UNIVERSITY OF CALIFORNIA, SAN DIEGO

Computational Modeling of Adult Neurogenesis in the Dentate Gyrus

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Specialization in Computational Neuroscience

by

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The Dissertation of James Bradley Aimone is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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2009

DEDICATION

To my beautiful wife Lindsey

EPIGRAPH

There are places I'll remember
All my life though some have changed
Some forever not for better
Some have gone and some remain
All these places have their moments
With lovers and friends I still can recall
Some are dead and some are living
In my life I've loved them all

--In My Life

Lennon/McCartney

After taking every detour
Getting lost and losing track
So that even if I wanted
I could not find my way back
After driving out the memory
Of the way things might have been
After I'd forgotten all about us
The song remembers when

--The Song Remembers When

Hugh Prestwood

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LIST OF ABBREVIATIONS

BC	Basket cells
BrdU	Bromodeoxyuridine
CCK	Cholecystokinin
DA	Dopamine
DG	Dentate gyrus
EC	Entorhinal cortex
lEC	Lateral entorhinal cortex
mEC	Medial entorhinal cortex
Env	Environment
FE	Familiar environment
GC	Granule cells
GCL	Granule cell layer
HI	Hilar interneurons
IEG	Immediate early genes
LTD	Long-term depression
LTP	Long-term potentiation
MAM	Methylazoxymethanol acetate
MC	Mossy cells
ML	Molecular layer
MWM	Morris water maze
NDP	Normalized dot product
NE	Novel environment
NG	Networks grown with neurogenesis
No NG	Networks grown with neurogenesis halted on Day 120

OB	Olfactory bulb
PCA	Principal components analysis
PCs	Principal components
PP	Perforant path
lPP	Lateral perforant path
mPP	Medial perforant path
PV	Parvalbumin
SGZ	Subgranular zone
STDP	Spike-timing dependent plasticity

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Chapter VIII, in part, includes material that is being prepared for publication. Mu, Yangling; Aimone, James B, and Gage, Fred H. The dissertation author was the primary investigator and author of those aspects of this manuscript that are included in this chapter.

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PUBLICATIONS

"Computational Influence of Adult Neurogenesis on Memory Encoding" Aimone JB, Wiles J, and Gage FH – *Neuron*, 61(2), January 2009

"Neurogenesis." Jessberger S, Aimone JB, and Gage FH - *Learning and Memory: A Comprehensive Reference* (Chapter 42) Elsevier Limited, Oxford UK ed. John H. Byrne. 2008

"Adult Neural Progenitor Cells in CNS Function and Disease" Jessberger S, Aigner S, Aimone JB, and Gage FH – *CNS Regeneration, 2nd Edition*. ed. Jeffrey Kordower & Mark Tuszynski Elsevier, London UK 2008

"Adult Neurogenesis." Aimone JB, Jessberger S, and Gage FH – *Scholarpedia*. 2(2):2100. 2007

"Computational Modeling of Adult Neurogenesis." Aimone JB and Wiskott L – *Adult Neurogenesis* (Chapter 22) Cold Spring Harbor Press, Cold Spring Harbor, NY, 2007

"Mecp2 deficiency leads to delayed maturation and altered gene expression in hippocampal neurons" Smrt RD, Eaves-Egenes J, Barkho BZ, Santistevan NJ, Zhao C, Aimone JB, Gage FH, and Zhao X - *Neurobiology of Disease,* 27(1), April 2007

"Synapse formation on neurons born in the adult hippocampus." Toni N, Teng EM, Bushong EA, Aimone JB, Zhao C, van Pragg H, Martone ME, Ellisman MH, and Gage FH – *Nature Neuroscience.* 10(6), June 2007

"Potential Role for Adult Neurogenesis in the Encoding of Time in New Memories." Aimone JB, Wiles J, and Gage FH – *Nature Neuroscience*, 9(6), June 2006

"Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation." Barkho BZ, Song H, Aimone JB, Smrt RD, Kuwabara T, Nakashima K, Gage FH, and Zhao X - *Stem Cell and Development*, 15(3), June 2006

"Cholinergic Input is Required during Embryonic Development to Mediate Proper Assemby of Spinal Locomotor Circuits." Myers CP, Lewcock JW, Hanson MG, Gosgnach S, Aimone JB, Gage FH, Lee KF, Landmesser LT, and Pfaff SL – *Neuron,* 46(1), April 2005

"Spatial and Temporal Gene Expression Profiling of the Contused Rat Spinal Cord" Aimone JB*, Leasure JL*, Perreau VM*, Thallmair M* and the Christopher Reeve Paralysis Foundation – *Experimental Neurology,* 189(2), October 2004

"Unbiased Characterization of High-densisty Oligonucleotide Microarrays Using Probe-Level Statistics" Aimone JB and Gage FH – *Journal of Neuroscience Methods*, 135(1-2), May 2004

"IGF-1 Instructs Multipotent Adult Stem Cells to Become Oligodendrocytes" Hsieh J, Aimone JB, Kaspar BK, Kuwabara T, Nakashima K and Gage FH– *Journal of Cell Biology,* 164(1). January 2004

"Routes to Calcified Porous Silicon: Implications for Drug Delivery and Biosensing" Coffer JL, Montchamp JL, Aimone JB, and Weis RP – *Physica. Status. Solidi. (a)* 197, No.2. 2003

ABSTRACT OF THE DISSERTATION

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The incorporation of new neurons into the adult brain is a form of plasticity that has only recently been appreciated in neuroscience. The localization of adult neurogenesis to the dentate gyrus (DG) area of the hippocampus is of particular interest, given the hippocampus's observed role as a structure crucial for memory formation. In the rodent, thousands of new neurons are born daily, and research into this process has revealed a complex maturation process, with newborn neurons showing unique physiological and anatomical features at different stages of development. Despite these findings regarding the biology of the neurogenesis process itself, a functional role for new neurons has remained elusive.

This dissertation will describe the design, implementation, and testing of a biologically driven computational model of the adult neurogenesis process in the DG. In this model, new neurons are incorporated into the DG circuit according to the process revealed by previous biological studies. Ultimately, this work provides evidence for three separate functions for new neurons that are both novel and experimentally testable. The first hypothesis is that immature

neurons are more likely to contribute to the encoding of new memories than mature neurons. This increased activity provides a pattern integration component to the global pattern separation function of the DG. The second hypothesis is that the continuing maturation of these young neurons makes this pattern integration role temporally dependent. As such, memories encoded close in time will be associated, while memories encoded far apart in time will be separated. Third, the results suggest that continual neurogenesis results in a DG network that is a cumulative representation of events experienced throughout life.

Finally, this dissertation will discuss how neuromodulation may affect the neurogenesis process and its relationship with hippocampal function and will include a description of how modulatory neurotransmitters can be considered in the computational model, and the specific example of dopamine will show how preliminary data regarding the effects of modulators on neurogenesis can be incorporated into the modeling framework.

CHAPTER I: INTRODUCTION

Does the adult brain generate new neurons? For much of the 20th century, this was a greatly debated topic. While biology textbooks claimed that no neurons were born after childhood and most neuroscientists operated under that assumption, there were an increasing number of observations of the very process not believed to exist.

Today, the question is no longer whether new neurons are generated in the brain after development, but what those new neurons do and what they represent. From a clinical point of view, adult neurogenesis represents a new opportunity - the adult brain is not fundamentally averse to regeneration. The difficulty in repairing other brain regions may be a challenge specific to the local environment, not a basic property of central nervous system tissue itself. Furthermore, the generation of new neurons implies a source of multipotent neural stem cells in the postnatal brain, a population of cells already located within the brain that may represent an ideal target for regenerative therapy. The clinical applications derived from adult neurogenesis and adult neural stem cells are still years away, but their promise continues to grow.

In contrast, the answer to the question of what the neurons born into the adult brain do has still remained elusive. The circumstances of the adult neurogenesis process are appealing to neuroscientists. New neurons are only incorporated into two regions of the brain: the olfactory bulb and the hippocampus. The latter region, in particular, excites the imagination. The hippocampus has been referred to as the "gateway to memory," and is believed to be essential to the formation of episodic memory. All memories of past events in one's life, from childhood friends to restaurants visited last week, would pass through the very region that is incorporating these new neurons. Do these new cells each encode a new memory? Are they maintaining old memories or helping to forget them?

Unfortunately the biological examination of neurogenesis function has struggled to assign a role to these newly born neurons. Our understanding has grown – experimental observations have suggested potential contribution of new neurons in cognition, such as learning and memory and affect, including antidepressant action. There is little mechanistic evidence to explain these general roles, however. While the biological details of the process can help frame the possible range of functions, these realities have only recently begun to be applied to exploration of function, and their application has been limited.

Difficulty in finding a function experimentally – a role for computational neuroscience

Biology has historically looked to the "knockout" experiment for confirmation of function. Whether a genetic knockout of a gene or the lesion of a brain region, observing that a system can no longer do something that it previously could has long been the "gold standard" in describing functionality. Adult neurogenesis is not an exception to this pursuit, and many methods for reducing or eliminating the birth of new neurons have been developed in the past decade. While there is room for improvement in these methods, the challenge appears to lie in the other half of the canonical "knockout" experiment – finding a function or behavior that is no longer present after neurogenesis is eliminated.

As is sometimes the case with functional studies relying on eliminating the process of interest, these studies of adult neurogenesis have hit a roadblock. While the general experimental structure of knocking out neurogenesis and testing animal behavior on specific tasks will likely prove effective, the challenge has become finding the appropriate behavioral tasks with which to test the neurogenesis knockout animals. In essence, the experiments designed to find a function are struggling for clear results because the function to test for is unknown. The behavioral assays designed to test hippocampal function have appeared either unaffected by elimination of neurogenesis or insensitive to the knock-down. In retrospect, this is unsurprising, since neurogenesis likely accounts for less than 1% of hippocampal neurons at any given time.

Regardless, the field continues to struggle with finding a task well-suited for the neurogenesis question.

Without a strong working hypothesis as a guide, many functional experiments in neuroscience, such as those looking for functions of neurogenesis, have been essentially relying on serendipity to find answers. The brain is a highly complex system, and in many ways our realization of this complexity is growing faster than our understanding of the system. The number of possible functions is so large and the noise of behavioral experiments so great that without reference to a clear theoretical framework, it is very difficult to glean useful information from experimental observations.

