

Workshop on

Neurogenesis and Aging

National Institute on Aging

March 16-17, 2020

Final May 27, 2020



This meeting summary was prepared by Bethany Stokes, Rose Li and Associates, Inc., under contract to Sigma Health Consulting and the National Institute on Aging. The views expressed in this document reflect individual opinions of the meeting participants and not necessarily those of Sigma Health Consulting or the National Institute on Aging. Review of earlier versions of this meeting summary by the following individuals is gratefully acknowledged: Maura Boldrini, Michael Bonaguidi, Dana Carluccio, Joseph Castellano, Amanda DiBattista, Ionut Dumitru, Rusty Gage, Shaoyu Ge, Rene Hen, Jenny Hsieh, Orly Lazarov, Mirjana Maletic-Savatic, Amar Sahay, Alejandro Schinder, Hongjun Song, Matt Sutterer, Kristyn Sylvia, Nancy Tuveesson, Saul Villeda, Molly Wagster, Ashley Webb, Brad Wise.

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Acronym Definitions

¹ HMRS	proton magnetic resonance imaging
ABLIM3	actin-binding LIM protein 3
AD	Alzheimer's disease
ATAC-Seq	assay for transposase-accessible chromatin using sequencing
β2M	beta 2 microglobulin
BDNF	brain-derived neurotrophic factor
BDZ	benzodiazepine
C ¹⁴	carbon 14
CA1	cornu ammonis 1
CA3	cornu ammonis 3
CCL11	C-C Motif Chemokine Ligand 11
Cis-MUFA	cis-monounsaturated fatty acid
CNS	central nervous system
CyPA	cyclophilin A
DCX	doublecortin
DG	dentate gyrus
DTI	diffusion tensor imaging
EE	environmental enrichment
EGF	epidermal growth factor
FAD	familial Alzheimer's disease
FFI	feed-forward inhibition
GABA	gamma aminobutyric acid
GCV	ganciclovir
Gpd11	glycosylphosphatidylinositol-specific phospholipase D1
GPCR	G protein-coupled receptor
Hopx	homeobox-only protein homeobox
HSC	hemopoietic stem cells
HNF4α	hepatocyte nuclear factor 4 alpha
IGF-1	insulin-like growth factor 1
KO	knockout
KI	knock-in
Klf9	kruppel-like factor 9
LEC	lateral entorhinal cortex
LRIG-1	Leucine-rich repeats and immunoglobulin-like domains protein 1
LTP	long-term potentiation
MADRS	Montgomery-Asberg Depression Rating Scale
MEA	multi-electrode array
MCI	mild cognitive impairment
MDD	major depressive disorder
MEC	medial entorhinal cortex
Mfge8	milk fat globule-EGF factor 8 protein

MS	mass spectrometry
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NIA	National Institute on Aging
NMDA	N-methyl-D-aspartate
NOR	novel object recognition
NSC	neural stem cell
OF	open field
PI3K	phosphatidylinositol-3 kinase
Prox1	Prospero-related homeobox 1
PSA-NCAM	polysialylated-neural cell adhesion molecule
qPCR	quantitative polymerase chain reaction
RNA-Seq	RNA sequencing
S1P	sphingosine-1-phosphate
Sox2	SRY (sex determining region Y)-box 2
SSRI	serotonin reuptake inhibitor
SST	somastatin
SVD	singular value decomposition
SVZ	subventricular zone
TIMP2	TIMP metalloproteinase inhibitor 2
TrkB	tropomyosin receptor kinase B
VEGFR2	vascular endothelial growth factor receptor 2

Executive Summary

The National Institute on Aging (NIA) held a virtual workshop on March 16-17, 2020, on Neurogenesis and Aging. Neurogenesis may contribute to many critical processes in the brain, such as memory formation, learning, and pattern separation, and this process is compromised during aging. Although many studies have assessed the impact of neurogenesis on aging and vice versa, the underlying mechanisms remain poorly understood.

The goal of this workshop was to review current findings, discuss knowledge and research gaps, and identify priorities for future research in neurogenesis and aging. The workshop was organized into three sessions: (1) neurogenesis in the adult human brain, (2) regulation of neurogenesis in the aging brain, and (3) functional significance of adult neurogenesis. A concluding discussion was also held to express final thoughts and identify addressable gaps and opportunities in the field.

The study of neurogenesis during aging and age-related neurodegenerative diseases requires characterization of the many cell types and factors involved. Because increased neurogenesis can be harmful under some conditions (e.g., epilepsy), further investigation is necessary to understand (1) the precise level of neurogenesis that provides benefits during normal physiological conditions, aging, and in brain diseases (e.g., Alzheimer's disease [AD]) and (2) whether the level of neurogenesis can be regulated through behavior or physiological intervention. Accurate characterization of neurogenesis in mouse models, non-human primates, and humans is essential to better understand the mechanisms underlying neurogenesis. Moreover, visualization of real-time neurogenesis processes is needed to move the field forward. Behavioral, genetic, and molecular interventions to regulate neurogenesis were discussed as strategies to improve cognitive outcomes during aging and in AD. Further assessment of how these interventions will translate to humans is required for wide-scale implementation.

Each session concluded with the identification of scientific gaps and opportunities to improve neurogenesis and aging research; two distinct topic areas emerged and are summarized below.

Mechanisms of Neurogenesis

- Impact of dysfunctional new neurons on the hippocampal circuit in aging and AD.
- Collective evaluation of the neurogenic niche (i.e., rather than separately assessing individual parts).
- Characterization of the neural stem cell niche in aging and AD (e.g., vascular, microglial, astrocytic, or peripheral factors).
- Identification of differences in neurogenesis between humans and model organisms.
- Exploration of both positive and potentially negative functional outcomes of newly integrated neurons in aging hippocampal circuits and in models of AD.
- Ideal balance of neurogenesis and mature neuron maintenance in aging and AD.

Experimental Tools and Resources

- New 'omics' or other markers for different stages of neurogenesis in the aging and AD brain, including those that distinguish between quiescent and active stem cells.
- Novel methods for tagging and monitoring newborn neurons, including molecular sensors, to study their incorporation into existing circuits that can be applied in aging and AD research.
- Standardization of tissue collection and processing procedures for studying neurogenesis.
- Live imaging approaches (i.e., magnetic resonance imaging, magnetic resonance spectroscopy, positron emission tomography, multimodal) to capture neurogenesis in humans and/or animal models to be applied for the study of both aging and AD.
- Multi-laboratory collaborations.

Meeting Summary

Introduction to Mammalian Adult Neurogenesis

Rusty Gage, PhD, The Salk Institute for Biological Studies

The field of neurogenesis, as well as many other neurological disciplines, originated from Smart and Leblond's studies in the 1960s, which developed the first method to label neural glial cells in the mouse brain through thymidine- H^3 injections. This approach facilitated many future studies, including work by Altman and Das, who reported the first histological observation of hippocampal neurogenesis in adult rats; Goldman and Nottebohm, who observed environmental-dependent cellular changes in the brains of songbirds; Greenough and colleagues, who found that granule cell maturation in the non-human primate dentate gyrus (DG) is significantly longer than those reported in rodent studies; and Ericksson and colleagues, who provided the first evidence for neurogenesis in humans.

The DG is one of the two main sites of neurogenesis. During the hippocampal neurogenic process, neural stem cells (NSCs) proliferate, undergo fate determination to become granule cells, migrate, and integrate by sending projections to the cornu ammonis 3 (CA3) subregion of the hippocampus. The subventricular zone is the other brain region that engages in neurogenesis, specifically in the generation of NSCs that migrate to the olfactory bulb to create synapses.

Neurogenesis is not a singular event, but a process that extends over approximately 8 weeks in the adult central nervous system (CNS) and that depends largely on the local environment in which the NSCs develop (i.e., NSCs within the DG become granule cells, while cells in the olfactory bulb and spinal cord do not). The process begins in the neurogenic niche, where NSCs are surrounded by glial cells, which secrete Wnt factors that play a role in neuronal fate instruction via NeuroD activity, and by DG vasculature. Once NSCs migrate from the niche, they begin to differentiate and integrate themselves within the inner layers of the dentate gyrus. Neurogenesis relies on the functioning of the hippocampal network, which is composed of synapses from the entorhinal cortex, cornu ammonis 1 (CA1), CA3, DG, and hilar interneurons. Neurogenesis is regulated by many neurotransmitters and neuropeptides, such as serotonin, dopamine, glutamate, and gamma aminobutyric acid (GABA), as well as environmental factors, such as exercise, environmental complexity, and stress.

Neurogenesis researchers rely heavily on the use of molecular markers that define each stage of the neurogenesis process (e.g., early neuroprogenitor cell markers, Sox 2 and nestin; and mature granule cell markers, NeuN and prospero-related homeobox [Prox1]). In addition, BrdU-labeling, retroviral-labeling, and transgenic mouse models are vital resources to study neurogenesis because these methods allow researchers to assess the age of specific cellular populations in vivo and in vitro in real time and over multiple timepoints. New methods, such as cellular carbon-dating and adeno-associated virus-mediated attenuation of neurogenesis via inverted terminal repeat sequences, are being integrated into the study of neurogenesis as well.

Gage and colleagues mapped the processes that regulate how newborn granule cells in the DG receive inputs from the cortex and how these cells eventually become integrated in the hippocampal network. Specifically, using a titanium implant in the mouse DG, Gage and colleagues performed two-photon microscopy to assess granule cell dendrite growth and integration over time, including overgrowth and pruning. They observed that all granule cells had similar numbers of dendrites by the final timepoint, suggesting the presence of a homeostatic mechanism to regulate the abundance of dendrites.

The majority of cells born in the adult DG are excitatory (and even hyperexcitable) granule cells, whereas those developed in the olfactory bulb are inhibitory; the behavior of these cells is believed to be environmentally dependent. Furthermore, Bischofberger, Song, and others observed a critical period for long-term potentiation hyperexcitability in newborn neurons followed by a period of increased inhibitory input during maturation, suggesting that hyperexcitability is critical for their ability to function in the circuit.

Neurogenesis can be regulated by many pathologies and behavioral interventions. For example, environmental enrichment (EE), exercise, and epilepsy increase neurogenesis, whereas stress, aging, Alzheimer's disease (AD), diabetes, and mitotic inhibitors reduce this process. Exercise, in particular, is the most commonly utilized behavioral intervention known to produce neurogenic benefits (e.g., increase the number of new neurons and learning and memory performance) even in aging mice.

Decades of neurogenesis research show that mature granule cells, new neurons, and NSCs play important roles in pattern separation, learning, memory formation, and brain plasticity. Translating findings from rodent and non-human primate models of neurogenesis to humans and developing the tools to study neurogenesis in living mice and humans in real-time are necessary to move the field of neurogenesis research forward. These steps will help identify ways to address age-dependent neurogenesis losses and to address other pathologies, such as anxiety and depression, that are causally linked to a loss in neurogenesis.

Session I: Neurogenesis in the Adult Human Brain

Adult Hippocampal Neurogenesis and Aging in Health and Disease

Maura Boldrini, MD, PhD, Columbia University

The ongoing debate surrounding the importance of neurogenesis has led many researchers to measure the levels of new cell growth during aging by characterizing cellular markers expressed during hippocampal neurogenesis. Quiescent neural progenitors express Sox2 and nestin; immediate progenitors express Ki-67, SRY (sex determining region Y)-box 2 (Sox2), and nestin; and immature granule neurons express doublecortin (DCX), NeuN, and polysialylated-neural cell adhesion molecule (PSA-NCAM). Using these markers, Boldrini and colleagues determined how the abundance of each cellular population (i.e., early and intermediate progenitors and immature neurons) changes with age and found that each cellular population was maintained in adults with normal aging, but not in adults with cognitive decline or psychiatric disease. This finding suggests the resilience of hippocampal neurogenesis in most adults.

