an enduring population of intermediate neural progenitors driving AHN in the SGZ[43]. Further immunohistochemical studies using improved tissue-processing methods supported the work by Boldrini and colleagues[44,84]. Based on experiments testing the influence of fixation time on the detection of markers of AHN in humans, Llorens-Martin's group limited their post-mortem interval to a shorter delay ranging from 2.5 to 10 hours and restricted their fixation time to 24 hours before applying their adapted slicing procedure, which minimizes tissue damage[44,84]. To ensure the specificity of the DCX+ signal, the authors assessed and selected the most specific antibody, a polyclonal goat anti-DCX antibody, and demonstrated the expression of several differentiated markers in a subset of DCX+ cells, including neuronal nuclei (NeuN), calretinin (CR), PSA-NCAM, calbindin (CB), and prospero homeobox 1 (Prox1)[44,84]. Using a well-defined immunofluorescence and optimized autofluorescence/background elimination approach on eight neurogenic markers, the researchers provided convincing evidence of fre-

quent AHN in humans by detecting thousands of DCX+ immature neurons with unambiguous neuronal morphologies in the DG up to the ninth decade of human life[44,84]. These findings reinforced the validity of their methodology, indicating the presence of mature and functional neurons originating from adult neurogenesis in the healthy human DG[44,84]. After conducting neuropathological assessments and screening subjects for cognitive impairment and Alzheimer's disease at different stages, the presence of persistent neurogenesis in the SGZ was observed throughout the tenth decade of life, as evidenced by the presence of DCX+ neuroblasts, immature neurons, and Nestin+/Sox2+/Ki67+ neural progenitor cells, in both elderly individuals and patients with Alzheimer's disease[44,93]. However, it ture neurons, and Nestin+/Sox2+/Ki67+ neural progenitor cells, in both elderly individuals and patients with Alzheimer's disease[44,93]. However, it was also noted that compared to healthy individuals, patients with Alzheimer's disease exhibited a progressive decline in the number and maturation of these neurons as the disease advanced[44,93]. Although the quantity of cells detected greatly varied between patients, and the proliferative capacity of DCX+ cells was somewhat inconsistent in findings[93,94], these studies effectively refuted prior research and established the persistent occurrence of AHN in humans across the lifespan, regardless of health status or age. They employed advanced immunofluorescence analyses and improved methodologies to provide strong evidence of the presence of immature neurons and neural progenitor cells in the SGZ. However, this also highlights the necessity of a standardized protocol to avoid inconsistencies and increase reliability in future studies. These findings provide additional implications for understanding the role of neurogenesis in cognitive function and aging. The relationship between neurogenesis and AD will be discussed in a later section to further explore the implications of the findings.

Many factors may have played a role in the discrepancies of these findings; the incapacity to identify pronounced emergence of newborn neurons in postnatal humans could be attributable to inconsistencies in methodologies for tissue fixation and storage of samples in long-term conditions84. By analyzing effects of post-mortem delay/interval; fixative use and its duration; antibodies used for immunohistochemistry; and other parameters, Llorens-Martin's group addressed the technical issue of fixation in which detection of immature neuron marker DCX will dramatically decrease if over-fixation of tissue occurs after a 24 hour fixation[44,84]. Regarding immunohistochemistry, Dennis and colleagues analyzed samples with post-mortem delay up to 90 hours[90], and Sorrells and colleagues included tissues with post-mortem delay up to 48 hours[42]. On the other hand, Boldrini and colleagues limited their post-mortem interval up to 26 hours[43], and Moreno-Jiménez and colleagues applied an even shorter delay fewer than 10 hours[44]. The change in delay could contribute to the absence of detection in the human DG considering degradation and undetectable DCX epitope after 24 hours post-mortem delay before fixation[42,84,90]. The use of a narrow post-mortem interval, as employed by Boldrini et al. and Moreno-Jiménez et al., may provide a more accurate representation of the extent of adult neurogenesis in human brains, as it prevents protein degradation and other factors that may affect the detection of neurogenic markers. However, while the tissue processing procedures used in the studies above may provide more scientifically sound evidence for the persistence of AHN in adult humans, the topic remains heavily debated and underscores the need for an agreed upon protocol to minimize conflicting results in future research.