Computational neuroscience is one of several theoretical subfields of neuroscience that seek to improve the conceptual understanding of the brain. Computational models of neuroscience differ in scope, goals, and structure; but their general approach is to use computational tools to explore the biological system's capabilities. Whether the contribution is to predict novel behaviors or to demonstrate a system's ability to perform a given function, a computational model is often a valuable tool in investigating systems too complex to monitor or to manipulate biologically. The development of a strong conceptual framework involves the consolidation of many aspects of the biological system, including the underlying anatomy of the neural networks, the physiology of individual neurons, and an understanding of the system's function.

Importantly, the rise of computational neuroscience is not intended to supplant experimental biology, but rather is intended to complement it. Ultimately, it is unlikely that any computational study can demonstrably "prove" hypotheses about neuroscience in the same manner for which classical biological approaches are often well-suited. The interaction between computational and biological approaches to studying neuroscience will hopefully be one of

synergy, with the strengths of each complementing one another to accelerate the understanding of the brain.

Computational modeling of adult neurogenesis

Adult neurogenesis is in many ways an ideal system to apply computational neuroscience in the search for a function. The hippocampus is among the best understood regions of the brain, with both the anatomy and physiology being well characterized. The dentate gyrus, the subregion where new neurons integrate, has a relatively simple architecture as well as straightforward inputs and outputs. Both the hippocampus and dentate gyrus have long been examined computationally, and these models are often considered success stories for the computational field. Additionally, while the neurogenesis process has only been a recent subject of study, many aspects of the neurogenesis process have been described experimentally.

Neurogenesis does provide unique challenges to computational modeling, however. The biggest challenge is developing modeling frameworks to allow new neurons to enter into the network. Similar to much of neuroscience, most computational approaches have been developed without regard for the possibility that new neurons are incorporated into the network. Most biologically-inspired neural network models utilize a static architecture, with synaptic weight changes being the only form of plasticity typically incorporated. Development, if considered at all, is treated as a process unique from the functional phase. While the hippocampus has been a model system for computational models, the widely-held theories of hippocampal function have generally developed without acknowledgment of the presence of adult neurogenesis in the dentate gyrus.

This dissertation describes the development of a computational neural network model of the dentate gyrus that incorporates new neurons. The model is biologically derived, with most of the architecture and functional parameters designed to approximate the biological system as closely as possible. While a simplification of the actual dentate gyrus system, the model is

capable of capturing much of the previously theorized pattern separation function of the dentate gyrus, and by incorporating neurogenesis it allows computational experimentation to investigate functions otherwise essentially impossible to test biologically.

The goal of this computational work was to generate novel hypotheses for the function of adult neurogenesis in the dentate gyrus. Ideally, these hypotheses will be the foundation for future experimental work seeking a functional role of new neurons. Three distinct possible functions for new neurons will be described, each of which has cognitive and behavioral implications. The first hypothesis is that immature neurons contribute to the pattern separation function of the dentate gyrus by adding similarity between memories, a process referred to as pattern integration. The second is that this pattern integration function is temporally dependent – new memories are only associated if they are temporally proximal. Such temporal separation may be responsible for adding "when" information to episodic memory formation. The third potential function concerns the long-term role of neurogenesis, whereupon new neurons survive to integrate into the network in a specialized manner, creating a highly specialized dentate gyrus network over a lifetime.

In addition to the generation of functional hypotheses, the model was used to explore the effects of the modulatory regulation of neurogenesis. Both alteration of neurogenesis rates and direct modulation of the dentate gyrus physiology were able to affect the functional contribution of new neurons in the computational model. These results provide a perspective for how modulatory neural systems can impact cognition by interacting with the adult neurogenesis process.

CHAPTER II: OVERVIEW AND BACKGROUND

Section I: The neurobiology of the dentate gyrus and hippocampus

Overview

The potential importance of new neurons to cognition is determined by the type of new neurons born and the region into which they incorporate. The observation that neurogenesis is generally limited to two regions of the adult mammalian brain suggests that there is something unique about those regions that either requires or benefits from new neurons. It is possible that these two regions (the olfactory bulb and the dentate gyrus) perform a similar computation that benefits from neurogenesis, but it is just as plausible that their functions are different yet both require new neurons. However, it is clear that there is something about these regions that sets them apart from the majority of the brain, including other sensory systems and cognitive regions.

The dentate gyrus (DG) is the more studied of these two neurogenic regions, and is a key part of the hippocampus, which is historically one of the most heavily studied regions of the brain. Although the cortex is generally considered the site of cognition and thought, the hippocampus is widely believed to be the critical site for memory formation. Studies in the 1950's on the famous patient H.M., whose treatment for epilepsy consisted of a temporal lobe lesion [1], followed by decades of studies on other lesion patients have reliably shown that individuals lacking a hippocampus and neighboring regions have dramatically impaired memory [2]. In addition to observations in human psychology, the hippocampus has been a focus for physiology research. Long-term potentiation (LTP) - still considered the major substrate of learning in the brain - was first observed in the dentate gyrus [3, 4]. In vivo physiology was pioneered in the hippocampus, and was accelerated by the discovery of place cells [5]. Also, the

relationship between interneurons and principal neurons has been most heavily characterized in the hippocampus, particularly in the CA1.

Ultimately, the functional relevance of adult neurogenesis will be shaped by how the dentate gyrus affects hippocampus function, and the role of the hippocampus itself in cognition and memory formation. The following sections will summarize several of the theories of hippocampus and dentate gyrus function that represent the framework with which the results of subsequent neurogenesis model will be interpreted.

Hippocampus

The hippocampus is physically located at the developmentally most extreme end of the nervous system. The entorhinal cortex flows into the subiculum and ultimately into the CA regions of the hippocampus. Due to the curvature of the brain, however, in rodents the hippocampus is internal to the cortex – occupying the space above the thalamus and posterior of the striatum. In primates, the hippocampus lies in the medial temporal lobe, along with the entorhinal and perirhinal cortices.

The hippocampus receives direct inputs from the cortex, but these are almost entirely from the entorhinal cortex (EC). The EC receives inputs from throughout the rest of cortex, both directly and indirectly. The EC also receives inputs from the amygdala, thalamus, and other forebrain regions [6]. Layers II and III of the EC project to the hippocampus, and layer V receives direct hippocampal inputs from the CA1 and indirect inputs through the subiculum.

In addition to this cortical input, there is a substantial direct subcortical input to the hippocampus. Much of this input passes through the fornix, which connects the hippocampus to basal forebrain regions such as the septum, diagonal band of Broca (DBB), mammary bodies, and the hypothalamus. There is a substantial cholinergic projection from the septum and the DBB to the hippocampus. Likewise, many other modulatory systems project to the hippocampus,

including dopaminergic (ventral tegmental area), serotonergic (dorsal and median raphe), and noradrenergic (locus coerelius).

For the purposes of this work, the hippocampus is defined as the DG and the CA fields (CA3, CA2, and CA1). This system (with the exclusion of CA2) is the basis for the classic "tri-synaptic circuit," where information ideally flows unidirectionally through the DG, into CA3, and then into CA1, before being passed back into cortex. This description is a significant oversimplification, but represents a first-order approximation of the network.

Hippocampus function: Behavior, Physiology, Computational Studies

The hippocampus has been studied functionally from several different perspectives. Psychological studies on human patients with hippocampal lesions, such as those on H.M., have focused on the hippocampus (and the medial temporal lobe in general) as key structures for episodic memory. Patients with lesions in the hippocampus and neighboring areas show potent amnesic effects, with severe lesions leading to complete inabilities to form new declarative memories [1, 2].

Behavioral and electrophysiological studies on rodent models have tended to focus on the hippocampus's role in spatial cognition. This function was originally motivated by the discovery of "place cells" in the rat hippocampus and their observation in other species [5]. CA1 and CA3 neurons, when isolated during in vivo recording, show a strong activity preference for particular regions of space, known as a place field. Neurons within other structures of the hippocampal formation, including the entorhinal cortex, DG, and subiculum, have place behaviors as well, though their place fields are not as structured or as well understood as those in CA3 and CA1 [7-11]. The spatial function of the hippocampus has been extensively studied using a range of behavioral tasks designed to test spatial cognition, such as the Morris water maze (MWM) [12], the Barnes maze [13], radial-arm maze, and object-place association tasks.

Finally, the anatomical observation that the hippocampus is highly connected to limbic regions such as the amygdala, hypothalamus, and other subcortical regions, has led to possible consideration of the hippocampus in affective processing. The ventral hippocampus, in particular, is believed to be involved in and affected by stress, depression, and other affective disorders.

These three functions for the hippocampus (episodic memory, spatial cognition, and affective processing) have often been studied in isolation, but they are likely each a different perspective on a general role for the hippocampus in processing information for memory formation. For example, information regarding the affective state of an animal must be incorporated into memory along with spatial information.

General computational framework for hippocampus

Computational models have long been used in studying the hippocampus. Marr's model in 1970 integrated much of the anatomical knowledge of the hippocampus into a general functional theory that still influences hippocampal modeling [14]. The key to Marr's theory of hippocampal function was the recurrent network structure in the CA3 and its ability to store discrete memories. Subsequent models of the hippocampus have continued to assign a memory storage or formation role to the CA3 network due to this associative connectivity. These include models by McNaughton and Morris [15], Treves and Rolls [16-18], Hasselmo and colleagues [19], and many others, which have all treated the auto-associative network of the CA3 as the key feature of hippocampus function.

The mathematical considerations of the CA3 as an associative network was advanced by the work of Hopfield in his analysis of fully recurrent neural networks [20]. The CA3 is one of the brain's regions whose connectivity is most similar to an ideal "Hopfield network," which is an abstract network containing full connectivity between all of the neurons. Hopfield's approach

demonstrated how the memory storage properties of associative networks could be quantified analytically. By demonstrating the mathematical structure of attractor states in a network, this work allowed the requirements and limitations of recurrent networks during memory formation to be better understood [21].

While the CA3's presumed function has been generally consistent across most computational models, the conjectured functions of the DG and CA1 have been more varied, and often the differences between models can be attributed to different functional roles assumed for these subregions. Functions for these subregions have been dissociated by several modeling, physiology, and behavioral studies [7, 22].

Dentate Gyrus

As described above, the DG is considered the primary input layer to the hippocampus, although there are significant direct cortical inputs to the other layers as well. Although most theoretical functions for the DG are related to its location as the input to the CA3, there have been several differing propositions for what role the DG plays in hippocampal function. The next few sections will describe the architecture and key features of DG anatomy and describe the possibilities regarding the functional relationship between the DG and CA3.