Boldrini and colleagues also validated the timeline of marker expression by differentiating DG granule neurons in cell culture. They found that early neuron progenitors first express Sox2 and nestin; as the early neuron progenitors age into neuroblasts, they express TUJ1 and microtubule-associated protein 2 (MAP2); and once the neuroblasts become mature granule neurons, they express Prox1.

Using neuronal age-dependent markers and stereology (a method that quantifies the number of cells in a 3D structure based on the thickness and region of interest), Boldrini and colleagues mapped the molecular layer, granule cell layer and subgranular zone along the anterior–posterior axis. They found that the abundance of Sox 2/nestin⁺ cells and DCX/PSA-NCAM⁺ cells remained stable during aging in the anterior DG, and DCX⁺ cells (i.e., immature neurons) did not change in the mid or posterior DG. However, the number of Sox 2/nestin⁺ cells (i.e., early progenitors) and PSA-NCAM/NeuN⁺ cells (i.e., mature granule neurons) decreased in the posterior DG. Interestingly, DCX/NeuN⁺ cells were decreased in the DG of patients with AD (approximately 300–600 cells), compared to age-matched controls (6,000 cells).

In addition, Boldrini and colleagues found that quiescent neural progenitors colocalize with capillaries in the DG, suggesting that angiogenesis and neurogenesis may be interdependent processes within the DG niche. Specifically, Boldrini's research team found that reduced capillary area and capillaries with shorter and fewer branches are associated with fewer PSA-NCAM⁺ cells in the DG. Additionally, aging is associated with fewer vascular endothelial growth factor (VEGFR2)-expressing cells in the middle and anterior DG, suggesting that vascular plasticity may also affect neurogenesis. Kruppel-like factor 9 (Klf9), a regulator of dendritic spine development, is also reduced in aging adults without neuropsychiatric disease.

While aging affects neurogenesis, many drugs are also known to impact the process. For example, some stimulants (e.g., nicotine), opiates (e.g., hydrocodone), and psychoactive drugs (e.g., benzodiazepine [BDZ]) can negatively affect neurogenesis, while some antipsychotics (e.g., clozapine, olanzapine) and tricyclic antidepressants (e.g., agomelatine) can positively impact neurogenesis; other antipsychotics (e.g., haloperidol) and anesthetics (e.g., ketamine) can have no effect. Building on these effects, Boldrini and colleagues sought to study the impact of depression and treatment with selective serotonin reuptake inhibitors (SSRIs) and/or BDZ.

Boldrini and colleagues found that in response to BDZ treatment, mitotic cells remained consistent but neural progenitor cells were reduced. Previous studies by the Boldrini lab indicated that patients with depression have fewer neurons in the anterior and mid DG compared to controls. Moreover, Boldrini's research team found that patients more responsive to SSRI treatment for major depressive disorder (MDD) have more DCX⁺ cells in the DG, especially in the subgranular zone. Further, DCX⁺ cells are reduced in patients with untreated depression (largely in the anterior hippocampus) and in depressed subjects not responding to SSRI treatment. DCX mRNA levels in control and MDD patients responding to treatment, however, are similar. Further proteomic analysis revealed that patients with MDD (either untreated or treated) display specific expression profiles composed of critical proteins for neuronal cell maturation, and the expression level of these proteins correlate with the number

of cells found during different stages of differentiation in the neuronal maturation cascade (i.e., nestin, DCX, NeuN).

The hippocampus of individuals with cognitive decline (e.g., AD) and psychiatric disorders (e.g., MDD) have distinct cellular compositions. These differences suggest that both aging and angiogenesis are critical to the process of neurogenesis and require further investigation to elucidate the mechanisms mediating their interdependence (e.g., molecular regulators of neurovascular plasticity, glial and neuronal differentiation in disease).

The Role of Hippocampal Neurogenesis in Aging-Linked Cognitive Deficits and Alzheimer's Disease

Orly Lazarov, PhD, University of Illinois at Chicago

AD is the most prevalent type of dementia in the older adult population, with approximately 95 percent of affected individuals experiencing late-onset sporadic disease and 5 percent experiencing the genetic, early-onset disease (e.g., mutations in amyloid precursor protein [APP], or presenilin-1,2). Thus, familial AD (FAD) has well-established causation, whereas the causes of sporadic AD are not fully understood. Furthermore, researchers have had difficulty correlating the level of hallmark pathologies of AD (i.e., tau hyperphosphorylation, neurofibrillary tangles, and A β accumulation and deposition) with the degree of cognitive decline and memory loss commonly experienced.

To investigate the mechanisms by which sporadic AD progresses, Lazarov and colleagues used three pieces of information to drive their research: (1) newborn neurons facilitate memory formation, (2) neurogenesis is impaired in AD, and (3) hippocampal neurogenesis is critical to ameliorating memory loss. Lazarov and colleagues employed mouse models of FAD (i.e., *APP^{Swe}/PS1 Δ E9* and *5XFAD*), which display a reduction in neural progenitors and new neurons in the DG compared to wildtype (WT) mice and thus suggest an impairment of hippocampal neurogenesis. Transgenic mice that overexpress FAD-linked mutant APP or presenilin-1 show neurogenesis impairments as early as 4 months of age; however, overexpression models may be impacted by off-target transgenic effects. To address that, *APP^{NL-G-F}* knock-in mice were examined.

In collaboration with Rush University's Alzheimer's Disease Research Center, Lazarov and colleagues studied 18 postmortem brain samples from individuals ages 79–99 with a clinical diagnosis of normal cognitive functioning, mild cognitive impairment (MCI), or AD at time of death to assess neurogenic-relevant cellular changes during neurodegenerative disease states. Researchers detected both nestin/Sox 2 and nestin/Sox 2/Ki67⁺ cells, which represent both new and intermediate NSCs, respectively. High variability between individuals was noted but was not associated with individuals' clinical diagnoses. For example, some individuals without cognitive impairment unexpectedly displayed reduced neurogenesis, which may have been caused by the small cohort size.

Recent studies from Lazarov and colleagues of the role of NSCs in aging and neurogenesis indicate that the number of NSCs in aging individuals and in those with AD or MCI are

comparable, suggesting that cognitive status does not impact NSC abundance. However, neural progenitor cells are reduced in patients with AD and MCI, suggesting that neurogenesis is failing in these patients. Quantification of DCX⁺ cells in the subgranular and granular layers of the DG is substantially reduced in patients with AD and MCI, suggesting that the number of new neurons and neuroblasts produced is directly correlated with cognitive deficits (i.e., more new neurons leads to an improved clinical diagnosis). Further, detected neuroblasts colocalized with synaptic markers, such as SNAP-25, which suggests an additional association between clinical diagnosis and plasticity. Interestingly, AD and MCI patients' neurogenesis levels were not associated with multiple hallmark pathologies of AD (e.g., A β deposition, Braak stage, and fibrillary tangles).

Lazarov and colleagues hypothesize that augmenting neurogenesis will ameliorate memory deficits observed in AD. To investigate this theory, researchers crossed *Bax^{ff}-nestin-CreER^{T2}* and *5XFAD* mice, creating a mouse model to drive expression of nestin upon tamoxifen administration. In FAD mice without tamoxifen, DCX⁺ cells are reduced; however, after tamoxifen administration, the number of surviving DCX⁺ cells increase in both the control and FAD mouse models, suggesting an enhancement and maintenance of neurogenesis.

Lazarov's research team subsequently used contextual fear conditioning to investigate whether *Bax^{ff}-nestin-CreER^{T2}:5XFAD* mice displayed changes in memory processing. Although tamoxifen administration did not improve neurogenesis in control mice, episodic memory improved in FAD mice, suggesting that memory can be enhanced through neurogenic activity. Next, Lazarov and colleagues labeled neurons that help to encode engrams (i.e., unit of cognitive information in memory processing) and found that (1) the number of new neurons expressing NeuN is higher in the tamoxifen-inducible model of FAD compared to the uninduced and control mice and (2) this enhancement leads to an increase in new neurons, specifically new neurons expressing *Egr-1*, recruited to engram formation. To further understand the mechanisms regulating new neuron recruitment, memory formation, and AD, Lazarov and colleagues plan to investigate the role and transcriptomic signature of these new neurons within the engram.

Neurogenesis in the Postnatal and Adult Human Brain

Ionut Dumitru, PhD, Karolinska Institute

Aboveground nuclear bomb testing in the 1950s and 1960s led to an increase in carbon-14 (¹⁴C) levels in the atmosphere, which has since gradually decreased as it is absorbed by the biotope. Because the atoms in DNA molecules are not exchanged outside cell division and mature neurons do not divide, the concentration of ¹⁴C present in the neuronal DNA mirrors the ¹⁴C levels in the biotope and the atmosphere at the moment when the neurons were generated. Most neurons in the human CNS are born during embryonic development; to determine whether a neuron was generated after birth, the level of ¹⁴C in DNA is extracted and compared to the atmospheric ¹⁴C levels at the individual's time of birth. If the DNA ¹⁴C concentration mirrors the atmospheric levels experienced at birth, the neurons were generated during the embryonic development or shortly after birth. However, a ¹⁴C concentration in the genomic DNA that does not correspond to atmospheric levels at birth is an indication that the neurons must have been generated later in life.

The neurogenic brain regions shown to harbor adult neurogenesis in rodents are the subventricular zone (SVZ) of the lateral ventricles and the DG. Neuroblasts generated in SVZ migrate to the olfactory bulbs where they integrate and mature into adult neurons. To assess whether the neurons in the adult human olfactory bulbs present turnover, Frisé and colleagues assessed the ^{14}C in the DNA extracted from neuronal and non-neuronal cell populations in the olfactory bulbs. They found that human olfactory bulb neurons did not experience cellular turnover. However, after testing individuals ages 19–92, Frisé and colleagues found that neurogenesis is ongoing through life in the human hippocampus. Mathematical modeling of the ^{14}C data showed that the results are best described by a two-population model: one population of neurons undergoing constant turnover and a second population without turnover. The neuronal population showing ongoing turnover accounted for 35 percent of all the neurons in the hippocampus, which corresponds to the percentage of DG neurons. The mathematical model also finds that neurogenesis is ongoing throughout adult life in the hippocampus, with a minor decrease with aging, and indicated an average turnover of 1.75 percent per year at the level of DG neurons. Frisé and colleagues compared these new data with previously published histological quantifications of neuroblasts in the DG and found that both data sets indicate ongoing neurogenesis throughout the lifespan with similar rates of neuronal turnover. These findings established the foundation for studying cellular turnover rates in specific disease pathologies (e.g., depression, substance abuse disorder).

In addition to the observation that adult neurogenesis occurs in the human hippocampus, ^{14}C -dating also showed that adult neurogenesis occurs in the human striatum. The DARPP32⁺ medium-spiny neurons in the striatum do not turnover; however, ^{14}C data show that DARPP32⁻ striatal interneurons are constantly turning over in adult humans. Preliminary investigations regarding the anatomical subdivisions of the human striatum determined that adult neurogenesis takes place in the neuronal populations of the human nucleus accumbens, nucleus caudatus, and putamen.

After characterizing neurogenesis in the human hippocampus and striatum with ^{14}C -dating, Frisé and colleagues investigated whether neurogenesis occurs after a single-hemisphere neocortex stroke, using the unaffected contralateral corresponding regions as a control. In both the affected and the control cortical regions, turnover was observed in the glial population. However, no turnover was observed in the neuronal populations after neither acute nor chronic stroke. The ^{14}C values identified in the cortical regions affected by stroke corresponded to the control contralateral cortex regions and mirrored the atmospheric ^{14}C values at the birth of the individual.