Aside from immunohistochemistry, the manner by which different studies recognize the region of interest —SGZ—may also contribute to contradictory findings[42,45,85,90]. Based on DCX expression in the DG, Knoth and colleagues did not observe a sharp hilar border of the GCL as distinct as in rodents, and there was no apparent SGZ to be easily distinguished[85]. Similarly, Sorrell and colleagues described a less defined SGZ in fetal or juvenile brains regarding their Ki67+ cells and a coalesced region with isolated cells marking an absence of continuous SGZ in the cohort of brains[42]. By contrast, SGZ was defined by Moreno-Jiménez and colleagues as the part of the GCL closer to the hilus and having a thickness of one to two cells[44]. As not all literature clearly reported their quantitative or qualitative definition of the SGZ region, it would be difficult for researchers to remain on the same page and the opposite findings regarding the persistence of AHN may be attributed, in part, to the varying definitions of the SGZ locations. With the latest progress in single-cell genomic analysis, innovative technologies may riddle out the considerable debate on AHN presence and pave the way for future studies[37-41]. For instance, considering the heterogeneity of cell lineages within neurogenic niches[37-40], single cell-RNA sequencing can help map cell heterogeneity and gene expression associated with stem cell functions that have been previously observed in mice. This method has been applied in both the developing and adult human cerebral cortex[95-97], so researchers potentially can validate the optimal markers and antibodies for identifying cell subtypes in the SGZ of humans through single-cell RNA sequencing in the future.The extent to which AHN occurs in diseased versus healthy individuals can be further explored, while minimizing confounding variables and maximizing signal specificity for more congruous views[47,53,92].

Disease-induced changes in AHN & Therapeutics

Major Depressive Disorder & Antidepressants

Individuals with mood disorders, such as major depressive disorder (MDD) and bipolar disorder, exhibit altered hippocampal volume and circuitry[98-100]. With fluorescence-based immunohistochemistry, Walton and colleagues further characterized bipolar patients as having an "immature dentate gyrus," demarked by increased numbers of calretinin-positive immature neuronal progenitors compared to the mature DG marker calbindin[101]. While Walton's group found differences between the SGZ of bipolar patients and healthy controls, they were unable to detect any statistically significant immunohistochemical differences in calretinin, calbindin, or PCNA expression in MDD patients[101]. Comparatively, Reif and colleagues, using sections of the anterior hippocampus from bipolar and MDD patients for IHC, detected no change in Ki67+ cells between MDD patients and controls[102]. In both studies, the changes in proliferative capacity marked by Ki67 and PCNA expression in SGZ of bipolar patients and controls were considered non-significant[101,102]. Additionally, Lucassen and colleagues recognized a significant reduction in Mcm2+ cells but not in PH3+ cells from post-mortem brains of MDD patients[103]. They concluded that progenitors or putative stem cells decrease in number and proliferation is unchanged[103]. One factor that can account for the differing data in bipolar disorder and MDD is the phasic nature of mood disorders, which contributes to the inability to examine whether patients suffered from the active symptoms of the mood disorders at the time of death if no further case details are provided[104,105]. Based on the studies reviewed, it appears that there is no significant change in the proliferative capacity of NSCs within the SGZ in patients with MDD. These findings suggest that the reduced hippocampal volume observed in patients with MDD may be not due to a decrease in neurogenesis. However, it is important to note limitations including small sample sizes and differences in the methods used to measure neurogenesis. Further research is needed to fully understand the role of neurogenesis in the pathophysiology of MDD and its potential as a therapeutic target.

Another factor to take into account is the consumption of antidepressants and its effect on altering AHN in bipolar and MDD patients. Some studies found no correlation between the use of antidepressants and altered AHN[102,103]. In contrast, Boldrini and colleagues collected autopsy post-mortem samples and compared medication-free MDD, medicated MDD, and nonpsychiatric control subjects, with the medicated group separated by tricyclic antidepressant (TCA) or selective serotonin reuptake inhibitor (SSRI) perscriptions[106,107]. Through immunohistofluorescence for Nestin and Ki67, they observed increased progenitor cells (Nestin-immunoreactive) and proliferation (Ki67+) in medicated MDD patients. Also, differential effects of SSRIs and TCAs were observed: MDD subjects treated with SSRI (MDDT-SSRI) exhibited greater DG volume and a greater number of Nestin+ neural progenitor cells than MDDT-TCA subjects; whereas, MDDT-TCA subjects demonstrated significantly more Ki67+ proliferative cells than MDD-SSRI subjects[106,107]. One proposed mechanism is that these drugs enhance the expression of growth factors such as brain-derived neurotrophic factor (BDNF) which is known to promote neurogenesis[108,109]. Although Boldrini and colleagues observed increased neurogenic proliferation in medicated MDD patients, with distinct effects of SSRIs and TCAs on progenitor cells, the conflicting results, again, can be attributed to various factors such as individual differences in neuro-