Anatomy of the Dentate Gyrus

The dentate gyrus, sometimes referred to as fascia dentata, is so named due to its 'V' or 'U' shaped structure that has the appearance of a tooth [23]. As with other hippocampal regions, the shape of the DG occurs in most views of the hippocampus. In the following description of the DG, 'outer' will refer to the outside of the 'V', and 'inner' refers to the inside of the 'V'. Although most brain slices will reveal this 'V' shape, in those preparations that do not, 'inner' refers to the area of the DG near CA3. The DG is typically divided into three regions, listed here

from outside inward: the molecular layer (ML, stratum moleculare), the granule cell layer (GCL, stratum granulosum), and the hilus (polymorphic layer). Both the ML and the GCL are often further subdivided, as discussed below.

Molecular Layer (MOPP Cells)

The ML region of the DG is principally important because it is the location of most synaptic transmission onto mature granule cells. The name "molecular" derives from the fact that it contains few cell bodies, and is thus very sparsely labeled under the majority of histological stains. Mostly the space is filled by the dendrites of granule cells and perforant path axons from the entorhinal cortex and mossy cells (as well as other hilar neurons).

The ML is divided into the outer, middle, and inner thirds, which correspond to different axon termination zones in the rodent. The outer third is the termination zone of the lateral perforant path (lPP). Axons in the lPP originate from glutamatergic neurons in layer II of the lateral entorhinal cortex (lEC). Similarly, the middle third of the ML receives input from the medial perforant path (mPP), which originates from Layer II of the medial entorhinal cortex (mEC) [24]. While perforant path axons are restricted to the outer two-thirds of the ML, the inner molecular layer also has many axon terminals. These axons arise from mossy cells located in the hilus and are known as the commisural/associative pathway. Interestingly, these mossy cell projections are mostly from distant locations along the dorsal/ventral axis of the DG.

Although the ML is predominantly filled by neuronal processes, the ML is not completely devoid of neuron soma – there are several small populations of interneurons that reside there. Of particular interest are the MOPP cells ("molecular onto perforant path"), which are GABAergic interneurons whose axons and dendrites stay in the molecular layer [25, 26]. These MOPP cells appear to be well situated to contribute to feed-forward inhibition to the network.

Granule Cell Layer (Granule Cells, Basket Cells)

The GCL is the principal neuron layer of the DG, and the two terms (DG and GCL) are often used interchangeably. While some studies break the GCL into similarly defined sublayers, the most typically identified subregion of the GCL is the subgranular zone (SGZ) which is important as both the location of several interneuron classes as well as the presumed location of adult neural stem cells.

There are several different cell types located within the GCL. The principal neuron of the layer, and of the DG in general, is the granule cell neuron. They are small neurons (~10 μm in diameter) and are densely packed into the GCL (~ 1 million per DG in rat). Structurally, granule cells appear to follow the classic design for neurons – they are highly polarized, with an apical dendrite extending into the ML where it ramifies into several branches, and a single axon that goes the opposite direction through the hilus and into CA3. The dendrites are very spiny, and each granule cell receives thousands of glutamatergic synaptic inputs, mostly from the perforant path of the entorhinal cortex.

Perhaps the most distinguishing anatomical characteristic of granule cells are their axons, known as mossy fibers. Mossy fibers give rise to three types of synapses, depending on the post-synaptic target. The most recognized are the "thorny excrescences," the large axon terminals that contact the proximal dendritic regions of CA3 pyramidal cells. These boutons often encompass several spines, and have unique physiological properties, including a strongly facilitating release probability and significant potency after a burst of spikes [27]. Although the thorny excrescences are often considered the main output of the DG, there are relatively few of them per granule cell. More common are en passant axon terminals onto interneurons in the CA3 and hilus. These smaller synapses have distinct physiological properties from the thorny excresences; in particular these are depressing synapses that appear more effective at transmission for single spikes as opposed to bursts. Finally, mossy fibers make a separate form of synapse onto distal dendrites of

mossy cells (in addition to thorny excrescences proximal to mossy cell somata). Several studies have considered the relative effects of these different types of granule cell axon terminals[28, 29].

Physiologically, granule cells appear to be very quiet, believed to be caused by both the physical properties of the cells as well as a high level of tonic inhibition. In slice preparations, recordings of granule cells are difficult in the absence of GABA blockade. In vivo recordings and immediate early gene studies have confirmed that granule cells are not very active in the network [30, 31], though the nature of their selectivity is debated [11, 32].

There are several classes of interneurons that reside in the GCL [26]. These include several types of basket cells and chandelier cells. Basket cells are of significant computational interest because they appear to receive both feed forward input from perforant path axons as well as feedback excitation from granule cells. The axons of basket cells terminate very close to the soma of granule cells and are well positioned to provide a very potent inhibitory signal. Different populations of basket cells have been identified based on histological staining; the two principal types are parvalbumin-positive (PV+) basket cells and cholecystokinin-positive (CCK+) basket cells. While basket cells connect to one another by both synapses and gap junctions, the networks generated by these two classes (PV and CCK) appear to be distinct. Furthermore, these classes receive distinct afferent inputs: PV+ basket cells receive strong cholinergic inputs from the septum, yet little other modulatory drive. CCK+ basket cells have been shown to be modulated by many other factors, including serotonin and cannabinoids [33].

Chandelier cells, which are sometimes referred to as axo-axonic interneurons, appear to comprise a sparser population in the GCL, and have not been as well studied physiologically. Anatomic observations show that their axon terminals are near the axon hillock of granule neurons, suggesting that these neurons can be powerful suppressors of granule cell output [34, 35].

Hilus (Mossy Cells, Hilar Interneurons)

The hilus, which is periodically referred to as the polymorphic layer or CA4, is the region internal to the 'V' formed by the GCL. While the hilus is not densely packed with neurons like the GCL, it has substantially more neuron somata than the ML. Most granule cell axons pass through the hilus, and it is likely that these mossy fibers provide the bulk of the excitatory drive to the region. In addition, the hilus is the target of many extrinsic projections. While there is no direct cortical input to the hilus, projections from many subcortical regions like the septum and raphe nuclei heavily ramify in the region. There is also a "backprojection" from the CA3 which appears to target hilar neurons [36].

There are many different neuronal populations in the hilus. The one class that has been identified as glutamatergic are the mossy cells [37, 38]. The dendrites of mossy cells appear to be limited to the hilus, though there are examples showing branches extending into the ML. Their axons have a very unusual projection pattern; while they avoid the longitudinal region of the DG, they project heavily to distal regions of the DG. The axons terminate within the inner molecular layer. Because of their unusual topography, there remains a significant debate whether the primary downstream targets of mossy cells are basket cells or granule cells; thus it is unclear whether their activation has a net inhibitory or excitatory effect on the DG [39]. Importantly, in rodents mossy cells contribute to a significant commissural projection to the other half of the brain, but it appears that in primates their axons are unilateral.

Physiology studies suggest that mossy cells are likely to be considerably more active in the network than granule cells, though there is no in vivo data available to draw conclusions about their behavioral activity. In addition, they appear to be sensitive to input from modulatory neurotransmitters, such as acetylcholine. Their position in the network as well as their axonal distribution suggests that their function may be important in modulating or controlling overall DG activity, though future work is necessary to elucidate how they affect DG function.

In addition to the excitatory mossy cells, the hilus is the location of several different GABAergic interneuron classes. These interneurons represent a broad category, as efforts to label different populations have had mixed results. Anatomically, there are different populations whose axons project to the outer two-thirds of the molecular layer (HIPP) and those that project to the IML (HICAP). Histologically, there are substantial populations that are labeled with somatostatin, NPY, calbindin, along with several other markers. These chemically defined populations are neither exclusive nor overlapping with each other or with the anatomical classes, though it appears that many of the HIPP cells are somatostatin positive. There have been several observations of these interneurons projecting axons to extra-hippocampal areas, which is relatively uncommon for GABAergic populations.

Generally, the dendritic arborizations of these hilar interneuron populations suggest that they receive most of their excitatory input in the hilus, either from granule cells or mossy cells. Their axons likely project widely throughout the molecular layer, suggesting they serve a feedback inhibition role in the network. In addition, as with basket cells, it appears that hilar interneurons synapse onto each other in a specific manner [40], suggesting that there are distinct interneuron "meshes" in the DG.

Function of the Dentate Gyrus

Although the functions of many regions of the brain were initially understood from experimental observations, the dentate gyrus is a region where computational models and theories have driven much of the biological exploration.

Computational modeling of DG – pattern separation

As described above, the dentate gyrus has often been treated as the initial component of the hippocampal tri-synaptic circuit [14]. One of the first models to give the DG a more specialized role was developed by McNaughton and Morris in 1987 [15]. The McNaughton

model of the DG treated granule cells as “detonator neurons” that are important for loading memories into the CA3.

Treves and Rolls expanded on this function considerably in 1992 by demonstrating how sparse coding by the DG with powerful synapses onto the CA3 is ideally suited to load memories into the associative CA3 network [16-18]. The Treves and Rolls framework considered the dual inputs to the CA3 layer, the direct perforant path inputs from EC and the indirect pathway through the DG. Because the direct EC synapses onto CA3 pyramidal neurons are individually weak but numerous, they are unable to provide sufficient signal over the “noise” contributed by the feedback recurrent CA3 projections. Essentially, the divergent EC projection is incapable of forming a new attractor in the CA3 without disrupting existing attractors. However, according to the Treves and Rolls theory, the very powerful yet sparse DG projection is well suited for selecting a small subset of CA3 neurons with which to store a memory. This activation of a CA3 subset will be accompanied by heterosynaptic plasticity of both the CA3 recurrent network and the EC-CA3 projection. During recall, the now trained EC-CA3 projection is sufficient to pattern complete to the original memory trace, whereas partial activation of the DG-CA3 projection will not work, due to its sparsity.

O’Reilly and McClelland expanded on the pattern separation function by the DG, demonstrating that the sparse coding in the DG is capable of considerably reducing network similarity in memories formed in downstream areas [41]. According to the O’Reilly and McClelland framework, the properties of the DG and its mossy fiber projection to CA3 are well suited to optimally separate inputs to the hippocampus in a two tier fashion – the EC to DG provides a potent initial separation and this is compounded by the subsequent DG to CA3 projection. Although their modeling results are generally consistent with the Treves and Rolls framework discussed above, they do not rule out a possible role for DG in recall, suggesting that

LTP in the DG region may allow the DG to contribute to the pattern completion function generally assigned to the CA3 [41].