To more closely investigate NSCs and progenitor populations in the human hippocampus, single nuclei RNA sequencing (RNA-Seq) was performed on tissue from children ages 0, 1, and 5 who died from sudden infant death syndrome or other non-neurological causes. Approximately 35,000 nuclei were analyzed, and small populations of neural stem cells, neuroblasts, and early neural progenitors were identified. Frisé and colleagues found increased levels of CALB2⁺ and EOMES⁺ in the neuroblasts and of TFAP2C and ASCL1 in the stem cell population. Neuroblast and NSC counts in samples from 0- and 1-year-old children were similar, whereas cell counts for

these populations decreased by approximately 10-fold—by age 5. Frisé and colleagues found that neuroblasts and NSCs were found in the 5-year-old human hippocampus at the rate of 2.5 to 3 nuclei per 10,000 sequenced nuclei. This low frequency explains why these cells were not identified in previous studies, in which less than 20,000 single cells/nuclei from the adult human hippocampus were analyzed. To mitigate these challenges in future experiments and increase the probability of identifying these cells, investigators must develop methods to enrich the progenitor population within their samples or sequence a higher number of cells.

Solving Human Neurogenesis in Vivo: Toward Better Understanding and Therapy of Brain Disorders

Mirjana Maletic-Savatic, MD, PhD, Baylor College of Medicine

Detection of molecular signatures associated with hippocampal neurogenesis could allow researchers to (1) monitor diseases associated with impaired neurogenesis in adult and aging brains, (2) perform targeted modulation of neurogenic processes, and (3) quantify the response of neuroprogenitor cells to external stimuli or therapeutic agents.

All proliferating cells depend on lipid metabolism and fatty acid synthesis to generate energy and cell membrane; hippocampal NSCs and progenitor cells are no different. Maletic-Savatic and colleagues used in vitro and ex vivo nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS) imaging, and in vivo proton magnetic resonance spectroscopy (^1H -MRS) to characterize, validate, and apply metabolome biomarker signatures, respectively, in an array of model systems (e.g., primary cell cultures, mouse models, and healthy and pathological human brain). They found that NSCs display a metabolomic signature distinct from neurons and glial cells, with an abundance of molecules resonating at 1.28 ppm on an NMR spectrum. Signals resonating at this frequency are associated with the backbone of fatty acids, and further analysis identified the signal's source as cis-monounsaturated fatty acid (cis-MUFA). Using MS, researchers then mapped the distribution of cis-MUFA in the mouse brain and found that it was particularly abundant in the neurogenic niche of the DG. The concentration of this MUFA increased following running, an activity known to stimulate neurogenesis. These findings led researchers to postulate that these particular molecules might be important for NSC biology; thus, they pursued further studies of the human brain toward detecting these molecules as biomarkers of neurogenesis.

To determine whether the neurogenic fatty acid signal is detectable in the live human hippocampus, Maletic-Savatic and colleagues used ^1H -MRS to assess many metabolites, but the neurogenic signal was embedded within a noisy spectral region and conclusions could not be reliably drawn. In collaboration with Li and colleagues at Shanghai Jiao Tong University, Maletic-Savatic produced an automated singular value decomposition (SVD) algorithm (SVD-Bank) to detect and quantify signals of low intensity, such as the neurogenic signal in the human hippocampus. Then, in collaboration with multiple groups all around the globe, they used SVD-Bank to analyze the neurogenic signal in the human brain. The signal was localized to the hippocampus, declined in an age-dependent manner, and correlated with performance on pattern separation tasks (e.g., Stark test), a known function of newborn neurons. The signal was

also diminished in adolescents diagnosed with the first depressive episode and responded to antidepressant treatment. Further investigations in collaboration with Valenzuela and colleagues at the University of Sydney, Australia determined that the neurogenic signal can predict both increases in hippocampal volume and favorable behavioral outcomes (e.g., Montgomery-Asberg Depression Rating Scale [MADRS] scores) in patients with medication-resistant depression treated with electroconvulsive therapy. These findings indicated that cis-MUFA could be a reliable biomarker for human hippocampal neurogenesis.

Maletic-Savatic and colleagues then investigated whether cis-MUFA can act as a surrogate biomarker of neurogenesis and whether it plays a biologically important role in NSCs. Fatty acids are critical for many processes throughout the human body. They are well-known signaling molecules and can bind to nuclear receptors, a large group of receptors that regulate metabolism, growth, circadian rhythm, and other processes. One such receptor, TLX, is expressed by adult mammalian neural stem and progenitor cells. To study MUFA's impact on TLX, researchers performed a variety of physico-chemical analyses and found that a single MUFA species can activate the receptor. Mice administered this ligand displayed increased proliferation of NSCs, and increased production of neuroblasts and mature granule cells at all ages. This effect was dependent on TLX activation, as TLX-knockout mouse models did not display MUFA-specific enhancement of neurogenesis. Therefore, researchers postulate that MUFA binds to the TLX receptor to activate cell cycle and neurogenic genes and enhance neurogenesis. These findings provide an informational foundation toward the development of small molecules to target NSCs and neurogenesis; specifically to prevent the decline of neurogenesis during aging, and to accelerate development of precision therapies for diseases with impaired neurogenesis, such as depression and AD.

Discussion of Session I

Moderator: Brad Wise, PhD, DN, NIA

Experimental Approaches, Tools, and Materials

Studies of neurogenesis and aging currently depend on the use of mouse models and human postmortem brain tissue. Several speakers noted that creating a live-imaging approach to identify and monitor neurogenesis that is translatable to humans would allow researchers to perform experiments with multiple timepoints—compared to the single timepoint possible in postmortem studies—and to study neurogenesis as it occurs in living adults. Additional timepoints may allow researchers to assess neurogenesis as a dynamic process and to characterize the process in stages. Discussion also highlighted that improvement of current imaging spectroscopy methods, coupled with development and incorporation of live imaging, could allow researchers to further characterize human neurogenesis. Participants suggested that the combination of MRS, MRI, and diffusion tensor imaging (DTI) technologies in humans may allow researchers to fully assess neurogenic processes, including niche factors, such as vasculature changes, that regulate neurogenesis. Several attendees emphasized that validation of imaging methods remains problematic. Comparative studies will be needed and pose considerable practical and conceptual challenges, for example, many imaging protocols used for humans cannot be easily translated back to rodents.

Finally, several speakers noted that most approaches currently used in the fields of neurogenesis and aging do not follow standard protocol guidelines, causing difficulty when comparing results across modalities. Standardizing the capabilities of each imaging and experimental approach to identify benefits and challenges would accelerate the field's ability to compare results and accurately display findings.

Models of Human Neurogenesis

Models such as rodents or non-human primates are vital resources that have provided critical insights into human processes, such as neurogenesis. However, these models are limited and may lack the ability to observe critical periods of human development. Participants hypothesize that the critical periods in, and protracted time of, development in humans (i.e., neoteny) compared to mice and rats suggest that other models should be explored. Participants proposed that the composition of the mouse's neurogenic niche may not be identical to that of humans and that the field should explore other model organisms.

Participants also observed that new molecular and cellular markers to identify different stages of neurogenesis (e.g., quiescent or active NSCs, cell migration and maturation) may be needed to visualize potential "missing" timepoints of neurogenesis or different cellular compositions in mice and humans. Live-imaging approaches (e.g., MRI, MRS, PET, multimodal) in humans could allow researchers to observe critical periods in human neurogenesis, and better neurogenic markers would capture and assess the cellular changes that may occur during those periods. Additional human cell-based models (e.g., organoids models) may be explored to study mechanisms of neurogenesis in humans and mice.

Tissue Standardization and Centralized Tissue Resources

Postmortem tissue is an irreplaceable resource to researchers in the neurogenesis and aging fields; however, several speakers noted that researchers have little control over tissue collection and processing methods. For example, many researchers analyze whole tissue samples (e.g., the entire hippocampus) because microdissection is not possible at tissue collection centers. The use of whole tissue samples prevents researchers from selectively assessing specific cellular populations. Attendees explained that tissue collection and processing methods should be used to answer specific questions, such as can single-cell transcriptomics define cell types involved in neurogenesis.

Many participants noted that developing and widely disseminating tissue collection and processing standards will reduce inconsistencies and create a uniform tissue pool. These standards will eliminate the possibility of poor communication regarding collection techniques between researchers and tissue collectors and will create a centralized resource for the field. In addition, standardizing or reporting the clinical assessments and medical history of each human tissue collected will improve the level of information available to researchers, resulting in enhanced validation of experimental results across studies.

Extent of Neurogenesis Required

Researchers across the fields of neurogenesis and aging have contemplated if either the number of new neurons produced or the connectivity of a limited set of neurons is important

for functional outcomes of neurogenesis in aging. Participants suggested that other neurological circuits, such as those involved in respiration and maintenance of circadian rhythm, are robust but rely on very few neurons. The production of new neurons is clearly important to hippocampal function. Several speakers agreed that further study may be required to understand the balance between new neuron formation and the functioning of the circuit with and without new neurons. But the question remains: how much neurogenesis is needed for a relevant clinical outcome? Computational approaches to understand how many new neurons and/or connections are needed to see functional outcomes in rodent models might be applied to human tissue to better grasp the dynamics of neurogenesis.

Session II: Regulation of Neurogenesis in the Aging Brain

Regulation of Neurogenesis in Aging and Disease

Michael Bonaguidi, PhD, University of Southern California, Los Angeles

Neurogenesis is negatively impacted by aging and many neurological pathologies (e.g., MCI, AD, epilepsy). Many studies have shown that neurogenesis can be restored and enhanced through therapeutic interventions in models of aging and neurological disease. Epilepsy is a pathology characterized by abnormal excitation of neural networks and can be caused by both genetic and environmental factors. Approximately 10 percent of the global population experiences spontaneous seizures, and about 1 percent will develop epilepsy. The most common form of the disease is mesial temporal lobe epilepsy (MTLE), which affects 60 percent of epilepsy patients, and most epilepsy patients are pharmacoresistant.

In mouse models of epilepsy, animals start to experience recurrent seizures at the same time as they experience enhanced and aberrant neurogenesis. However, as the seizures continue to occur, the NSC pool in the DG becomes depleted, neurogenic activity decreases, and mice begin to display memory deficits. These findings suggest that altered neurogenesis plays consequential roles in epilepsy and comorbidities within mouse models. Bonaguidi and colleagues employed a multi-pronged approach (i.e., histology, cell culture, and multi-electrode array [MEA] studies) in humans to (1) characterize adult neurogenesis in MTLE patients, (2) understand how cell proliferation is affected in MTLE progression, and (3) identify whether new born cells are impacting epileptiform activity.

Using histological methodologies on resected tissue from human epilepsy patients, Bonaguidi's research team found that DCX/Prox1⁺ cells appear both inside and outside the DG's granular layer, indicative of neurogenesis. Neurogenesis is observed in 50 percent of patients with epilepsy in Bonaguidi's study, and patients that display neurogenesis are typically younger (i.e., ≤40 years of age) and have shorter disease durations (i.e., patients who have lived with the disease for less time). Whereas neurogenesis decline is correlated with aging, this decline is more strongly associated with disease duration—neurogenesis is only evident in patients with a disease duration shorter than 20 years. Meanwhile, astrogenesis persisted in all investigated patients with MTLE up to 60 years. Neurogenesis and astrogenesis were also observed in cell culture derived from MTLE patients. Using MEA, Bonaguidi and colleagues isolated abnormal

neuronal discharges (i.e., interictal activity) between seizures in hippocampus *ex vivo*. They found that epileptiform activity in the DG was associated with granule cell activity, whereas a lack of hyperactivity in the DG occurred with higher rates of glial cell activity. Bonaguidi and colleagues' findings suggest that, in addition to neurogenesis, newborn astrocytes modulate human epileptiform activity.