anatomy and sample size differences[106,107]. For example, individuals with smaller hippocampal volumes may have a reduced capacity for neurogenesis, which could affect their response to antidepressant treatment[110]. Additionally, differences in the functional connectivity of the hippocampus with other brain regions, such as the prefrontal cortex, may also play a role in determining the effects of antidepressants on neurogenesis[111]. Furthermore, the double labeling of Ki67/Nestin is not adequate to delineate neural versus non-neural lineages or to extrapolate conclusions on neurogenesis. Regardless, the studies on neurogenic progenitors and proliferation provide valuable insight into the complex interplay between neural stem cells, their microenvironment, and the effect of various factors, such as medication, on the pathophysiology of depression. Specifically, they shed light on the mechanisms of adult neurogenesis in the hippocampus and how it may be altered in individuals with MDD. As the hippocampus is implicated in mood disorders, a more in-depth understanding of neurogenesis is necessary to develop more effective therapies and treatment. While AHN may play a role in MDD, it is important to keep in mind that depression is a complex multifaceted disorder with many factors at play beyond specific biological brain regions. Therefore, we should view AHN as just one piece of the puzzle, rather than a definitive explanation for the development or treatment of MDD.

Alzheimer's Disease & Neurodegeneration

Alzheimer's disease (AD) is a debilitating, relentlessly progressive neurodegenerative disease characterized by memory and cognitive impairments, affecting millions of people worldwide[112]. AD is often marked by early neuron loss and cell death in the hippocampus as a pathological feature, so SGZ neurogenesis may be impacted in AD progression and implicated for AD prevention[113]. However, early studies investigating AHN in AD have reported conflicting results. Some studies found increased neurogenesis in the SGZ of AD patients through heightened expression of AHN immunohistochemical markers. For example, Jin and colleagues observed elevated expression of NeuroD, PSA-NCAM, DCX, and TUC-4 (a protein expressed in early neuronal differentiation) in fourteen post-mortem AD brains based on double-label immunohistochemistry[32]. DCX, PSA-NCAM, and TUC4-positive cells also co-expressed cleaved caspase-8, suggesting that AHN is upregulated in AD brains to compensate for proliferating neuronal precursors that underwent caspase-dependent programmed cell death during degeneration[32]. Another study of progressive chronic neurodegeneration described an increased number of non-microglial Ki67+ cells and calretinin+ cells in ten AD SGZs, consistent with the previously proposed notion of increased neurogenesis during the progression of AD to counteract the effects of chronic neurodegeneration[114]. Contradiction arose in experiments by Lovell and colleagues that show lower viable Ki-67+/Musashi-1+ precursor cells isolated from three AD patient post-mortem tissue samples compared to NSCs isolated from healthy controls, and senescence was faster reached in AD cells compared with controls[115]. Later in 2006, a study using single IHC documented a reduction in the amount of progenitor cells in seven AD patients' SVZ regarding the decrease in Musashi-1 immunoreactivity, but an increase in GFAP-negative and Nestin+ astrocyte-like stem cells with progenitor characteristics[116]. It should be noted that earlier studies examining the relationship between AD and AHN are limited by sample availability and differences in methodologies due to the lack of advanced technology. Furthermore, differences in the stages of AD progression studied may also play a role in these conflicting findings. As the understanding of the complex interplay between AD and AHN continues to evolve, further research with standardized methodologies and larger sample sizes may provide more definitive insights.