Behavioral exploration of DG function

The function of the DG has been explored behaviorally primarily using lesion studies in rats. Granule cells are selectively sensitive to colchicine, a natural toxin, allowing it to be used for targeted DG knockouts in many of these studies. Rats with lesioned DG (DG-KO rats) have impaired performance on several tasks. Consistent with its location as the main structural input to the hippocampus, DG-KO rats have been shown to have impaired performance on many tasks associated with hippocampal function, including working memory in the 8-arm radial maze and as well as in the MWM [42-44]. However, tasks showing that a DG lesion is at times comparable to a hippocampal lesion are not sufficient to describe DG function, rather that it is an important part of the hippocampus.

To dissociate DG function from global hippocampal function, Kesner and colleagues developed spatial tasks designed to measure pattern separation, the suggested function of DG emerging from many computational models of the hippocampus [45]. In one task, rats were tested on a delayed-match-to-sample paradigm where the animal was first exposed to an object in one location, and on the test phase exposed to two identical objects, one at the previous location and one at a novel location some distance away. Rats without a DG had difficulty determining the familiar location when the objects were spatially close to one another, while all animals could determine the familiar location for distant objects [46].

In addition to the pattern separation function, many models of hippocampus have postulated that the main role of the DG is in the encoding phase of memory formation, and that it is not necessary during retrieval. A series of tasks by Lee and Kesner [47-49] have demonstrated that DG-KO rats show impairments in the acquisition phase of certain tasks, including the Hebb-

Williams maze and context fear conditioning. When rats were lesioned after the acquisition phase of these tasks, however, their performance was comparable to controls on the test phases.

Molecular techniques have been used to control DG function. Mice with a conditional knockdown of the NMDA receptor in granule cells have impaired synaptic plasticity [50]. Presumably, DG function that is not dependent on this plasticity is maintained, though neurogenesis is also likely affected in these animals. Behavioral studies on these transgenic mice showed normal learning on many hippocampal tasks, such as MWM and context fear conditioning, but impaired performance on a context fear conditioning task that required the animals to dissociate between safe and unsafe contexts.

Activity measures of DG function

The activity of DG granule cells during animal behavioral tasks has been measured by both in vivo electrophysiology techniques as well as histological labeling of immediate early genes (IEGs), such as c-fos, arc, and zif [30]. In vivo recordings of neurons in the GCL have confirmed that granule cells are sparsely active. Monitoring place behavior of recorded cells revealed that the firing rates of isolated neurons, presumably granule cells, are generally low [11, 31]. Furthermore, these studies qualitatively observed that these neurons are difficult to find and to record from. The sparse coding and high information content of active neurons observed by Jung is consistent with a pattern separation function [31]. The Leutgeb results were less clear - if correlations between neurons are considered for different spatial locations, recorded DG neurons are effective at separation. However, unlike neurons in CA3 (and contrary to most DG theories), the population of active neurons does not change significantly between environments [11].

The second requirement for the pattern separation function of the DG is the ability for granule cells to be powerful activators of CA3 pyramidal neurons even when sparsely active. The Buzsaki laboratory demonstrated that a single granule cell could induce activity in a target pyramidal neuron during in vivo conditions [27]. This is consistent with the McNaughton and

Morris "detonator" synapse idea, and along with the in vivo observations about sparse coding, suggestive of the Treves and Rolls framework. The mossy fiber pathway does appear considerably more complicated than a simple detonator synapse, however. Future work will be necessary to understand the nature of the relationship between mossy fibers and CA3 and hilar interneurons.

Section II: The Neurobiology of Adult Neurogenesis

Overview and History

The controversy over whether new neurons functionally integrate into the adult brain has been largely settled in the past decade. Neurogenesis in the dentate gyrus (DG) was extensively characterized during the 1990's, and capped off by the observation by Eriksson et al. (1998) that adult neurogenesis occurs at significant levels humans [51]. Although the debate over neurogenesis has continued in other regions, such as frontal cortex [52-56], hypothalamus [57], and piriform cortex [58], adult neurogenesis in the DG has proved a robust finding that has resulted in considerable widespread interest.

Prior to this burst of studies on neurogenesis during the mid 1990's, research into adult neurogenesis was sparse and was often met with considerable skepticism. Perhaps the earliest to systematically study the adult neurogenesis phenomenon was Joseph Altman, who in a series of papers throughout the early 1960's [59, 60], characterized the development of the DG using tritiated thymadine (Thy-H3), a radioactive nucleotide analogue which incorporated into dividing cell nuclei allowing them to be subsequently birthdated. Altman believed these radio-labeled cells were new neurons, and continued to characterize the process as such, though his work did not separate adult neurogenesis from postnatal neurogenesis, but rather as simply a developmental process that continued well into adulthood. However, the lack of histological proof that the labeled cells were neurons limited the widespread acceptance of the observation.

Other work followed in subsequent years, a key example being that of Michael Kaplan, who showed that the Thy-H3 labeled cells were neurons using electron microscopy [61-63]. This work too was generally ignored by the neuroscience community [64]. Eventually, the isolation of neural stem cells from the adult hippocampus [65], along with studies investigating the regulation of proliferation in the hippocampus after stress [66-69] and with learning, exercise, enrichment, and aging [70-73] in the 1990s, brought research regarding adult neurogenesis to the forefront of neuroscience. The observation of adult neurogenesis in the human hippocampus and later demonstration of functional integration into the hippocampal circuitry were significant steps in the general acceptance of the process.

Biological Process of Dentate Gyrus Adult Neurogenesis

Today, two regions of the adult brain are widely accepted as having significant neurogenesis – the olfactory bulb (OB) and the dentate gyrus (DG). The process by which new neurons emerge from neural stem cells is fundamentally different between these two regions. The new neurons that enter into OB circuitry are actually born in the subventricular zone (SVZ). Neural progenitors born in the SVZ pass through several stages of differentiation that are closely associated with the nearby ventricle. Once committed to a neuronal lineage, these neural precursors migrate a long distance from the ventricle to the OB via the rostral migratory stream. Once in the OB, these cells differentiate into several different types of neurons: GABAergic granule cells and periglomerular cells that release GABA and/or dopamine.

While migration is a major feature of the SVZ/OB neurogenesis process, DG neurogenesis is localized to the inner part of the granule cell layer and the subgranular layer (SGL). Although the origin of new neurons is not fully understood, one working model is that radial glial cells residing in the SGL are the ultimate source of new neurons. These relatively quiescent radial glial cells are similar to astrocytes and have a unique morphology including a

process that extends into the molecular layer, where they presumably monitor local activity. By some unknown method, these radial glial cells give rise to a smaller rapidly dividing neural progenitor cell, which then divides several times symmetrically. These neural precursors may then migrate small distances into the inner granule cell layer, where they complete their differentiation into neurons and begin maturation.

Once a precursor cell differentiates into the neuronal lineage, it begins a slow maturation process that will ultimately result in a mature granule cell. These states each appear important for their integration into the network and ultimate survival.

Neuroblast stage – Less than 1 week old

It is difficult to demarcate exactly when a progenitor cell becomes committed to the neuronal lineage, since cell type is essentially defined by its future – once "differentiated" it will no longer divide and eventually become a neuron if it survives. These neuron precursors, or neuroblasts, initially show little neuronal morphology, and are typically localized in the SGZ near the stem cell population. Initially, these young neurons lack the polarity and processes typical of neurons. Within several days they begin to extend neurites and by the end of the first week they orient themselves appropriately in the network (with proto-dendrites extending toward the molecular layer)[74].

These very immature neurons likely have no functional impact on the network, since it is very unlikely that they have any efferent connections. Nonetheless, they appear to be sensitive to the activity of the local environment. In particular, GABA appears to be a critical neurotransmitter at this stage [75, 76]. Although these neurons do not receive any direct synaptic inputs, they are sensitive to bath application of GABA as well as local activity, suggesting that they are responsive to GABA diffusing from nearby synapses [77]. Because these neurons and their neurites tend to be localized within the inner region of the GCL at this stage, if the source of

this GABAergic depolarization is local, it likely originates from one of the basket cell populations.

GABA depolarization and early growth – 1 - 2 weeks old

After the second week of age, the immature neurons have a distinct neuronal morphology, but are still considerably smaller than mature GCs. Their dendrites approach the molecular layer, but have minimal branching and lack spines and Their axon begins to extend into the hilus [74].

Electrophysiologically, neurons at this stage are still depolarized by GABA, but the GABAergic input is now predominantly synaptic [77-79]. There is still little glutamatergic input, which is consistent with the observation that they lack spines on their dendrites. Direct current injection is capable of inducing action potentials, which are broad and incapable of bursting like mature GCs. It is unclear whether GABAergic depolarization is sufficient to induce an action potential, or whether the neurons form any downstream connections at this point.

Dendritic expansion and axonal expansion – ~2-4 weeks old

By about 16 days after the final cell division, immature neurons are beginning to integrate into the network. By this time, their general morphology is certainly neuronal, and spine formation is beginning [74]. By 21 days, the immature neurons will have roughly 25% of the number of spines as a mature neuron. In addition to spine development, the dendrite elaborates considerably by 21 days. Electron microscopy (EM) shows that immature neuron filopodia (the precursor to spines) appear to grow toward existing synapses [80].

The timescale of axon outgrowth is similar to that of dendritic development. Although axons can be observed early in development, they do not extend fully into the CA3 until roughly two weeks of age, which is comparable to when spine formation is beginning in the molecular layer [74].

Electrophysiologically, neurons change considerably between two and four weeks of age. GABA becomes inhibitory during this time, and the glutamatergic input onto neurons begin to develop. Spike-shapes become more similar to mature neurons, and by four weeks, neurons are bursting similarly to mature granule cells. Membrane resistance drops during this time and capacitance increases, consistent with the increasing surface area and number of receptors on young neurons. The resting potential of neurons begins to drop as well, from the relatively high levels of immature neurons towards the very hyperpolarized resting state of mature granule cells [77, 81].

In addition to having different basic electrophysiological properties, immature neurons have a different capacity for LTP [82]. Under several different paradigms, LTP is easier to induce in young neurons, either birthdated with GFP [83] or labeled with PSA-NCAM [84]. The changes in LTP are likely related to the increased spine dynamics seen in microscopy studies [74, 80]. Increased LTP by young neurons is also consistent with observations that animals with neurogenesis removed by irradiation have significantly impaired in vivo LTP [85].

Later maturation - >4 weeks old

After about one month of age, immature neurons have morphologies and physiological properties generally consistent with those of mature GCs, but still have some differences [74, 77, 81]. The physiology of neurons continues to develop for several months, though this development has not been as systematically explored. Neurons between 4-6 weeks old continue to show a stronger LTP phenotype than mature neurons, with easier acquisition than mature neurons and higher amplitude than younger neurons [83].