Next, Bonaguidi and colleagues sought to elucidate the mechanism resulting in age-dependent loss of NSCs and neurogenesis. Mouse models provided evidence that the most dramatic loss of NSCs occurs between the ages of 3 to 6 months (i.e., when a mouse reaches maturity). Previous studies in the Bonaguidi laboratory have shown that the DG neurogenic niche contains NSC heterogeneity; NSCs typically differentiate quickly and generate neurons or a multipotent NSC that can generate further NSCs, astrocytes, and neurons. Single-cell lineage tracing of more than 1,500 NSC clones in the subgranular zone of tamoxifen-inducible mice resulted in the largest decline in nestin⁺ cells at ages 4 to 5 months, with well-maintained NSC populations from ages 2 to 4 months. These data suggest that a cellular mechanism is likely driving reduction in neurogenesis during this critical period.

The observed loss in NSC homeostasis during aging is caused by an imbalanced cellular differentiation and renewal rate. NSCs are depleted in a linear fashion during aging, whereas the NSC renewal rate expands linearly in young adult mice, but slows at 4 months, suggesting that a failure in NSC self-renewal is likely causing an NSC deficit. Bonaguidi and colleagues found that NSCs become more quiescent over time despite an age-associated increase in symmetric cellular division. Using single-cell RNA-Seq, researchers found that NSC populations increase quiescence and display many hallmarks of aging in mice aged 4.5 months compared to mice aged 2 months. These findings suggest that NSCs undergo molecular aging as they enter a deep quiescence as they age.

Using gene network analysis of single-cell RNA-Seq, Bonaguidi and colleagues identified *Abl1* as an NSC aging factor. *Abl1* transcripts in quiescent NSCs decrease with age, whereas *Abl1* protein increases by middle age. *Abl1* suppression using imatinib, a small molecule kinase inhibitor, in middle-aged mice leads to an acute increase in NSC activation. At later time points after imatinib treatment, NSCs cells become quiescent, but are not depleted in the DG niche, revealing an uncoupling of NSC activation and their differentiation.

NSC subpopulations asynchronously decline, and an increase in quiescence is the most likely mechanism for age-related NSC homeostasis loss. NSCs undergo molecular aging in the mature brain, and targeting molecular aging can partially restore NSC function. Further study is required to fully understand the mechanism that regulates loss of neurogenesis observed during aging and the differences between biological and chronological aging.

Enhanced Plasticity of New Neurons in the Aging Hippocampus

Alejandro Schinder, PhD, Fundación Instituto Leloir

Upon their generation, new granule cells integrate with glutamatergic and GABAergic synapses in a complex circuit, as observed in mouse models. In young adult mice, new granule cells will

(1) receive depolarizing dendritic GABAergic input for the first 14 days, (2) then receive dendritic glutamatergic input at day 21, and (3) finally receive hyperpolarizing perisomatic GABAergic input by day 28 post-generation. By 42 days, neurons are fully integrated within the synaptic circuit.

Neurogenesis can be impacted by many factors, such as the quantity and quality of NSCs with neurogenic capacity, rate of new neuron generation, modalities of development, level of integration within the circuit, neuronal survival, ability to process information, remodeling capacity, and behavior-induced regulation. Schinder and colleagues sought to understand these regulatory mechanisms in the context of the aging brain. They studied the maturation of new neurons in 2-month-old and 8-month-old mice for 56 days and found that neurons in 8-month-old mice did not mature as quickly and developed less dendritic spines than 2-month-old mice.

To explore how changes in behavior impact neurogenesis in middle-aged mice, Schinder and colleagues examined how exercise, which has a well-known association with improved neurogenesis, impacted granule cell morphology and density. They found that mice allowed to run developed new granule cells with longer dendrites and increased spine density, suggesting that running accelerates the formation of synaptic inputs in 8-month-old mice. They also hypothesized that dysfunctional neurotrophin signaling during aging may induce age-related dendritic phenotypes observed in new granule cells. Neurotrophin signaling is mediated by brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) activity, which is negatively regulated by leucine-rich repeats and immunoglobulin-like domains protein 1 (LRIG-1). Therefore, shRNA-mediated knockdown of LRIG-1 was used as a model of enhanced neurotrophin signaling. Researchers found that new granule cells expressing the shRNA for LRIG-1 displayed increased dendritic length, suggesting that enhanced neurotrophin function may counteract the effects of aging in new neurons. Conversely, LRIG-1 overexpression prevents the effects of running behavior on neuronal growth.

Schinder and colleagues also found that running-specific neurogenic effects (i.e., increase in the number of new neurons and faster integration of these neurons within the circuit) persist only while active, and effects decline when running ceases. EE, which includes supplemental toys and objects for exploration but not running wheels, promotes integration of new neurons into the brain's circuitry. Exposing mice to EE for 1 week was sufficient to improve dendrite growth in middle-aged mice. In addition, EE promotes maturation of mossy fiber boutons, which denote output capabilities of the synaptic connections. Neurons in mice not exposed to EE require approximately 70 days to develop the same level of connectivity as those exposed to EE for 21 days. In addition, EE neurons display significantly increased responsiveness to input activity compared to sedentary mice without EE. These findings suggest that EE induce rapid functional integration of new neurons in middle-aged mice.

To examine whether EE-induced integration of new neurons in the 8-month old mouse brain is also observed in the 2-month old mouse brain, Schinder and colleagues performed retrovirus labeling. They found that neurons required a very low threshold of EE (i.e., 2 days) to promote (1) rapid integration of neurons, (2) increased dendritic spine length, and (3) improved

responsiveness to input stimuli. These findings led to the hypothesis that activity within the granule cell layer is critical to the experience-mediated recruitment of developing granule cells. Schinder and colleagues tested the effects of GABAergic interneuron activity on developing granule cells and found that an overexpression of this activity increased dendritic integration of granule cells. Conversely, silencing of interneuron activity prevents EE-mediated granule cell integration. Therefore, interneuron activity is critical to integration of new neurons into the DG circuit in the adult mouse model, but further investigation is required to determine whether these effects are seen in young adult mice.

Embryonic Origin and Maintenance of Adult Neural Stem Cells

Hongjun Song, PhD, University of Pennsylvania

Decline in adult hippocampal neurogenesis is a hallmark of aging, and determining how adult NSCs are generated and maintained prior to aging is critical to understanding how modulation during aging could improve neurogenesis. To study neurogenesis prior to the onset of aging, Song and colleagues sought to characterize neurogenesis and to identify markers of this process in embryonic, neonatal and adult mouse models. Song and colleagues observed multiple cell types and their respective cellular markers using single-cell RNA-Seq in mice. Milk fat globule-EGF Factor 8 Protein (Mfge8) is a marker of quiescent adult NSCs, and its expression declines when the NSC is activated. Interestingly, deletion of Mfge8 in mouse NSCs induces their activation and subsequent depletion in cell culture, which is followed by an increase in astrocyte proliferation. Deletion of Mfge8 causes NSC activation and increased neurogenesis during early murine postnatal stages in vivo (i.e., by 15 days after birth) and leads to decreased neurogenesis in adult animals. These results suggest that Mfge8 maintains NSCs in a quiescent state during development but acts to preserve the NSC pool in the hippocampus during adulthood.

Further, previously the field of neurogenesis utilized two models of the embryonic origin of adult NSCs: (1) the “sequential” model, which states that neurons, glia, and adult-born neuroprogenitor cells are produced separately during embryonic, early postnatal, and adult stages, respectively, and (2) the “set-aside” model, which states that NSCs are created during embryonic stages but are deactivated and kept in a quiescent state until adulthood. Song and colleagues sought to address these models during their investigation of hippocampal neurogenesis during embryogenesis.

To investigate quiescent adult NSCs, Song and colleagues utilized the homeodomain-only protein homeobox (Hox) marker and the *Hox-CreER^{T2}* mouse line, which selectively labels quiescent NSCs. They found a significant NSC population within the DG, and Hox⁺ quiescent NSCs represent the majority of neuroprogenitor cells in the developing DG, contributing to both neurogenesis and gliogenesis. Song and colleagues labeled Hox⁺ NSCs to perform lineage tracing and found that E10.5 mouse embryo Hox⁺ cells give rise to radial glial cell-like NSCs that begin to migrate away from the ventricular zone toward the DG during E15.5 to E18.5. By E18.5, the majority of these cells have differentiated into neurons, but small portions of cells have become astrocytes and radial glial cells. Incorporating Edu-labeling in the *Hox-CreER^{T2}*

mouse allows researchers to assess whether NSCs are continuously generating from embryonic to postnatal stages. Conflicting with the “set-aside” model of embryonic NSCs, Song and colleagues found that Edu/Hopx⁺ neuroprogenitor cells are generated during embryonic stages and proliferation only increases after birth. These cells start to become quiescent during early postnatal stages (i.e., between P3 and P7), which is reminiscent of the quiescence acquired radial glial cells.

Together, these findings led Song and colleagues to propose the “continuous” model, whereby (1) Hopx⁺ neural precursor cells are generated in the dentate neuroepithelium during embryogenesis, (2) differentiation into DG neurons occurs during embryonic stages and into post-natal periods, (3) deactivation of NSCs begins in early postnatal stages, and (4) lineage specification occurs consistently throughout development. Further, RNA-Seq analysis of Hopx⁺ progenitors revealed a common epigenomic molecular signature: mRNAs involved in mRNA processing, DNA replication, and RNA splicing were enriched in the progenitor population. These cells also display distinct molecular changes when transitioning between each developmental stage. For example, embryonic progenitors express mRNAs relevant to lipid metabolism, angiogenesis, and hypoxia responsiveness, whereas up-regulated mRNAs are involved in oxidation-reduction processes, protein and ion transport, and metabolic processes and downregulated mRNAs are associated with translation, cell cycle processes, and RNA splicing in adults. Assay for Transposase-Accessible Chromatin using sequencing (ATAC-Seq) methods used by Song’s research team resulted in similar chromatin accessibility signatures for Hopx⁺ progenitors throughout development, suggesting conservation of these signatures.

Song and colleagues are applying these findings to human hippocampus-like organoids derived from pluripotent stem cells to model and assess human hippocampal neurogenesis. Transferring the human organoid into mouse models induces neuronal projection migration to the hippocampus. These models will allow researchers to study human hippocampal neurogenesis through marker staining, lineage tracing, and sequencing of the resulting cellular connections and products. In addition, Song and colleagues are performing single-nucleus RNA-Seq of human dentate gyrus across different stages to identify the molecular landscape of newborn dentate granule neurons in humans.

Blood-borne Regulators of the Hippocampal Neurogenic Niche

Joseph Castellano, PhD, Icahn School of Medicine at Mount Sinai

Aging is the strongest risk factor for AD and other neurological disorders and results in declines in cognitive, cellular, and molecular domains of the brain. With the aged population growing dramatically, understanding how critical processes are impacted by aging will provide the potential for researchers and clinicians to modulate the effects of aging with therapeutic interventions.