Recent studies have consistently shown impaired SGZ neurogenesis in AD through a reduced number of cells positive for various neurogenic markers, such as GFAP, PH3, PSA-NCAM, Ki67, PCNA, DCX, Sox2, Nestin, Prox1, NeuN, βIII-tubulin, and calbindin[44,94]. Furthermore, Moreno-Jimenez and colleagues showed a persistence of SGZ neurogenesis until the tenth decade of life by evaluation of forty five patients with AD between 52 and 97 years of age[44]. However, they observed a tendency for DCX+ cells to have impaired maturation and decreased density compared to healthy aged subjects as AD advances, based on their analysis of immunohistochemical colocalization of DCX and the lineage markers listed to demonstrate deterioration[44]. A separate study assessed cognitive diagnosis to determine disease progression stages in AD patients and discerned a decreased quantity of DCX+PCNA+ cells in patients with mild cognitive impairments–illustrating an association between neurogenesis and cognitive status[93]. In addition, patients with AD exhibited lower counts of DCX+/PCNA+ neuroblasts with a significant drop in neurogenesis even at early stages of dementia development[93]. Terreros-Roncal and colleagues studied post-mortem human tissues from patients with neurodegenerative diseases such as Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and frontotemporal dementia[117]. They highlighted aberrant morphologies of DCX+ immature dentate granule cells and variations in the immunoreactivity of DGC differentiation biomarkers in these neurodegenerative diseases, suggestive of altered homeostasis of DG neurogenic niche functions and vulnerability of the AHN to neurodegeneration in humans[117]. These studies collectively demonstrate the dynamic nature of both AD and neurogenesis, as they show how the processes of neurogenesis and disease progression are closely intertwined and how the impairment of SGZ neurogenesis contributes to the progression of neurodegenerative AD. Furthermore, the studies emphasize the complex and evolving nature of both AD and neurogenesis, highlighting the need for ongoing investigation into their interplay.

Targeting AHN in Alzheimer's Disease

Our analysis of surface albedo numbers in the literature revealed that the atIt was established that therapeutics targeted at alleviating the neurodegenerative process were not successful[118]. Taking into consideration the negative correlation between AD progression and neurogenesis, improving neurogenesis has drawn attention as a new therapeutic target for AD[118]. One proposed approach to upregulating neurogenesis in the hippocampus proposed preventing microglial activation during neuroinflammation, with the anti-inflammatory drug minocycline[119-121]. Wadhwa and colleagues demonstrated that impaired neurogenesis can be improved via minocycline administration at different developmental stages: proliferation (more Ki-67+/BrdU+ DG cells), phases of differentiation (increased DCX+ cells) and growth factor (restored level of BDNF proteins)[119]. Comparably, the usage of retinoic acid derived from vitamin A can be a potential therapeutic to induce unspecialized stem cell differentiation and reinstate neurogenesis in AD patients[122]. The proposed mechanism is that retinoic acid, through direct activation of retinoid X receptors (RXRs) and retinoic acid receptors (RARs), impedes the pathogenesis of AD in mice by suppressing the release of pro-inflammatory cytokines and chemokines in glia cells, including astrocytes and microglia[122]. As an antioxidant, retinoid acid also attenuates Aβ plaque accumulation in APP/PS1 mice, an animal model for AD, while restoring spatial learning and memory deficits in treated mice[123].

As it was well-documented that excessive oxidative stress could inhibit neurogenesis in the SGZ of the hippocampus[124-126], researchers adopted the therapeutic potential of antioxidants to target reactive oxygen species (ROS) and alleviate pathogenesis of AD. Montiel and colleagues revealed that nerve-end lesioning and enhanced lipoperoxidation (LPO) elicited by Aβ administration in the hippocampus of rats was efficiently prevented with antioxidant α-tocopherol (vitamin E)[127]. Since vitamin E is known for its protective antioxidant effects against free radicals[128], another epidemiological prospective cohort study concluded that participants with diets abundant in vitamin E may carry a modestly lower long-term risk of AD and dementia[129]. Another vitamin with known antioxidant effects and feasibility to lower the risk of AD is vitamin C[130,131], which was also shown to attenuate Aβ oligomerization alongside lower cerebral oxidative damage and to restore behavioural deficits associated with AD progression in mouse models[132]. Another antioxidant implicated in AD therapeutics is curcumin: a curry spice found in turmeric with radical scavenging activity and anti-inflammatory activities[133,134]. In a transgenic mouse model of AD, low-dose curcumin significantly suppressed interleukin-1β (a proinflammatory cytokine), lowered oxidized proteins, and diminished soluble/insoluble amyloid accounting for the overall plaque burden, which suggests that curcumin contributes to AD prevention[135,136].