Regulation of Adult Neurogenesis

Unlike much of embryonic and postnatal neural development, which is guided by the environment but intended to be robust to many external environmental conditions, adult

neurogenesis is highly regulated by the animal's experience. Regulation of neurogenesis can occur at both the proliferation stage and the maturation stage. Proliferation can be either accelerated or slowed, and in doing so affects the rate of new neurons entering the population. Many factors have been shown to increase or decrease neurogenesis rates, and these factors tend to be systemic and relate to the general state of the animal. Some of the most potent regulators of neurogenesis rates are physical activity, stress, and aging [67, 70, 72, 73].

Regulation of the maturation and survival of new neurons appears to be more specific to experience and the information being processed by the hippocampus. A large proportion of adult-born neurons die under standard laboratory conditions [86]. The survival of new neurons can be dramatically altered by many factors, and many of these factors suggest that as these neurons develop, their behavior will ultimately determine whether they live or die. Experience, particularly by the use of enriched environments, has been demonstrated to be effective at inducing the survival of adult born neurons [72, 87]. This survival appears to be dependent on NMDA activation [88].

An important observation about neurogenesis is that the effects of regulatory signals will be temporally delayed. Since new neurons are likely not affecting function for several weeks after they begin differentiation, changing the rate of neurogenesis at the proliferation stage probably will not have an effect for days or weeks. Regulation of survival likely has a more immediate effect on the system and is likely more specific to individual neurons.

Behavioral models of neurogenesis function

Several biological techniques have been developed to investigate neurogenesis function. The first section will review the techniques that have been developed to reduce proliferation and will summarize their efficacy and drawbacks. The second section will then discuss the different behavioral assays that have been examined using these various knockdowns.

Methods for knocking out neurogenesis

Three general types of approaches have been used to knockout neurogenesis: anti-proliferative drugs, irradiation, and molecular techniques. Almost every knockdown technique has been designed to act at the proliferation stage of the neurogenesis process, meaning that any behavioral effect of the knockdown would be expected to occur sometime after treatment. Each of these methods has advantages and disadvantages, and the search for the ideal knockdown continues.

The first study to knockout neurogenesis used methylazoxymethanol acetate (MAM), which is a chemotherapy drug that blocks cell proliferation, including neural stem cells in the DG [89, 90]. MAM has the advantage that it is easy to deliver and can be administered to rats. It is also fast-acting – unlike other knock-out methods, MAM treated animals can be tested anytime after treatment – and fairly effective (80% knockdown of BrdU labeled cells after treatment). The principal drawback to MAM is its nonspecificity. Because it impairs cell division, many other proliferative populations of cells are affected as well, and can cause subject animals to become ill or die. Indeed, experimental evidence suggests that doses of MAM high enough for a more complete knockdown of neurogenesis are too high for the overall health of the animals, thus complicating the interpretation of behavior [91].

The second approach to knocking out neurogenesis has been to use irradiation. Both mice and rats have been used in irradiation studies [92-98]. In addition to directly affecting the neural progenitor population, irradiation alters the local microenvironment of the SGZ, greatly attenuating the capacity for future neurogenesis. Irradiation is a very effective knockdown technique, almost entirely eliminating the number of new neurons produced. However, there are several significant drawbacks to its use as a behavioral tool. First, there is no clear evidence that the side effects of the process are not impacting function. Even when irradiation is focally limited to the DG, post-radiation inflammation may affect local circuit behavior [98]. Second,

animals are typically given a considerable amount of time to recover after irradiation, essentially limiting the experimentation to the study of a chronic neurogenesis knockdown.

The specificity concerns regarding irradiation and MAM have led to several transgenic mouse models that can conditionally eliminate neurogenesis. One strategy that has been used is to develop mice that express the viral thymadine kinase (TK) gene under a stem cell specific promoter, such as GFAP [92]. When animals are given ganciclovir, it is phosphorylated by TK and ultimately induces cell death. Mice have also been generated with the *Tlx* gene conditionally removed using a *CreER* system [99]. When given tamoxifen, the *Tlx* gene is removed from cells throughout the brain, essentially blocking neurogenesis, which is dependent on *Tlx*. A third molecular knockdown model uses bigenic *Tet-Bax/nestin-rtTA* mice [100]. When these animals are given doxycylcine, *Bax*, a pro-apoptotic gene, is specifically expressed at high levels in *nestin*-positive cells.

The main drawback to these genetic models is that they are essentially limited to mice. The range of assays available for testing behavior in mice is considerably more limited than for rats. For example, the majority of the tasks that investigate pattern separation were designed for rats [46] and their utility in a mouse model is unclear. The second drawback to genetic models is that their levels of knockdown are considerably lower than what is observed after irradiation. Despite these concerns, molecular models provide an improved level of control for a knock-out model. While they are usually not 100% specific, there are fewer concerns about side effects than with irradiation or drugs.

One possible alternative to transgenic implementation of molecular knockdown is the use of viruses to deliver genes that decrease neurogenesis. The proliferation of neural stem cells is dependent on the family of extracellular signaling Wnt proteins. If stem cells are saturated with a dominant-negative version of Wnt (dnWnt), proliferation is markedly decreased [101]. Because dnWnt is a secreted molecule, it can be delivered virally to animals, including rats [102]. If

expressed at high enough levels, dnWnt can block neurogenesis. The main drawbacks to this viral system are the extent of knockdown, which is highly variable, and specificity. *Wnt* genes are also believed to be important in other forms of plasticity, making it difficult to interpret whether the effects of dnWnt are specific to the lack of new neurons.

Behavioral results of neurogenesis knockdown

A wide range of tasks have been investigated with these neurogenesis knockdowns. The bulk of these studies have investigated the hippocampus's role in memory and spatial processing, and as such have utilized tasks designed to measure general hippocampal function. The MWM has been one of the most widely used tasks in examining neurogenesis's role in spatial memory. A wide range of knockdown models have been tested on the MWM task with highly variable results. Several studies have observed deficits on the MWM task, though impairments were observed either at the learning phase [99, 100] or during the memory phase when tested with probe trials [103]. Other studies have been unable to see any deficit in MWM [89, 94-96]. Learning in MWM has been correlated to neurogenesis levels [104], though another group has argued that this effect is independent of neurogenesis [96].

Context fear conditioning is the other task most commonly investigated in knockdown models. Like MWM, some laboratories have observed a significant deficit [94, 95] whereas other groups have failed to see an effect [89, 99, 100], although other fear conditioning paradigms were implicated by Shors [90].

Not all tasks have shown impairments in neurogenesis knockdowns. Using a working memory paradigm, Saxe and colleagues showed that irradiated mice had increased performance on a working memory version of the radial arm maze, where animals were trained to choose the novel arm when forced to select between two arms, one of which had been previously visited [92].

The wide variation between results emphasizes several concerns with the utility of these behavioral tasks for examining neurogenesis. First, it appears as if the method of knockdown is of considerable importance, with no behavioral result proving to be consistent across knockdown approaches. This is not particularly surprising, given that the different techniques have widely varying levels of reduction of proliferation, and are testing either a chronic or acute loss of neurogenesis. Second, tasks such as MWM and context fear conditioning are highly dependent on experimental conditions, making experimenter and location differences an important factor. Finally, these tasks are likely not sufficiently sensitive for examining neurogenesis. Most of these tasks were developed for understanding memory using hippocampal or other large scale lesions, and there is no strong a priori rationale for why neurogenesis may be playing a role in any of these tasks. Therefore, negative or noisy results leave several possibilities: that neurogenesis is not involved in that task, that its contribution is minor and below the noise inherent in the behavioral data, or that neurogenesis is used, if available, but other compensatory mechanisms can be recruited given sufficient time without new neurons.

Computational models of adult neurogenesis

Over the past ten years, there have been several computational studies that have modeled the neurogenesis process. These models range in their incorporation of biological detail, level of study, and ultimate conclusions. This section will summarize the existing models of dentate gyrus neurogenesis, organizing them generally by their motivation and level of abstraction.

Abstract models of neuronal replacement in memory networks

The first models designed to investigate the functional role of neurogenesis focused on the role of replacing neurons in multi-layered, feed-forward networks. Two similar studies presented models along these lines in 2004.

The Chambers study, as well as its follow-up studies [105-107], investigated the role of neurogenesis in the learning of letters of alphabets in an artificial neural network. In their model, the shapes of different letters of the Roman alphabet were presented as the input to their three-layer network, and the network was trained using backpropagation to provide a readout in the final layer representing the letter position (i.e., 'A' as an input activates Neuron #1 in the output; 'B' activates Neuron #2, etc). The middle 'hidden' layer represents the DG, and the input and output are the EC and CA3, respectively.

The authors show that after training the network to accurately map the letter shape to letter number, the network is very slow in subsequently learning a new alphabet with different mappings, in this case the Greek alphabet. However, when there is neurogenesis (which is modeled by simple replacement of a DG layer neuron or resetting of synaptic weights to and from a random DG layer neuron), the network can more quickly learn the new Greek alphabet. Importantly, the old Roman alphabet is forgotten faster.

The results of this study were followed by several others that increased the sophistication of the model and investigated the role of different neurogenesis parameters in function. The Crick and Miranker study incorporated lateral inhibition, biologically realistic Hebbian learning, and non-random apoptosis. In addition to replicating many of the Chambers findings in a more biologically realistic network, their results showed that functionally-directed apoptosis allows more effective learning [106]. Chambers and Conroy later used the model to show that neurogenesis rates can be optimally tailored to the difficulty of the network's task. Re-training a network to learn a second distinct alphabet, such as Hebrew after learning Roman, required higher rates of neurogenesis than learning an alphabet more similar to the first one [105].

At roughly the same time of the first Chambers study, Deisseroth and colleagues included a computational model in a larger paper about the activity dependence of neural progenitor proliferation [108]. While the general structure of the two models is the same (learning of

patterns using three-layered networks with neural replacement), the Deisseroth study was both considerably larger (500 'DG' neurons as opposed to less than 32) and also used unsupervised Hebbian learning. Despite these differences, the results were generally the same: neural replacement in a hidden layer increases learning of new memories and clearance of old memories.

Although the relationship of these abstract studies to DG function is not clear, they provide strong evidence that neurogenesis can have a potent effect on network function, and that under certain conditions it is more effective at acquiring new information than simple synaptic plasticity. The results regarding increased learning rates and forgetting were robust to architecture and network size. Importantly, however, the utility of these results is unclear in the context of more general hippocampal theories, where the function of the DG is typically not one of memory storage, rather one limited to memory encoding.