To mitigate declines in cognitive, cellular, and molecular function in the brain, Castellano and colleagues seek to leverage blood-borne factors related to aging. Previous studies have established that communication between blood and organ systems occurs and is altered during aging. Specifically, Rando and colleagues found through use of the parabiosis model (i.e., the

vasculature systems of two mice are connected and shared) that skeletal muscle of an older aged mouse is rejuvenated by exposure to blood from a younger mouse; similar findings in other organ systems, including the brain, have been found using the parabiosis model. Heterochronic parabiosis describes a mouse model in which blood is shared between a young mouse and an older mouse, whereas isochronic parabiosis involves the connection of two mice of the same age.

Using the heterochronic parabiosis model system to assess cell proliferation and regeneration, Rando and colleagues found that, when exposed to younger blood, older mice displayed increased hepatocyte proliferation, and cultured skeletal muscle satellite cells from the older mice displayed regenerative capabilities (i.e., they appeared to be returned to a youthful state). While in the Wyss-Coray laboratory, Villeda and colleagues observed that older mice in the heterochronic model also display increased neurogenesis. The neurogenic effect of young blood is not limited to the subgranular zone niche; this effect was also found by the Rubin lab in the subventricular zone (i.e., the location in which neurovasculature was also rejuvenated by young blood). Conversely, Villeda and colleagues found that C-C Motif Chemokine Ligand 11 (CCL11) and $\beta 2$ microglobulin ($\beta 2M$) act as pro-aging, anti-neurogenic factors (i.e., promote the aging process).

In assessing the specific effects on synaptic plasticity, Villeda and colleagues found that exposure to young blood increases long-term potentiation (LTP) of synapses in the aged hippocampus, and through these improvements, mice show improved learning and memory task performance. Further studies indicate that older mice given young plasma perform fear-conditioning tasks well compared to control-treated old mice, whereas older mice given young heat-denatured blood do not exhibit plasma-mediated rejuvenation. These results suggest that the effects of young blood on cognition in aged mice are likely protein-mediated.

To investigate which proteins in the blood influence cognition, Castellano and colleagues performed proteomics analyses of young and aged mouse and human plasma. They found distinct age-dependent expression patterns in plasma of both species, and relative tissue inhibitor of metalloproteinase inhibitor 2 (TIMP2) levels were higher early in life compared to later. Supplementing older mice with recombinant TIMP2 over 2 weeks led to a dramatic improvement in learning and memory functioning; and aged mice given TIMP2-depleted plasma do not exhibit plasma-induced cognitive improvements.

Because TIMP2 is depleted during aging, most importantly in the hippocampus, Castellano's research team sought to investigate whether loss of TIMP2 influences brain function. They found that, on average, *TIMP2*^{KO} mice performed worse than their matched controls in multiple cognitive performance tasks. However, TIMP2 deletions do not impact working memory or motor function. Interestingly, *TIMP2*^{KO} mice display an impaired coordination phenotype, validating the high expression of TIMP2 in the mouse cerebellum. Results from a protein microarray analysis of plasma from *TIMP2*^{KO} mice indicate that TIMP2 regulates many extracellular matrix- and neurogenesis-related blood-borne proteins, suggesting that neurogenesis is impacted by the deletion of TIMP2.

To further investigate this hypothesis, Castellano and colleagues examined BrdU-labeled cells in the DG of *TIMP2^{KO}* mice and found reduced numbers of proliferating cells in the subgranular zone and reduced neural progenitor cell numbers. In addition, these mice appear to display increased microglia and astrocytes in the DG; however, ongoing work in the lab will clarify whether microglia and astrocytes increased in number or changed morphologically and the extent to which cellular communication within the neurogenic microenvironment plays a role in TIMP2's regulation of these phenotypes.

These findings suggest that blood-borne factors (e.g., TIMP2) heavily regulate aging-mediated effects in the neurogenic niche, as well as synaptic plasticity and cognitive function. Further investigations into the aspects of neurogenesis that respond to these blood-borne factors and how to take advantage of these potential neurogenic benefits of circulating blood-borne factors is necessary.

Transcriptional and Epigenetic Regulation of Neural Stem Cell Quiescence and Activation

Ashley Webb, PhD, Brown University

The process of quiescent NSC activation is poorly understood. During aging, these cells typically become more quiescent and less active. Quiescent NSCs proliferate slowly, contain low RNA content and low rates of protein synthesis, slow autophagic processes, and employ less oxidative metabolism, whereas all activated NSC processes are the opposite and therefore lead to increased neurogenesis. Understanding the increased difficulty in activating quiescent cells during aging is critical to determining potential ways to increase cell activation through intervention.

As quiescent NSCs undergo activation, they upregulate their mitochondrial content, rapidly turnover lysosomes, and alter their transcriptome. Webb and colleagues hypothesized that the transcriptional remodeling during activation is mediated by chromatin alterations and epigenetic regulation. To investigate this hypothesis, they performed cell culture experiments in which primary NSCs were treated with BMP4, which promotes NSC cell cycle exit. They found that the presence of this transcriptional factor induced a reversible state of quiescence that could be removed by the presence of epidermal growth factor (EGF). Further, using ATAC-Seq, they assessed chromatin accessibility in activated and quiescent NSC and found that distinct signatures of dynamic chromatin accessibility were associated with each NSC state. Specifically, highly dynamic regions of chromatin were associated with enhancers, which actively bind key transcriptional factors to regulate gene expression. Webb and colleagues also analyzed functional signatures of these enhancer regions and found that dynamic chromatin-associated enhancers are associated with neurogenic genes (i.e., genes involved in axonal guidance, CREB signaling, and LTP). The stable and constitutively accessible chromatin sites that do not change during NSC activation are associated with basal homeostasis processes (i.e., translation, proliferation, metabolism, and stress responses).

Webb and colleagues then sought to identify specific transcriptional regulators of chromatin accessibility during the NSC activation or inactivation process. The insulin/insulin-like growth factor 1 (IGF-1)/phosphatidylinositol-3 kinase (PI3K)/AKT pathway induces downstream regulation through FOXO proteins, which are critical to NSC homeostasis. FOXO3 is highly expressed in quiescent NSCs, compared to activated NSCs. Webb and colleagues performed RNA-Seq on quiescent and activated NSCs isolated from adult and aged mice with and without FOXO3 deletions. Activated WT NSCs and quiescent FOXO3^{-/-} NSCs from aged mice are enriched with oxidative phosphorylation genes, suggesting that FOXO3 maintains NSCs in a quiescent state through suppression of pathways that upregulate mitochondrial and ribosomal genes. Further, researchers found that a loss of FOXO3 increases mitochondrial content and increases quiescent NSC activation during aging. Interestingly, the loss of FOXO3 appears to upregulate activation-specific markers, such as ASCL1, yet it does not downregulate markers of NSC quiescence, suggesting that another signal is required to fully activate NSCs out of quiescence. Consistent with these findings, mice with active FOXO3 activity appear to reduce NSC activation through repression of ASCL1 activity.

Together, these findings illustrate the dynamic cellular, epigenetic, and transcriptional changes that occur during NSC activation. The genomic sites under dynamic regulation according to the activation state are largely occupied by critical regulators of neurogenesis. These sites are impacted by key transcriptional factors, such as FOXO3, which actively maintains the metabolic stability of quiescent NSCs during aging in vivo.

Adult Neurogenesis and the Neurobiology of Individuality

Gerd Kempermann, MD, German Center for Neurodegenerative Diseases

Neurogenesis contributes to individualization of the brain. Some individuals display more resilience than others during aging, which led Stern and colleagues to hypothesize the presence of a cognitive brain reserve. Kempermann and colleagues chose to study the impact of neurogenesis on individuality through the EE paradigm. The classic model of EE postulates that phenotypes are the product of genetics and environment. Through the use of mouse models, researchers can maintain a relatively constant genome and assess the direct impact of EE on phenotype. The same approach can, however, also be used to study whether EE impacts variance of phenotypes and, hence, individuality.

The use of EE and subsequent behavior analyses allow researchers to understand the direct impact of environment on mammalian cognitive functioning. On average, rodents exposed to EE travel longer distances during the first trial of the open-field (OF) locomotion tasks; however, upon the second trial, they travel less because of habituation. During the novel object recognition (NOR) task, EE-exposed mice display higher variance in the duration of object exploration compared to control mice, suggesting that EE mediates inter-individual variability. Similarly, variance also increased during rotarod tasks in mice exposed to EE. Most importantly, however, EE-mediated performance of the NOR tasks correlated positively with roaming entropy, a longitudinal measure of exploratory behavior, recorded over the experimental period of 3 to 6 months.

In an unpublished pilot study, Kempermann and colleagues analyzed volume changes in multiple regions of the mouse brain before and after EE. The DG increased significantly in volume, whereas cortical regions, such as the cingulate cortex and motor cortex, remained the same after EE. These findings are in some apparent contrast to previous findings by Diamond and colleagues, who had suggested that linear measures of cortical changes (i.e., cortical thickness) are evident after EE. Kempermann and colleagues also observed systemic responses to EE, including moderate decreases in liver and adrenal gland weight and triglyceride cholesterol levels. Further, associations among behavior, neurogenesis phenotypes, and bodily phenotypes in EE-exposed mice differ from those in control mice, which may be due to the individualizing capabilities of the brain.

EE induces increased adult hippocampal neurogenesis. Adult neurogenesis and the survival of new neurons, both in terms of mean differences and variance were greater in mice exposed to EE compared to those in control environments, further suggesting that EE can induce individuality as adult neurogenesis increases. These effects may be mediated through a feedback loop in which the plasticity of the DG is altered by learning and the behavioral experiences resulting from EE, which contribute to individualized hippocampal function. Kempermann and colleagues tested this theory by assessing inbred animals (i.e., controlled genetics) in stable shared environments and variable non-shared environments. Overall activity decreased, yet variance between EE-exposed animals was significantly increased, suggesting that genetically similar animals displayed very different behavior within the multi-cage system. These behavioral trajectories correlate with individual levels of adult neurogenesis, which is at least partially a potential result of roaming entropy differences.

Kempermann and colleagues then performed an experiment in which mice were either exposed to EE for 6 months or exposed to EE for 3 months and then a control environment (i.e., the withdrawal group). Mice in the withdrawal group exhibited increased variance in behavior during the first 3 months that persisted during their remaining time in the control environment; however, neurogenic activity did not. Nevertheless, behavioral trajectories of the withdrawal group were associated with stable individualized differences in adult neurogenesis, originating from the first 3 months with EE. The EE-exposed group displayed the highest level of inter-individual variance in behavior, although the withdrawal group displayed significant variance as well. Interestingly, EE-induced methylation is sustained in the withdrawal group, suggesting that EE incurs long-lasting effects on plasticity-related epigenetics in the hippocampus.

Neurogenesis must be studied as the sum of many individual variables within an organism's environment, behavior, connectivity, and cellular composition to accurately identify the phenotypic differences between two genetically similar organisms. Addressing these differences can be challenging because of the interconnectivity of the mechanisms involved in neurogenesis. Collectively, Kempermann's findings provide evidence that adult neurogenesis is an individualizing trait, and incorporating this concept into future studies will inform understanding of individualism during aging.

Discussion of Session II

Moderator: Amanda DiBattista, PhD, DN, NIA

Research Needs to Assess the Whole Niche

Participants indicated that improved imaging modalities and approaches to better assess neurogenesis and the vascular components of the neurogenic niche could greatly improve researchers' ability to view neurogenesis as a complete process rather than the sum of its parts. The neurogenic niche is composed of many cells, including neurons, NSCs, glial cells, and blood vessels; studying these cellular populations in isolation will not provide an all-encompassing view of neurogenesis and, therefore, studying these populations in a dynamic, connected model may be advantageous. A key subject that remains a challenge is to understand the balance between stem cell quiescence and activation, including its regulation by extrinsic factors. Single-cell RNA sequencing data suggest substantial heterogeneity of niche populations and the necessary refinement of conventional, linear and unidirectional concepts.