Exercise as an alternative and adjunct treatment has been illustrated to enhance cell proliferation and neurogenesis in DG of adult mice, where running doubled the quantity of surviving newborn cells[137]. Van Praag and colleagues reported improved LTP and spatial learning in exercised mice with more BrdU+ cell numbers, which signify elevated AHN in the hipp-

ocampal DG[138]. Other literature has connected the brain-derived neurotrophic factor (BDNF) to exercise-mediated neurogenesis[139,140]. A study using AD mouse models demonstrated that exacerbation of DG neuron loss and cognitive impairment could be induced by blocking AHN[140]. They noted that induction of neurogenesis alone showed no significant effect in improving cognition in AD mice[140]. However, simultaneous induction of neurogenesis and increased levels of BDNF mimics the effect of exercise-mediated AHN and could alleviate cognitive deficits observed in AD mice[140].

Therapeutic interventions that enhance neurogenesis in neurodegenerative diseases such as AD are a fruitful area of investigation but face many ongoing challenges due to the multifaceted nature of these diseases[141]. The translational limitations of animal models to humans and conflicting findings on the pathology of neurological disorders involving AHN can be frustrating in the context of treatments and interventions. Through increased insight on the topic of disease-induced changes on AHN and interventions targeting AHN, future therapeutic approaches can be devised in order to improve cognitive abilities in aging populations if AHN as a target is further explored. For example, a deeper understanding of the mechanisms involved in AHN and how they relate to cognitive decline in conditions such as Alzheimer's disease could lead to the development of targeted therapies aimed at enhancing neurogenesis and improving cognitive function. In addition, continued research into the relationship between AHN and other factors such as environment, diet, and stress could provide valuable insights into how lifestyle interventions may help to support healthy neurogenesis and promote cognitive health in aging populations. Ultimately, by gaining a better understanding of the complex interplay between AHN and cognitive function, researchers may be able to devise more effective strategies for promoting healthy brain aging and mitigating the effects of age-related cognitive decline.

Future Directions

In the past, most of our understanding of AHN came from rodents, and researchers agreed on the presence of AHN, which involves the proliferation and differentiation of neural stem cells into neurons through distinct phases of neurogenesis. While AHN is a simple process mechanistically, it plays an essential role in disease formation and maintenance, as well as in scholars' views of cognitive processes. Early findings from rodent models of AHN paved the way for translational studies aimed at humans, but discrepancies arise when studying post-mortem tissues in a manner similar to exploring mice. More recent studies have utilized molecular markers, such as DCX, to confirm the existence of AHN throughout the entire lifespan, but with a decline due to aging. Although the lack of standardized methodologies has led to reports of both absence and persistence of AHN; overall, modern research utilizing advanced techniques has provided strong support for the presence of AHN in the adult human hippocampus, which is negatively impacted by aging. The implications of AHN have been investigated in other fields, including mood disorders such as MDD, neurodegeneration such as AD, and various therapeutics. However, without a clear understanding of its fundamental existence, further research on these topics would only create more confusion and contradiction.

Presently, there is a pressing need for consensus on the presence of AHN in humans and a more coherent comprehension of defective AHN implicated in aged and diseased brains. This neuropharmacological field has fallen short in part due to technological hindrance and the shortage of post-mortem human tissues, but the emergence of a unified understanding of AHN is plausible in the future. Our current knowledge about how AHN works and how it interacts with the rest of the human brain is still quite limited. Our knowledge about the role of AHN in aging, neurodegeneration or psychiatric disorders is also impacted by heterogenous manipulation procedures. Recent development in transcriptomic methodologies, such as RNAscope and single cell-RNA sequencing, allows for precise collection of cell profiles from both human tissue and genetically altered disease-specific mouse strains, allowing for increased insight into disease mechanisms/consequences[142]. The highly sensitive in-situ hybridization method known as RNAscope permits multiplex detection for up to four target genes and ensures visualization of genes with low expression levels[143]. This allows for a more precise colocalization profile, which in turn facilitates the accurate identification of the cell type and potential quantification of mRNA levels in each cell. The RNAscope approach is particularly useful in unraveling the heterogeneity of neurogenic lineages in the SGZ. Although funding can be restrictive depending on the scope of the project, new approaches combined with the latest findings would improve our understanding of AHN in humans. Ultimately, this knowledge would help produce potential treatments for devastating diseases such as AD, dementia, and psychiatric illnesses.

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