Neurogenesis models designed to examine specific hypotheses

Several of the models of neurogenesis were designed specifically to examine a priori hypotheses for neurogenesis function. In general, because these models were constructed with specific questions in mind, they contain biologically realistic components, but the degree to which they can predict other functionality is unclear.

Wiskott and colleagues explored the possibility that the continuous addition of new neurons into the system enables it to avoid catastrophic interference in memory storage [109]. Catastrophic interference is basically a process by which the memory attractors in feed forward networks collapse due to overloading [110, 111]. If the number of memories trained into a network exceeds its capacity, the attractors in the network will begin to collapse, even if the information encoded by each attractor is unrelated. This will lead to failure in both the encoding of new memories as well as the elimination of old memories. New neurons added to the DG/CA3 network provide locations for new attractors that are independent from previous states, keeping new memories from interfering with old memories.

A different hypothesis has been pursued in a series of studies by Butz and colleagues [112-114]. Their goal was to investigate how new neurons provide a continuous pool of newly available synapses in a synaptic homeostatic system. Synaptic homeostasis, according to this framework, allows a recurrent network to eliminate and add synapses to offset changes in the strength of inputs. Their models of these homeostatic systems, which range from abstract recurrent networks to biologically motivated DG-like networks, are capable of maintaining stable levels of activity without neurogenesis and cell death if the variation of inputs is limited. However, in responses to very large shifts in input activity, the high levels of synaptic plasticity provide an effective substrate for absorbing or amplifying synaptic inputs.

Hippocampal models with neurogenesis

Although there is an extensive history of neural network modeling in the hippocampus, few of these biologically derived models implement neurogenesis. One example of a hippocampus model with neurogenesis is that of Becker in 2005 [115]. In a full model of the hippocampus (EC->DG->CA3->CA1->EC), Becker investigated the role of new neurons (along with a novel implementation of synaptic plasticity) in a model that emphasized the DG's role in memory encoding as opposed to memory storage. With random connectivity, the network's capacity showed that the presence of neurogenesis in the DG allows the system to better encode similar information in the network without interference in downstream layers.

While the Becker model has some interesting insights regarding neurogenesis function, its relevance to the biological process is unclear. The function for which neurogenesis had the most value – encoding similar data – was benefited most by complete turnover in the network. Since biological neurogenesis is occurring at a much lower rate, and appears to be an addition process as opposed to a turnover process, it is possible that there is some aspect of DG function that is not adequately captured by the Becker model. Despite these questions, the Becker model

is useful in demonstrating that neurogenesis can affect processing when measured at the hippocampal level.

In a later theoretical work, Becker and Wojtowicz extended the interpretation of these results in consideration of the observation that new neurons are born in temporal clusters, and that this integration of several new neurons for a given environment allows different features of the environment to be encoded in parallel and independently [116]. They argue that neurogenesis may be important for associations of positive contexts to new memories, a process that is impaired in affective conditions such as depression, where neurogenesis is thought to be low.

Summary of computational models

The computational models of adult neurogenesis described in this section demonstrate several possible roles new neurons may play in artificial neural networks designed to represent the hippocampus. One general feature that is consistent across many models is that new neurons allow networks to form new memories easier, often without disrupting existing memories. The Wiskott, Chambers, and Deisseroth models each demonstrate how new neurons, whether by replacement or addition, can help the network form new memories, and in some cases delete old memories, without dramatically affecting the remaining memory structure. Similarly, the Becker model demonstrates how neurogenesis's presence at the encoding phase of hippocampal memory formation can enable the system to store new information in downstream hippocampal areas.

While these approaches have provided several insights regarding how new neurons can improve memory capacity in neural networks, there are several aspects where these existing models fall short in describing the DG neurogenesis process. The first is their relevance to the hippocampal system. With the exception of Becker's model of the hippocampus, most of the models investigate the role of neurogenesis in more abstract network, and are investigating network memory as the principal feature that neurogenesis may optimize. Given the DG's

proposed role in memory encoding and pattern separation, it is not clear that memory storage by the DG is a proper readout for neurogenesis function.

The second limitation to the existing computational models is their minimal inclusion of the biological details of the neurogenesis process itself. It is increasingly clear that the integration of new neurons into the network is considerably more sophisticated than the simple replacement or addition processes used in these studies. The slow time-scale of the neurogenesis process suggests that there may be aspects of new neuron maturation that should be considered functionally important [74, 77]. In addition, the presence of immature and mature neurons within the same network suggests that the DG network is actually far more heterogeneous that typically considered [79, 82]. While some of the computational models have considered certain aspects of new neuron maturation (e.g., Wiskott's young neurons are more plastic; the spine dynamics of young neurons are different in the Lehman and Butz models), for the most part these models do not emphasize the details of the neurogenesis process.

Overall these studies are modeling the functional significance of replacing or adding new neurons into artificial neural networks as opposed to modeling the specific question regarding what the gradual incorporation of GC into the DG means to hippocampal function. One goal of the work described in this dissertation is to complement these existing studies on what neurogenesis may be doing in the abstract situation by focusing the model directly on the DG and its purported pattern separation function and by specifically modeling the specific features of the neurogenesis process.

References

1. Scoville, W.B. and Milner, B., *Loss of recent memory after bilateral hippocampal lesions.* J Neurol Neurosurg Psychiatry, 1957. **20**(1): p. 11-21.

2. Squire, L.R., Stark, C.E., and Clark, R.E., *The medial temporal lobe.* Annu Rev Neurosci, 2004. **27**: p. 279-306.

3. Lomo, T., *The discovery of long-term potentiation.* Philos Trans R Soc Lond B Biol Sci, 2003. **358**(1432): p. 617-20.

4. Bliss, T.V. and Lomo, T., *Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path.* J Physiol, 1973. **232**(2): p. 331-56.

5. O'Keefe, J. and Dostrovsky, J., *The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat.* Brain Res, 1971. **34**(1): p. 171-5.

6. Kerr, K.M., Agster, K.L., Furtak, S.C., and Burwell, R.D., *Functional neuroanatomy of the parahippocampal region: the lateral and medial entorhinal areas.* Hippocampus, 2007. **17**(9): p. 697-708.

7. McNaughton, B.L., Battaglia, F.P., Jensen, O., Moser, E.I., and Moser, M.B., *Path integration and the neural basis of the 'cognitive map'.* Nat Rev Neurosci, 2006. **7**(8): p. 663-78.

8. Leutgeb, S., Leutgeb, J.K., Treves, A., Moser, M.B., and Moser, E.I., *Distinct ensemble codes in hippocampal areas CA3 and CA1.* Science, 2004. **305**(5688): p. 1295-8.

9. Hafting, T., Fyhn, M., Molden, S., Moser, M.B., and Moser, E.I., *Microstructure of a spatial map in the entorhinal cortex.* Nature, 2005. **436**(7052): p. 801-6.

10. Fyhn, M., Molden, S., Witter, M.P., Moser, E.I., and Moser, M.B., *Spatial representation in the entorhinal cortex.* Science, 2004. **305**(5688): p. 1258-64.

11. Leutgeb, J.K., Leutgeb, S., Moser, M.B., and Moser, E.I., *Pattern separation in the dentate gyrus and CA3 of the hippocampus.* Science, 2007. **315**(5814): p. 961-6.

12. Morris, R.G., Garrud, P., Rawlins, J.N., and O'Keefe, J., *Place navigation impaired in rats with hippocampal lesions.* Nature, 1982. **297**(5868): p. 681-3.

13. Barnes, C.A., *Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat.* J Comp Physiol Psychol, 1979. **93**(1): p. 74-104.

14. Marr, D., *Simple memory: a theory for archicortex.* Philos Trans R Soc Lond B Biol Sci, 1971. **262**(841): p. 23-81.

15. McNaughton, B.L. and Morris, R.G.M., *Hippocampal synaptic enhancement and information storage within a distributed memory system.* Trends in Neurosciences, 1987. **10**(10): p. 408-415.

16. Rolls, E.T., *A theory of hippocampal function in memory.* Hippocampus, 1996. **6**(6): p. 601-20.

17. Rolls, E.T. and Treves, A., *Neural networks in the brain involved in memory and recall.* Prog Brain Res, 1994. **102**: p. 335-41.

18. Treves, A. and Rolls, E.T., *Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network.* Hippocampus, 1992. **2**(2): p. 189-99.

19. Hasselmo, M.E., Wyble, B.P., and Wallenstein, G.V., *Encoding and retrieval of episodic memories: role of cholinergic and GABAergic modulation in the hippocampus.* Hippocampus, 1996. **6**(6): p. 693-708.

20. Hopfield, J.J., *Neural networks and physical systems with emergent collective computational abilities.* Proc Natl Acad Sci U S A, 1982. **79**(8): p. 2554-8.

21. Amit, D.J., Gutfreund, H., and Sompolinsky, H., *Information storage in neural networks with low levels of activity.* Phys Rev A, 1987. **35**(5): p. 2293-2303.

22. Rolls, E.T. and Kesner, R.P., *A computational theory of hippocampal function, and empirical tests of the theory.* Prog Neurobiol, 2006. **79**(1): p. 1-48.

23. Amaral, D.G., Scharfman, H.E., and Lavenex, P., *The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies).* Prog Brain Res, 2007. **163**: p. 3-22.

24. Witter, M.P., *The perforant path: projections from the entorhinal cortex to the dentate gyrus.* Prog Brain Res, 2007. **163**: p. 43-61.

25. Halasy, K. and Somogyi, P., *Subdivisions in the multiple GABAergic innervation of granule cells in the dentate gyrus of the rat hippocampus.* Eur J Neurosci, 1993. **5**(5): p. 411-29.

26. Freund, T.F. and Buzsaki, G., *Interneurons of the hippocampus.* Hippocampus, 1996. **6**(4): p. 347-470.

27. Henze, D.A., Wittner, L., and Buzsaki, G., *Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo.* Nat Neurosci, 2002. **5**(8): p. 790-5.

28. Urban, N.N., Henze, D.A., and Barrionuevo, G., *Revisiting the role of the hippocampal mossy fiber synapse.* Hippocampus, 2001. **11**(4): p. 408-17.

29. Henze, D.A., Urban, N.N., and Barrionuevo, G., *The multifarious hippocampal mossy fiber pathway: a review.* Neuroscience, 2000. **98**(3): p. 407-27.