Participants stressed the need for new live imaging technologies to detect various cellular mechanisms related to neurogenesis, including the interaction of neural stem cells and the blood. These technologies could allow researchers to observe interactions in real time, which is currently not possible in humans.

Further, microglia are known to have both pro-neurogenic and anti-neurogenic functions, pointing toward a potential key function in niche control. Another consideration is that these cellular populations within the hippocampal neurogenic niche may not age at the same rate, which may cause a host of pathological challenges. For example, as inflammation develops, the neurogenic niche is altered. Speakers noted that additional imaging capabilities that allow researchers to assess the human brain in vivo, and to compare the younger and older human brain, could contribute to better understanding of these processes.

Most methodologies to assess individual cells involved in neurogenesis require sequencing many cells to cover cells of interest in human tissues. While single-cell RNA sequencing and methylomics approaches could provide important data, a limitation to consider is that the small number of cells undergoing neurogenesis in the adult brain means the number of cells sampled must be high. Participants commented that creation or use of novel alternate methods (e.g., spatial transcriptomics) that can accurately assess neurogenesis' transcriptomic signatures with fewer cells could facilitate more efficient studies.

Mouse Models

Participants noted that models of sporadic AD may be needed to further characterize neurogenesis. Most mouse models of AD are representative of FAD rather than the more common sporadic AD. Consequently, sporadic AD pathology is poorly understood in humans. In addition, hallmark pathologies of AD (i.e., tau and A β seeding) are difficult to recapitulate in mouse models because the pathology is wholly dependent on the location where seeding is initiated. Mouse models of seeding AD pathologies have been used to define cell mechanisms underlying AD, and (with further study) may become more reliable and comparable to human pathologies. Several speakers noted that human brain organoids generated from induced

pluripotent stem cells derived from patients with AD and matched controls could provide another approach.

Importantly, older mice appear to show a gradual decline in neurogenesis compared to younger mice. This is paralleled by cognitive decline. Because age is the greatest risk for AD, participants noted that age-related decline in neurogenesis is an area of opportunity for further exploration to complement the AD genetic models.

Mechanisms of Neurogenesis in Aging and Disease

Neurogenesis involves many interconnected mechanisms that lead to the production and maturation of granule cells. This process is influenced by aging; however, the effect may not be linear, but rather a complex web of feedback loops. Some processes could be asynchronous, but others may occur in concert. While deficits associated with aging may be caused by defects in the neurogenic niche, aging itself may also cause a decline in neurogenesis. Several speakers highlighted that the mechanism leading to the decline or maintenance of the niche during aging is not fully understood. Similarly, neurogenic behavior depends on the circuitry and resources in the hippocampus, and changes experienced during aging impact those resources. Dissecting age-related effects on each cell type and circuit within the hippocampus may allow researchers to understand the limiting factors associated with neurogenesis and aging to identify where challenges are most likely to arise.

Regardless of the exact contribution of neurogenesis to AD, participants noted that the scientists have an opportunity to understand whether stimulating neurogenesis improves functional deficits observed in AD models. Although, neuroinflammation is observed in early stages of AD, speakers indicated that further study on the specific cell types impacted by inflammation and how these cell types interact with one another may be needed to fully characterize the relationship between inflammation and neurogenesis.

Session III: Functional Significance of Adult Neurogenesis

The Function of New Neurons in Adult and Aging Hippocampal Neural Circuits

Shaoyu Ge, PhD, SUNY Stony Brook

New neurons are necessary for functional adult hippocampal circuitry, but the integration of new neurons into the circuit becomes challenging during aging. Ge and colleagues have observed and visualized the competitive selection of new neurons in the layers of the DG through retroviral labeling and endoscope implantation in live mice. Ge's research team studied this functioning by examining neurogenesis over the course of 3 to 26 days after injecting mice with retroviral labels. By day 7, they observed new neurons migrating into the DG cellular layer; by day 14, only a few new neurons had successfully integrated and no others survived. The newly integrated neurons displayed high excitability and rejuvenation capabilities, indicated by improved performance in contextual freezing tasks.

Ge and colleagues then designed an experiment to investigate the inhibitory circuit bias within the hippocampus. Researchers implanted a tetrode into the DG of a living mouse to record

cellular behavior in control and EE states and found individual populations of granule cells and interneurons in both control and EE-exposed mice. In the EE condition, granule cells decreased in activity but interneurons increased. These changes correlate with a decrease in excitatory activity and an increase in inhibitory activity, suggesting that the DG becomes more inhibitory when the animal is free to explore a novel and enriching environment. This bias toward inhibitory activity also produces excess energy, which is sufficient to produce new neurons in the DG.

During aging, interneuron-mediated inhibitory activity is higher than granule cell-mediated excitatory activity; however, the inhibitory firing rate in aged mice is significantly lower than in young mice, and excitatory firing is significantly higher in aged mice compared to young mice. These results suggest that the aged circuit becomes hostile toward excitatory neurons as aging progresses.

Metabolomic analysis revealed that lipid metabolism and associated metabolites are regulated by EE. For example, sphingosine-1-phosphate (S1P) depolarizes interneurons upon its phosphorylation and has no effect on DG neurons. Immunocytochemistry staining of S1P's receptor 2 (S1PR2) revealed that the presence of S1PR2 is required for inhibitory activity in interneurons. The mice with *S1PR2* knockdown do not display the EE-mediated spike in inhibitory activity; interestingly, the EE-mediated decrease in excitatory activity is also abolished. The mice with *S1PR2* knockdown have reduced EE-induced neurogenesis, suggesting that S1PR2 is required for behavior-induced survival of new neurons. These findings led Ge and colleagues to produce a computational model incorporating the ratio of excitatory to inhibitory activity before and after the experimental condition (i.e., abolished S1PR2 activity) that predicts the level of neurogenesis, which was reduced. This model can be used to predict other facets of neurogenesis as well (e.g., impact of epileptic seizures and aging).

Ge and colleagues have begun to investigate neurovascular coupling in the adult hippocampus and will soon assess these processes in a model of aging to more fully understand the neurogenic niche in the DG.

Blood: At the Interface of Aging and Adult Neurogenesis

Saul Villeda, PhD, University of California, San Francisco

The main hallmarks of aging are well established, but whether and how these hallmarks can be reversed through molecular- or drug-mediated intervention is not known. Villeda and colleagues seek to investigate whether two simultaneously occurring hallmarks—cognitive decline and neurodegenerative disease—can be delayed during the aging process. The researchers began by focusing on the hippocampus and the trisynaptic circuit within it, which is composed of connections between the entorhinal cortex, DG, CA3, and CA1 that are critical to many physiological functions, including connections with the immune system to maintain the integrity of the circuit.

Reduced neurogenesis in the hippocampus is the first hallmark of aging in mouse models. This reduction is quickly followed by an increase in proinflammatory microglia activity. Aging also

causes dendritic instability and an overall reduction in dendritic spines; however, cell death is not typically observed, which suggests that the decline in neurogenesis may be amenable to rescue. To study these effects during aging, Villeda and colleagues used the heterochronic parabiosis mouse model and determined that exposure to young blood in an older mouse can reverse hippocampal aging phenotypes (i.e., decreases in neurogenesis, synaptic plasticity, and cognition); young mice exposed to old blood display promoted aging phenotypes.

To study whether age-dependent effects could be mediated systemically through non-neural cell types, Villeda and colleagues transplanted hematopoietic stem cells (HSCs) from aged mice into young mice and assessed their ability to perform fear conditioning tasks. The aged HSCs decreased neurogenesis and impaired hippocampal-dependent learning and memory functioning in young mice. Proteomic analysis of the heterochronic parabiosis model identified cyclophilin A (CyPA) as a circulating pro-aging immune factor that increased in expression as cognitive abilities declined. CyPA expression is elevated in both human and murine blood compared to other tissues and is maintained at a high level during aging. Researchers also found that inhibiting CyPA using a neutralizing antibody increased neurogenesis and improved cognitive function in aged mice.

Because exercise as well as parabiosis and plasma transfer from young to aged animals can increase neurogenesis, improve synaptic plasticity, and reduce cognitive decline, Villeda and colleagues sought to investigate whether the neurogenic benefits of exercise could be transferred via blood. Blood from aged mice subjected to a 6-week voluntary running regimen was transferred into sedentary old mice. The old sedentary mice that received blood transfusions from mice who exercised showed increased neurogenesis compared to sedentary old mice that received blood from sedentary mice. Exercise-treated plasma also rescues age-related cognitive and memory impairments in recipient mice.

Many highly abundant compounds found through MS analysis on plasma collected from mice exposed to exercise were derived from the liver (e.g., the enzyme glycosylphosphatidylinositol-specific phospholipase D1 [Gpdl1] whose function is to cleave GPI-anchored proteins from the cell surface). Gpdl1 expression levels increase in mature and aged mice following exercise, as confirmed by quantitative polymerase chain reaction (qPCR) methods, and these increased levels correlate with improved cognitive performance in aged mice. Villeda and colleagues replicated these findings in active (i.e., $\geq 7,100$ steps per day assessed by FitBit data) older adult humans. Researchers then hypothesized that increasing the expression of Gpdl1 may recapitulate the benefits of exercise-induced plasma. Injecting mice with a vector to overexpress liver-derived Gpdl1 led to an increase in adult neurogenesis in the aged hippocampus and rescued cognitive impairment phenotypes in aged mice.

Interestingly, systemic Gpdl1 and exercise behavior can upregulate coagulation and downregulate complement signaling cascades, suggesting potential mechanisms by which Gpdl1 and exercise-induced factors produce neurological benefits in aged mice. Additional experimentation is required to assess whether administration of these factors can truly reverse the effects of aging in mice and humans.

Pathological Roles and Mechanisms of Aberrant Neurogenesis in Epilepsy

Jenny Hsieh, PhD, University of Texas at San Antonio

Increased incidence of temporal lobe epilepsy or other neurological diseases, such as AD, is observed during aging. New neurons are commonly believed to exert only benefits; however, Hsieh and colleagues hypothesize that an overabundance of new neurons may play a pathological role in these disorders. Adult neurogenesis improves learning, memory, pattern separation, spatial navigation, and mood regulation, but aberrant new neurons may contribute to epileptogenesis and cognitive impairment. Integration of aberrant new neurons can cause dramatic remodeling in the hippocampus and increase excitatory activity, and together with the death of interneurons after a severe brain insult, may contribute to the development of seizure activity.

Using a transgenic nestin-driving thymidine kinase mouse model, Hsieh and colleagues assessed the effects of new neuron ablation. They injected mice with ganciclovir (GCV) to cause specific ablation of the adult-born neuronal population and then administered pilocarpine by injection to induce seizure activity. Ablation of aberrant neurogenesis reduced chronic seizure activity compared to vehicle-treated mice and prevented cognitive decline. However, ablation reduced chronic seizures only when mice were treated with GCV before or greater than 4 weeks after the onset of acute seizures. Hsieh postulated that suppression of seizures did not persist as quiescent NSCs became activated and generated aberrant new neurons that subsequently contributed to seizure behavior.