30. Chawla, M.K., Guzowski, J.F., Ramirez-Amaya, V., Lipa, P., Hoffman, K.L., Marriott, L.K., Worley, P.F., McNaughton, B.L., and Barnes, C.A., *Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience.* Hippocampus, 2005. **15**(5): p. 579-86.

31. Jung, M.W. and McNaughton, B.L., *Spatial selectivity of unit activity in the hippocampal granular layer.* Hippocampus, 1993. **3**(2): p. 165-82.

32. Leutgeb, J.K. and Moser, E.I., *Enigmas of the dentate gyrus.* Neuron, 2007. **55**(2): p. 176-8.

33. Freund, T.F., *Interneuron Diversity series: Rhythm and mood in perisomatic inhibition.* Trends Neurosci, 2003. **26**(9): p. 489-95.

34. Soriano, E., Nitsch, R., and Frotscher, M., *Axo-axonic chandelier cells in the rat fascia dentata: Golgi-electron microscopy and immunocytochemical studies.* J Comp Neurol, 1990. **293**(1): p. 1-25.

35. Soriano, E. and Frotscher, M., *A GABAergic axo-axonic cell in the fascia dentata controls the main excitatory hippocampal pathway.* Brain Res, 1989. **503**(1): p. 170-4.

36. Scharfman, H.E., *The CA3 "backprojection" to the dentate gyrus.* Prog Brain Res, 2007. **163**: p. 627-37.

37. Henze, D.A. and Buzsaki, G., *Hilar mossy cells: functional identification and activity in vivo.* Prog Brain Res, 2007. **163**: p. 199-216.

38. Ribak, C.E., Seress, L., and Amaral, D.G., *The development, ultrastructure and synaptic connections of the mossy cells of the dentate gyrus.* J Neurocytol, 1985. **14**(5): p. 835-57.

39. Sloviter, R.S., Zappone, C.A., Harvey, B.D., Bumanglag, A.V., Bender, R.A., and Frotscher, M., *"Dormant basket cell" hypothesis revisited: relative vulnerabilities of dentate gyrus mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat.* J Comp Neurol, 2003. **459**(1): p. 44-76.

40. Larimer, P. and Strowbridge, B.W., *Nonrandom local circuits in the dentate gyrus.* J Neurosci, 2008. **28**(47): p. 12212-23.

41. O'Reilly, R.C. and McClelland, J.L., *Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off.* Hippocampus, 1994. **4**(6): p. 661-82.

42. Xavier, G.F., Oliveira-Filho, F.J., and Santos, A.M., *Dentate gyrus-selective colchicine lesion and disruption of performance in spatial tasks: difficulties in "place strategy" because of a lack of flexibility in the use of environmental cues?* Hippocampus, 1999. **9**(6): p. 668-81.

43. Walsh, T.J., Schulz, D.W., Tilson, H.A., and Schmechel, D.E., *Colchicine-induced granule cell loss in rat hippocampus: selective behavioral and histological alterations.* Brain Res, 1986. **398**(1): p. 23-36.

44. Sutherland, R.J., Whishaw, I.Q., and Kolb, B., *A behavioural analysis of spatial localization following electrolytic, kainate- or colchicine-induced damage to the hippocampal formation in the rat.* Behav Brain Res, 1983. **7**(2): p. 133-53.

45. Kesner, R.P., Gilbert, P.E., and Wallenstein, G.V., *Testing neural network models of memory with behavioral experiments.* Curr Opin Neurobiol, 2000. **10**(2): p. 260-5.

46. Gilbert, P.E., Kesner, R.P., and Lee, I., *Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1.* Hippocampus, 2001. **11**(6): p. 626-36.

47. Lee, I., Hunsaker, M.R., and Kesner, R.P., *The role of hippocampal subregions in detecting spatial novelty.* Behav Neurosci, 2005. **119**(1): p. 145-53.

48. Lee, I. and Kesner, R.P., *Encoding versus retrieval of spatial memory: double dissociation between the dentate gyrus and the perforant path inputs into CA3 in the dorsal hippocampus.* Hippocampus, 2004. **14**(1): p. 66-76.

49. Lee, I. and Kesner, R.P., *Differential contributions of dorsal hippocampal subregions to memory acquisition and retrieval in contextual fear-conditioning.* Hippocampus, 2004. **14**(3): p. 301-10.

50. McHugh, T.J., Jones, M.W., Quinn, J.J., Balthasar, N., Coppari, R., Elmquist, J.K., Lowell, B.B., Fanselow, M.S., Wilson, M.A., and Tonegawa, S., *Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network.* Science, 2007. **317**(5834): p. 94-9.

51. Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., and Gage, F.H., *Neurogenesis in the adult human hippocampus.* Nat Med, 1998. **4**(11): p. 1313-7.

52. Rakic, P., *Neurogenesis in adult primate neocortex: an evaluation of the evidence.* Nat Rev Neurosci, 2002. **3**(1): p. 65-71.

53. Kornack, D.R. and Rakic, P., *Cell proliferation without neurogenesis in adult primate neocortex.* Science, 2001. **294**(5549): p. 2127-30.

54. Gould, E., Vail, N., Wagers, M., and Gross, C.G., *Adult-generated hippocampal and neocortical neurons in macaques have a transient existence.* Proc Natl Acad Sci U S A, 2001. **98**(19): p. 10910-7.

55. Gould, E., Reeves, A.J., Graziano, M.S., and Gross, C.G., *Neurogenesis in the neocortex of adult primates.* Science, 1999. **286**(5439): p. 548-52.

56. Bhardwaj, R.D., Curtis, M.A., Spalding, K.L., Buchholz, B.A., Fink, D., Bjork-Eriksson, T., Nordborg, C., Gage, F.H., Druid, H., Eriksson, P.S., and Frisen, J., *Neocortical neurogenesis in humans is restricted to development.* Proc Natl Acad Sci U S A, 2006. **103**(33): p. 12564-8.

57. Kokoeva, M.V., Yin, H., and Flier, J.S., *Neurogenesis in the hypothalamus of adult mice: potential role in energy balance.* Science, 2005. **310**(5748): p. 679-83.

58. Bernier, P.J., Bedard, A., Vinet, J., Levesque, M., and Parent, A., *Newly generated neurons in the amygdala and adjoining cortex of adult primates.* Proc Natl Acad Sci U S A, 2002. **99**(17): p. 11464-9.

59. Altman, J. and Das, G.D., *Autoradiographic and histological studies of postnatal neurogenesis. I. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in neonate rats, with special reference to postnatal neurogenesis in some brain regions.* J Comp Neurol, 1966. **126**(3): p. 337-89.

60. Altman, J. and Das, G.D., *Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats.* J Comp Neurol, 1965. **124**(3): p. 319-35.

61. Kaplan, M.S. and Bell, D.H., *Mitotic neuroblasts in the 9-day-old and 11-month-old rodent hippocampus.* J Neurosci, 1984. **4**(6): p. 1429-41.

62. Kaplan, M.S., *Neurogenesis in the 3-month-old rat visual cortex.* J Comp Neurol, 1981. **195**(2): p. 323-38.

63. Kaplan, M.S. and Hinds, J.W., *Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs.* Science, 1977. **197**(4308): p. 1092-4.

64. Kaplan, M.S., *Environment complexity stimulates visual cortex neurogenesis: death of a dogma and a research career.* Trends Neurosci, 2001. **24**(10): p. 617-20.

65. Reynolds, B.A. and Weiss, S., *Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system.* Science, 1992. **255**(5052): p. 1707-10.

66. Cameron, H.A., Woolley, C.S., McEwen, B.S., and Gould, E., *Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat.* Neuroscience, 1993. **56**(2): p. 337-44.

67. Gould, E., Cameron, H.A., Daniels, D.C., Woolley, C.S., and McEwen, B.S., *Adrenal hormones suppress cell division in the adult rat dentate gyrus.* J Neurosci, 1992. **12**(9): p. 3642-50.

68. Gould, E., Woolley, C.S., and McEwen, B.S., *Adrenal steroids regulate postnatal development of the rat dentate gyrus: I. Effects of glucocorticoids on cell death.* J Comp Neurol, 1991. **313**(3): p. 479-85.

69. Gould, E., Woolley, C.S., Cameron, H.A., Daniels, D.C., and McEwen, B.S., *Adrenal steroids regulate postnatal development of the rat dentate gyrus: II. Effects of glucocorticoids and mineralocorticoids on cell birth.* J Comp Neurol, 1991. **313**(3): p. 486-93.

70. van Praag, H., Kempermann, G., and Gage, F.H., *Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus.* Nat Neurosci, 1999. **2**(3): p. 266-70.

71. Gould, E., Beylin, A., Tanapat, P., Reeves, A., and Shors, T.J., *Learning enhances adult neurogenesis in the hippocampal formation.* Nat Neurosci, 1999. **2**(3): p. 260-5.

72. Kempermann, G., Kuhn, H.G., and Gage, F.H., *More hippocampal neurons in adult mice living in an enriched environment.* Nature, 1997. **386**(6624): p. 493-5.

73. Kuhn, H.G., Dickinson-Anson, H., and Gage, F.H., *Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation.* J Neurosci, 1996. **16**(6): p. 2027-33.

74. Zhao, C., Teng, E.M., Summers, R.G., Jr., Ming, G.L., and Gage, F.H., *Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus.* J Neurosci, 2006. **26**(1): p. 3-11.

75. Ge, S., Goh, E.L., Sailor, K.A., Kitabatake, Y., Ming, G.L., and Song, H., *GABA regulates synaptic integration of newly generated neurons in the adult brain.* Nature, 2006. **439**(7076): p. 589-93.

76. Tozuka, Y., Fukuda, S., Namba, T., Seki, T., and Hisatsune, T., *GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells.* Neuron, 2005. **47**(6): p. 803-15.

77. Esposito, M.S., Piatti, V.C., Laplagne, D.A., Morgenstern, N.A., Ferrari, C.C., Pitossi, F.J., and Schinder, A.F., *Neuronal differentiation in the adult hippocampus recapitulates embryonic development.* J Neurosci, 2005. **25**(44): p. 10074-86.

78. Overstreet Wadiche, L., Bromberg, D.A., Bensen, A.L., and Westbrook, G.L., *GABAergic signaling to newborn neurons in dentate gyrus.* J Neurophysiol, 2005. **94**(6): p. 4528-32.

79. Ambrogini, P., Lattanzi, D., Ciuffoli, S., Agostini, D., Bertini, L., Stocchi, V., Santi, S., and Cuppini, R., *Morpho-functional characterization of neuronal cells at different stages of maturation in granule cell layer of adult rat dentate gyrus.* Brain Res, 2004. **1017**(1-2): p. 21-31.