Tonic GABAergic activity produces a depolarizing signal that promotes the maturation of new neurons, which led Hsieh and colleagues to hypothesize that GABAergic activity within new neurons may promote aberrant maturation. To test this idea, they retrovirally labeled *CAG-hM3Dq-GFP* mice and administered clozapine-N-oxide (CNO) at 0-1 weeks or 1-2 weeks after retroviral injection to increase canonical G protein-coupled receptor (GPCR) pathway. *CAG-hM3Dq-GFP* mice treated with CNO generated ectopic granule cells (EGCs) that aberrantly migrated, whereas mice retrovirally labeled with GFP alone did not. Further, only the *CAG-hM3Dq-GFP* mice treated with CNO developed seizures. When CNO was administered 8 weeks after the retroviral injection, *CAG-hM3Dq-GFP* mice did not develop seizures, suggesting that a critical period to develop epilepsy exists. Further, in the pilocarpine model of temporal lobe epilepsy, the retroviral *CAG-hM4Di* mouse model induces silencing of local synaptic transmission upon CNO treatment at 0-2 weeks after retroviral injection; these mice displayed reduced EGC migration and decreased spontaneous seizures. However, silencing mature neurons when CNO was given 8 weeks after the retroviral injection in the *CAG-hM4Di* mouse model did not affect spontaneous seizure frequency but increased seizure duration, suggesting that hyperactive new neurons may exacerbate epileptogenesis.

Through the use of these CNO-inducible mouse models, Hsieh and colleagues postulate that new neurons have the ability to rewire the hippocampal circuit. Hsieh's team used a rabies virus tracing system (i.e., labeling with mCherry) with *CAG-hM4Di-GFP* mice to determine changes to the neuronal input of adult-born granule cells. Neither PV nor somastatin (SST)

interneurons, the primary inhibitory cells in the dentate circuit, displayed changes. Researchers did not observe changes in CA1 neurons but did observe a reduction in CA3 neurons, the primary output of the dentate gyrus. The most dramatic increase was observed in input from the lateral entorhinal cortex, the primary excitatory input of the hippocampus circuit.

Hsieh and colleagues have validated that aberrant neurogenesis can rewire hippocampal circuitry (decreasing back projections from the CA3, a recurrent connection thought to be a major source of extra excitable activity into the dentate circuitry, and increasing cortical connections). Further, aberrant new neurons are not firing action potentials, yet they are engaging in GABAergic activity and increasing intracellular Ca^{2+} , which may alter neurogenic gene expression.

Despite their limited number, adult-born granule cells have a tremendous and unlimited pathological capacity to disrupt hippocampal circuitry. The field should aim to identify epilepsy therapeutic interventions and their targets to prevent the onset and progression of epilepsy.

Rejuvenating, Re-Engineering, and Restoring Aging Memory Circuits

Amar Sahay, PhD, Massachusetts General Hospital

The DG-CA3 circuit is thought to contribute to memory precision by decreasing interference between similar memories through circuit mechanisms such as pattern separation. Previous experiments by Bakker and colleagues have shown that patients with lesions in the DG experience memory interference impairments (i.e., they cannot distinguish between two similar items in an incidental encoding task). Patients also display increased memory interference during aging and when affected by MCI. Researchers have learned that, during aging, animals experience impaired remapping of CA3 neurons and elevated hyperexcitability in the aged CA3.

Sahay and colleagues found that reducing adult hippocampal neurogenesis, akin to that seen with stress and aging in mice increases memory interference in the context discrimination task. Conversely, Sahay and colleagues have shown that increasing neurogenesis in adulthood and aging is sufficient to reduce memory interference and improve memory precision. Convergent findings from different groups support a role for adult-born dentate granule cells (DGCs) in resolution of memory interference.

Sahay and colleagues propose that this cardinal function of adult-born DGCs supports hippocampal indexing. According to the Hippocampal Indexing theory, the hippocampus does not store memories; rather it registers experiences in sparse populations of neurons, engrams, that function as indexes for those experiences stored in spatial and temporal patterns of activity in association and sensory cortices. Based on work by many different groups, Sahay and colleagues posit that immature granule cells undergo a sensitive period, during which they experience synaptic competition to become input-specific and finely tuned mature granule cells that respond to specific features of previously encountered experiences. As such, different combinations of mature adult-born neurons are flexibly allocated into new engrams and recruit inhibitory microcircuits to promote memory consolidation and decrease interference in

connected cortical ensembles. Thus, adult neurogenesis expands the capacity for hippocampal indexing of memories. In conditions where these granule cells are compromised (e.g., during aging), indexing capabilities are also impaired.

Sahay and colleagues postulated that adult-born neurons face extensive competition with existing neurons for pre-synaptic inputs and integration into the hippocampus. To assess this theory, they engineered a model of biased competition, in which older neurons' dendritic spines were reduced in number through reversible Klf9 overexpression, which allowed adult-born neurons to gain a competitive advantage in synapse integration. This increase in integration of new neurons also caused a dramatic increase in the abundance of adult-born neurons within memory circuits and an increase in NSC activation. When the bias was removed (through reversal of Klf9 overexpression and rescue of the dendritic density of older neurons), the previously integrated new neurons retained their connections, non-integrated neurons lost their competitive advantage, and NSCs reverted to a quiescent state. These results suggest that rejuvenation of the aged hippocampal circuit is possible through biasing competition in favor of adult-born neurons. Rejuvenation of DG circuits, through biased new neuron competition, can improve context discrimination in aging mice. Enhancing neurogenesis can also promote population-based memory coding and reduce interference or overlap between ensembles of similar contexts.

Aging circuits in the DG can also be re-engineered through molecular specifiers to improve DG-CA3 connectivity and, subsequently, memory precision. Feed-forward inhibition (FFI) in DG-CA3 is theorized to govern burst firing of CA3 neurons and memory retrieval. Sahay and colleagues found that the ratio of FFI to feed-forward excitation decreases during adult-born granule cell maturation. Actin-binding LIM protein 3 (ABLIM3) is a cytoskeletal protein that regulates FFI within DG and CA3 circuits and is downregulated in response to learning. Downregulating ABLIM3 increases the connectivity with the DG-CA3 and enhances the effects of FFI in the CA3, which induces memory precision improvements in aged mice. Sahay and colleagues received a patent to assess the use of ABLIM3 as a target to improve memory and are investigating compounds to act as antagonists of ABLIM3.

During aging, the neurogenic niche undergoes many changes; in addition to experiencing age-dependent loss of neurogenesis and synapses, astrocytes and microglia also polarize into pro-reactive and inflammatory states, respectively. Sahay and colleagues hypothesize that synaptic loss induces secretion of pro-neurogenic niche factors. Using RNA-Seq, they identified upregulation of a DG-specific secreted factor, sPlA2g2f (phospholipase that increases in expression during aging), in response to synaptic loss. Viral injection of sPlA2g2f into the mouse DG induces NSC activation, and overexpression of sPlA2g2f reduces microglia and astrocyte densities in the DG. Conversely, viral deletion of sPlA2g2f increases microglial density, impairs granule cell maturation, and reduces adult-born neuron spine density in middle-aged mice. These findings led Sahay and colleagues to develop a model in which an aging-inducing neuronal signal dampens microglial, astrocytic, and stimulates SC activation in order to preserve hippocampal circuitry and support neurogenesis. Further investigations in the Sahay lab using this model will enable dissection of the impact of each of these cellular populations

(e.g., microglia, astrocytes) and how they can each undergo manipulation to improve neurogenesis in aging and AD.

Targeting Hippocampal Neurogenesis to Improve Pattern Separation in Aging

Rene Hen, PhD, Columbia University

Pattern separation capabilities depend on proper functioning of the hippocampal trisynaptic circuit; this circuit begins with the transmission of input information from the entorhinal cortex to the DG (believed to perform pattern separation), which then transmits information to the CA3, which performs pattern completion, and, finally, this information is integrated in CA1, which is the output structure. These processes depend on adult hippocampal neurogenesis, which typically produces 3,000 new cells per day. However, approximately 80 percent of these cells do not survive, and only 70 percent of the cells that do survive become mature neurons. Each step of neurogenesis depends largely on the organism's environment.

Human pattern separation tasks, such as the Stark task, assess the ability to discriminate between very similar objects. Some of the rodent tasks are derivatives of contextual fear-conditioning paradigms, which are hippocampus and amygdala dependent. Mouse models of fear conditioning pair an aversive stimulus (i.e., an electric shock) with an environment and assess the ability to discriminate between the environment paired with the aversive stimulus and a similar one. Typically, mice will progressively freeze less often in the similar environment because they learn they will not be shocked, which suggests that animals can separate patterns of their environment.

Neurogenesis decreases by approximately 10-fold during aging, and in aged mice, the decrease in neurogenesis occurs simultaneously with a decrease in pattern separation capabilities. However, if given the opportunity to learn pattern separation tasks, older mice do improve their abilities. Hen and colleagues further assessed these age-related changes through use of the *iBax* mouse model, which abolishes Bax-dependent apoptosis in young adult-born neurons and increases neurogenesis by 4- to 5-fold. Previous studies by Sahay and colleagues found that enhanced neurogenesis is sufficient to improve pattern separation. Hen and colleagues observed an improvement in pattern separation tasks in aged *iBax* mice similar to that observed in young mice. To verify whether this effect was a consequence of improved neurogenesis, they ablated neurogenesis in the *iBax* mice and observed a dramatic decrease in pattern separation abilities. Therefore, enhanced neurogenesis is the underlying mechanism of pattern separation improvements.

Hen and colleagues performed two-photon microscopy techniques to image mature DG cells in aged mice exposed to cues (i.e., the presence of a banana smell) while running on a treadmill. They observed that cues allow for the identification of cue-specific and place-specific DG cells. Cue-specific cells fired signals according to the position of the cue along the treadmill and ceased activity once the cue was removed. However, the place-specific cell activity persisted regardless of the cue's presence. This experiment was replicated in mice that underwent neurogenic irradiation; and researchers observed a decrease in remapping ability when mice were presented with a different context compared to sham mice.

Recent studies by Hen and colleagues have revealed that neurogenesis differentially impacts the lateral entorhinal cortex (LEC) and medial EC (MEC) inputs to the DG. An optogenetic strategy that silenced abGCs led to an increase in LEC-driven activity and a decrease in MEC-driven activity. Excitation and inhibition of mature GCs is mediated by N-methyl-D-aspartate (NMDA) and mGluRII glutamatergic receptors, respectively. High stimulation of these circuits led to excitatory activity, whereas low stimulation led to inhibitory signals. These effects are blocked by tetrodotoxin, which suggests that these connections between abGCs and mature GCs are monosynaptic.

Harnessing neurogenesis through these processes may allow researchers to treat neurological disorders, such as AD, or mitigate the effects of aging. For example, treatment with Bax antagonists (i.e., P7C3 and iMAC2) improves pattern separation in mice. Hen and colleagues seek to translate these findings into a clinical trial to assess the impact of these compounds and environmental manipulations (e.g., running) on pattern separation in a subset of patients who display pattern separation deficits or hippocampal dysfunction.

Discussion of Session III

Moderator: Molly Wagster, PhD, DN, NIA

Costs and Benefits to Increased Neurogenesis

Neurogenesis is typically viewed as a process that must increase in order to benefit a host; however, increasing neurogenesis is not necessarily always beneficial. Although new neurons are capable of reinvigorating and rejuvenating the hippocampal circuit to increase memory formation and learning under normal physiological conditions, speakers noted that the long-term effects of increased neurogenesis have yet to be studied. Most previous studies have observed the local and short-term impact of neurogenesis during normative and pathological development without observing downstream or compensatory changes. For example, long-term exposure to enriched environment, exercise, and modified healthy diets for entire lives with or without early ablation of neurogenesis could test the requirement of neurogenesis for healthy cognitive aging.

Participants suggested the potential for negative impacts of neurogenesis in aging. In AD, new neurons can be dysfunctional, and therefore global increases in neurogenesis may not always be beneficial. However, restoration of neurogenesis levels has been shown to ameliorate deficits in AD mouse models. In addition, increased neurogenesis has been linked to increased forgetful behavior. These observations suggest that the quality of new neurons may be more critical than quantity for proper functioning.

Participants stressed the importance of experimental context in understanding underlying neurogenesis mechanisms. The brain may benefit only from processes it needs; incorporating processes that are known to narrowly aid neurogenesis may not be beneficial if they are not imperative to the overall survival and functioning of an organism. For example, if the hippocampal network is not prepared to integrate new neurons or already contains enough neurons to properly function, then non-physiologically increased neurogenesis may have no effect, or possibly negative effects. Several studies have illustrated that exercise interventions

and enriched environments can increase neurogenesis in healthy animals without observed negative effects. However, if the network is dysfunctional due to a lack of neurons, increased neurogenesis might be critically beneficial, if proper integration can be achieved. Therefore, neurogenesis and the state of the network must be balanced for the system to properly function. Speakers identified that characterizing and assessing modulators of that balance can provide researchers with a better understanding of normative and disease mechanisms.

Mechanisms of Neurogenesis

Neurogenesis in young and old organisms is often compared using similar assessment tools, despite vast physiological differences between these populations. For example, older mice and humans display higher levels of inflammation and different metabolic patterns than young individuals, yet speakers noted it is unclear how these changes may impact the ability to record neurogenesis in research settings.

Participants indicated that the importance of timing in neurogenesis mechanisms requires further attention. Kempermann and colleagues reported that EE can induce life-long improvements in neurogenesis in mice when EE exposure occurs early in life. Additional studies are required to determine the sustained impacts of behavioral changes, such as exercise, that are presented during critical periods in an organism's lifespan.

Speakers also noted that further investigation into how pathologies (e.g., AD, epilepsy) impact or are impacted by neurogenesis may also be critical to developing interventions or techniques to mitigate negative changes. Genetic predispositions may regulate neurogenesis, and further study into the impact of genetic makeup on neurogenesis may highlight new neurogenic or anti-neurogenic processes.

Research Approaches and Models

For many neurological pathologies, diagnoses are often defined once the disease has reached a chronic or robust state that is not as amenable to molecular interventions as early-stage disease. Participants identified that research into these pathologies may benefit from more effective screening methods (either through imaging techniques or molecular markers) that allow researchers to monitor physiological and cellular changes that accompany the onset of AD, epilepsy, or other pathologies relevant to neurogenesis.

Mouse models allow researchers to view the physiological input of neurogenesis in a variety of disease conditions; however, neurogenesis researchers often aim to understand neurogenesis in humans to develop molecular or behavioral interventions to improve outcomes. Therefore, participants highlighted that the translation of findings from mouse models to non-human primates, human cells, and humanized organoids (studied either in culture or humanized mouse models) may greatly improve the translatability of research. For example, the effects of ablating or overexpressing neurogenesis and injecting young blood into aged animals has not been studied in non-human primates, which are the closest model organism to humans.

Within mouse models, the development of the APP^{KI} model of AD is seen by many as a great improvement from the APP overexpression models, which typically cause robust off-target

effects. Speakers indicated that continuing to improve ubiquitous models in neurogenesis research will facilitate continuous growth in the field.

Final Discussion: Gaps and Opportunities

Research Tools and Resources

Extensive human live-imaging approaches do not currently exist, which prevents researchers from viewing neurogenesis as it occurs under normal physiological and pathological conditions across multiple timepoints (i.e., during and throughout aging). Current imaging techniques such as MRI and PET-ligand studies do not provide adequate resolution to view sparse cells. Speakers noted that improving and developing these methods to observe neurogenesis in living humans could vastly improve a researcher's abilities to understand the mechanisms and dynamics of neurogenesis.

Several participants expressed that neurogenesis research would benefit from further study of the transcriptomic, proteomic, and epigenomic environments of the neurogenic niche in human brain. Most transcriptomic and epigenomic studies of neurogenesis have been performed in mice; however, researchers do not know whether the profiles identified in mice are comparable to those in humans. Therefore, the markers developed in mice may not assess the complete neurogenesis process in humans. Additional transcriptomic, proteomic, and epigenomic studies on human tissue will allow researchers to identify new human-specific markers and mechanisms or to validate previous and future mouse studies.

Tissue collection can be a varied and inconsistent process, partly because of a lack of standardized procedures for tissue retrieval, processing, and tissue fixing. These inconsistencies can result in downstream challenges to researchers who are unable to perform the experiment of interest because of inadequate fixative use, ultimately resulting in results that cannot be compared to those from other laboratories. Several participants identified that creation of a multi-center human tissue bank with streamlined collection and processing procedures could allow researchers across the United States, and eventually the world, to perform experiments on a uniform pool of tissues and directly compare results. However, participants stressed that the scientific objectives of interest must inform the methodological (e.g., immunocytochemistry or transcriptomics) and tissue collection procedures (e.g., whether tissue is fixed or fresh frozen); a 'one-size-fits-all' approach is likely inappropriate for all human neurogenesis studies.

Speakers noted that partnering with pharmaceutical companies can be challenging for researchers who have not been provided the opportunity to validate and de-risk their biological targets. Participants suggested that additional funding opportunities would greatly enhance researchers' ability to validate molecular compounds that have the potential to regulate or enhance neurogenesis, re-engineer connectivity, or restore the niche in clinical trials.

Consortia Collaborations

The field of adult neurogenesis research has become highly differentiated, and many key studies cover a large range of methods and experimental approaches. Because this is likely to

increase and complexity of the studies will grow, participants noted that establishing interdisciplinary collaborations is likely critical to the future development of neurogenesis and AD research. Virtual collaborations have become more common in recent years but not necessarily across research fields. Through multi-laboratory collaborations, researchers can combine their expertise to design studies that may not be possible in isolation because each laboratory may address neurogenesis from a different perspective. Speakers noted that by incorporating many different perspectives and findings into experimental study design, researchers can produce focused and effective studies that will result in critical scientific breakthroughs.

Appendix 1: Agenda

Workshop on Neurogenesis and Aging

March 16-17, 2020

Day 1: Monday, March 16, 2020

- 8:00 am** **Registration**
- 8:45 am** **Welcome and Introductions**
Marie Bernard, MD, NIA, Office of the Director
Eliezer Masliah, MD, NIA, Division of Neuroscience (DN)
Molly Wagster, PhD NIA, DN
- 9:00 am** **Overview of Adult Mammalian Neurogenesis in the Hippocampus**
Rusty Gage, PhD, The Salk Institute for Biological Studies
- Session I: Neurogenesis in the Adult Human Brain**
- 9:45 am** **Adult Hippocampal Neurogenesis and Aging in Health and Disease**
Maura Boldrini, MD, PhD, Columbia University
- 10:15 am** **The Role of Hippocampal Neurogenesis in Aging-Linked Cognitive Deficits and Alzheimer's Disease**
Orly Lazarov, PhD, University of Illinois at Chicago
- 10:45 am** **Break**
- 11:00 am** **Neurogenesis in the Postnatal and Adult Human Brain**
Ionut Dumitru, PhD, Karolinska Institute
- 11:30 am** **Solving Human Neurogenesis in vivo Toward Better Understanding and Therapy of Brain Disorders**
Mirjana Maletic-Savatic, MD, PhD, Baylor College of Medicine
- 12:00 pm** **Discussion of Session I**
Brad Wise, PhD, NIA, DN
- 12:30 pm** **Lunch**

Session II: Regulation of Neurogenesis in the Aging Brain

- 1:30 pm** **Regulation of Neurogenesis in Aging and Disease**
Michael Bonaguidi, PhD, University of Southern California, Los Angeles
- 2:00 pm** **Enhanced Plasticity of New Neurons in the Aging Hippocampus**
Alejandro Schinder, PhD, Fundación Instituto Leloir
- 2:30 pm** **Embryonic Origin and Maintenance of Adult Neural Stem Cells**
Hongjun Song, PhD, University of Pennsylvania
- 3:00 pm** **Break**
- 3:15 pm** ***Blood-borne Regulators of the Hippocampal Neurogenic Niche***
Joseph Castellano, PhD, Icahn School of Medicine at Mount Sinai
- 3:45 pm** **Transcriptional and Epigenetic Regulation of Neural Stem Cell Quiescence and Activation**
Ashley Webb, PhD, Brown University
- 4:15 pm** **Adult Neurogenesis and the Neurobiology of Individuality**
Gerd Kempermann, MD, German Center for Neurodegenerative Diseases
- 4:45 pm** **Discussion of Session II**
Amanda DiBattista, PhD, NIA, DN
- 5:15 pm** **Adjourn Day One**

Day 2: Tuesday, March 17, 2020**Session III: Functional Significance of Adult Neurogenesis**

- 9:00 am** **The Function of New Neurons in Adult and Aging Hippocampal Neural Circuits**
Shaoyu Ge, PhD, SUNY Stony Brook
- 9:30 am** **Blood: At the Interface of Aging and Adult Neurogenesis**
Saul Villeda, PhD, University of California San Francisco
- 10:00 am** **Pathological Roles and Mechanisms of Aberrant Neurogenesis in Epilepsy**
Jenny Hsieh, PhD, University of Texas at San Antonio
- 10:30 am** **Break**

- 10:45 am** **Rejuvenating, Re-Engineering, Restoring Aging Memory Circuits**
Amar Sahay, PhD, Massachusetts General Hospital
- 11:15 am** **Targeting Hippocampal Neurogenesis to Improve Pattern Separation
in Aging**
Rene Hen, PhD, Columbia University
- 11:45 am** **Discussion of Session III**
Molly Wagster, PhD, NIA, DN
- 12:15 pm** **Final Discussion: Gaps and Opportunities**
- 12:45 pm** **Adjourn Day Two**

Appendix 2: Speakers and Attendees

Workshop on Neurogenesis and Aging

March 16-17, 2020

Speakers

Maura Boldrini, MD, PhD, Columbia University, New York, New York
Michael Bonaguidi, PhD, University of Southern California, Los Angeles, Los Angeles, California
Joseph Castellano, PhD, Icahn School of Medicine at Mount Sinai, New York, New York
Ionut Dumitru, PhD, Karolinska Institute, Stockholm, Sweden
Rusty Gage, PhD, The Salk Institute for Biological Studies, La Jolla, California
Shaoyu Ge, PhD, SUNY Stony Brook, Stony Brook, New York
Rene Hen, PhD, Columbia University, New York, New York
Jenny Hsieh, PhD, University of Texas at San Antonio, San Antonio, Texas
Gerd Kempermann, MD, German Center for Neurodegenerative Diseases, Dresden, Germany
Orly Lazarov, PhD, University of Illinois at Chicago, Chicago, Illinois
Mirjana Maletic-Savatic, MD, PhD, Baylor College of Medicine, Houston, Texas
Amar Sahay, PhD, Massachusetts General Hospital, Boston, Massachusetts
Alejandro Schinder, PhD, Fundación Instituto Leloir, Buenos Aires, Argentina
Hongjun Song, PhD, University of Pennsylvania, Philadelphia, Pennsylvania
Saul Villeda, PhD, University of California San Francisco, San Francisco, California
Ashley Webb, PhD, Brown University, Providence, Rhode Island

National Institute on Aging

Marie Bernard, MD, Deputy Director
Eliezer Masliah, MD, Director, Division of Neuroscience (DN)
Amanda DiBattista, PhD, DN
Matt Sutterer, PhD, DN
Molly Wagster, PhD, DN
Brad Wise, PhD, DN

Science Writer

Bethany Stokes, Rose Li & Associates

Total Day One Registered Attendees: 178

Total Day Two Registered Attendees: 156