80. Toni, N., Teng, E.M., Bushong, E.A., Aimone, J.B., Zhao, C., Consiglio, A., van Praag, H., Martone, M.E., Ellisman, M.H., and Gage, F.H., *Synapse formation on neurons born in the adult hippocampus.* Nat Neurosci, 2007. **10**(6): p. 727-34.

81. van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., and Gage, F.H., *Functional neurogenesis in the adult hippocampus.* Nature, 2002. **415**(6875): p. 1030-4.

82. Wang, S., Scott, B.W., and Wojtowicz, J.M., *Heterogenous properties of dentate granule neurons in the adult rat.* J Neurobiol, 2000. **42**(2): p. 248-57.

83. Ge, S., Yang, C.H., Hsu, K.S., Ming, G.L., and Song, H., *A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain.* Neuron, 2007. **54**(4): p. 559-66.

84. Schmidt-Hieber, C., Jonas, P., and Bischofberger, J., *Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus.* Nature, 2004. **429**(6988): p. 184-7.

85. Snyder, J.S., Kee, N., and Wojtowicz, J.M., *Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus.* J Neurophysiol, 2001. **85**(6): p. 2423-31.

86. Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., and Gage, F.H., *Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice.* Development, 2003. **130**(2): p. 391-9.

87. Tashiro, A., Makino, H., and Gage, F.H., *Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage.* J Neurosci, 2007. **27**(12): p. 3252-9.

88. Tashiro, A., Sandler, V.M., Toni, N., Zhao, C., and Gage, F.H., *NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus.* Nature, 2006. **442**(7105): p. 929-33.

89. Shors, T.J., Townsend, D.A., Zhao, M., Kozorovitskiy, Y., and Gould, E., *Neurogenesis may relate to some but not all types of hippocampal-dependent learning.* Hippocampus, 2002. **12**(5): p. 578-84.

90. Shors, T.J., Miesegaes, G., Beylin, A., Zhao, M., Rydel, T., and Gould, E., *Neurogenesis in the adult is involved in the formation of trace memories.* Nature, 2001. **410**(6826): p. 372-6.

91. Dupret, D., Montaron, M.F., Drapeau, E., Aurousseau, C., Le Moal, M., Piazza, P.V., and Abrous, D.N., *Methylazoxymethanol acetate does not fully block cell genesis in the young and aged dentate gyrus.* Eur J Neurosci, 2005. **22**(3): p. 778-83.

92. Saxe, M.D., Malleret, G., Vronskaya, S., Mendez, I., Garcia, A.D., Sofroniew, M.V., Kandel, E.R., and Hen, R., *Paradoxical Influence of hippocampal neurogenesis on working memory.* Proceedings of the National Academy of Sciences of the United States of America, 2007. **104**(11): p. 4642-4646.

93. Wojtowicz, J.M., *Irradiation as an experimental tool in studies of adult neurogenesis.* Hippocampus, 2006. **16**(3): p. 261-6.

94. Winocur, G., Wojtowicz, J.M., Sekeres, M., Snyder, J.S., and Wang, S., *Inhibition of neurogenesis interferes with hippocampus-dependent memory function.* Hippocampus, 2006. **16**(3): p. 296-304.

95. Saxe, M.D., Battaglia, F., Wang, J.W., Malleret, G., David, D.J., Monckton, J.E., Garcia, A.D.R., Sofroniew, M.V., Kandel, E.R., Santarelli, L., Hen, R., and Drew, M.R., *Ablation*

of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. Proceedings of the National Academy of Sciences of the United States of America, 2006. **103**(46): p. 17501-17506.

96. Meshi, D., Drew, M.R., Saxe, M., Ansorge, M.S., David, D., Santarelli, L., Malapani, C., Moore, H., and Hen, R., *Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment.* Nature Neuroscience, 2006. **9**(6): p. 729-731.

97. Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., and Hen, R., *Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants.* Science, 2003. **301**(5634): p. 805-809.

98. Monje, M.L., Mizumatsu, S., Fike, J.R., and Palmer, T.D., *Irradiation induces neural precursor-cell dysfunction.* Nat Med, 2002. **8**(9): p. 955-62.

99. Zhang, C.L., Zou, Y., He, W., Gage, F.H., and Evans, R.M., *A role for adult TLX-positive neural stem cells in learning and behaviour.* Nature, 2008. **451**(7181): p. 1004-7.

100. Dupret, D., Revest, J.M., Koehl, M., Ichas, F., De Giorgi, F., Costet, P., Abrous, D.N., and Piazza, P.V., *Spatial relational memory requires hippocampal adult neurogenesis.* PLoS ONE, 2008. **3**(4): p. e1959.

101. Lie, D.C., Colamarino, S.A., Song, H.J., Desire, L., Mira, H., Consiglio, A., Lein, E.S., Jessberger, S., Lansford, H., Dearie, A.R., and Gage, F.H., *Wnt signalling regulates adult hippocampal neurogenesis.* Nature, 2005. **437**(7063): p. 1370-5.

102. Jessberger, S., Clark, R.E., Broadbent, N.J., Clemenson, G.D., Jr., Consiglio, A., Lie, D.C., Squire, L.R., and Gage, F.H., *Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats.* Learn Mem, 2009. **16**(2): p. 147-54.

103. Snyder, J.S., Hong, N.S., McDonald, R.J., and Wojtowicz, J.M., *A role for adult neurogenesis in spatial long-term memory.* Neuroscience, 2005. **130**(4): p. 843-52.

104. van Praag, H., Christie, B.R., Sejnowski, T.J., and Gage, F.H., *Running enhances neurogenesis, learning, and long-term potentiation in mice.* Proc Natl Acad Sci U S A, 1999. **96**(23): p. 13427-31.

105. Chambers, R.A. and Conroy, S.K., *Network modeling of adult neurogenesis: shifting rates of neuronal turnover optimally gears network learning according to novelty gradient.* J Cogn Neurosci, 2007. **19**(1): p. 1-12.

106. Crick, C. and Miranker, W., *Apoptosis, neurogenesis, and information content in Hebbian networks.* Biol Cybern, 2006. **94**(1): p. 9-19.

107. Chambers, R.A., Potenza, M.N., Hoffman, R.E., and Miranker, W., *Simulated apoptosis/neurogenesis regulates learning and memory capabilities of adaptive neural networks.* Neuropsychopharmacology, 2004. **29**(4): p. 747-58.

108. Deisseroth, K., Singla, S., Toda, H., Monje, M., Palmer, T.D., and Malenka, R.C., *Excitation-neurogenesis coupling in adult neural stem/progenitor cells.* Neuron, 2004. **42**(4): p. 535-52.

109. Wiskott, L., Rasch, M.J., and Kempermann, G., *A functional hypothesis for adult hippocampal neurogenesis: avoidance of catastrophic interference in the dentate gyrus.* Hippocampus, 2006. **16**(3): p. 329-43.

110. McCloskey, M. and Cohen, N.J., *Catastrophic interference in connectionist networks: The sequential learning problem.* The Psychology of Learning and Motivation, 1989. **24**: p. 109-165.

111. French, R.M., *Semi-distributed Representations and Catastrophic Forgetting in Connectionist Networks.* Connection Science, 1992. **4**(3): p. 365-377.

112. Butz, M., Teuchert-Noodt, G., Grafen, K., and van Ooyen, A., *Inverse relationship between adult hippocampal cell proliferation and synaptic rewiring in the dentate gyrus.* Hippocampus, 2008. **18**(9): p. 879-98.

113. Butz, M., Lehmann, K., Dammasch, I.E., and Teuchert-Noodt, G., *A theoretical network model to analyse neurogenesis and synaptogenesis in the dentate gyrus.* Neural Netw, 2006. **19**(10): p. 1490-505.

114. Lehmann, K., Butz, M., and Teuchert-Noodt, G., *Offer and demand: proliferation and survival of neurons in the dentate gyrus.* Eur J Neurosci, 2005. **21**(12): p. 3205-16.

115. Becker, S., *A computational principle for hippocampal learning and neurogenesis.* Hippocampus, 2005. **15**(6): p. 722-38.

116. Becker, S. and Wojtowicz, J.M., *A model of hippocampal neurogenesis in memory and mood disorders.* Trends Cogn Sci, 2007. **11**(2): p. 70-6.

CHAPTER III: THEORY BACKGROUND AND REVIEW: REPRINT OF "POTENTIAL ROLE FOR ADULT NEUROGENESIS IN THE ENCODING OF TIME IN NEW MEMORIES" – *AIMONE, WILES & GAGE; 2006*

The following is a reprint of the Perspective article "Potential Role for Adult Neurogenesis in the Encoding of Time in New Memories" published in *Nature Neuroscience* in June, 2006[1]. This article is theoretical in scope, describing how the details of the biophysical maturation of new neurons may affect the presumed pattern separation function of the dentate gyrus. The hypotheses described in this article were developed in parallel to the modeling work that is described in later chapters and illustrates the development of the theoretical framework used in the interpretation of the subsequent modeling results.

Abstract

The dentate gyrus in the hippocampus is one of two brain regions with lifelong neurogenesis in mammals. Despite an increasing amount of information about the characteristics of the newborn granule cells, the specific contribution that their robust generation plays in memory formation by the hippocampus remains unclear. We describe here a possible role that this population of young granule cells may play in the formation of temporal associations in memory. Neurogenesis is a continuous process; the newborn population is only composed of the same cells for a short period of time. As time passes, the young neurons mature or die and others are born, gradually changing the identity of this young population. We discuss the possibility that one cognitive impact of this gradually changing population on hippocampal memory formation is the formation of the temporal clusters of long-term episodic memories seen in some human psychological studies.

Introduction

The past decade has seen a dramatic increase in our understanding of the mechanisms involved in, and the extent of, the continuing addition of new neurons in the adult brain[2, 3]. The persistent lifetime incorporation of new granule cells into the dentate gyrus has been demonstrated in rodents, primates and humans[4, 5]. The effects of environment and behavior on the dynamics of the neurogenesis process are being revealed[6-8], but animal studies focusing on the impact of neurogenesis on behavior have been inconclusive[9].

In this Perspective, we have taken a somewhat different approach to investigate the function of adult neurogenesis. By applying recent findings about the developmental properties of newborn granule cells to what is known about the surrounding circuit, we have developed an idea for one role that continuous neurogenesis would have in the function of the dentate gyrus. Mounting evidence that immature granule cells are possibly more "excitable," with a stronger propensity for LTP than fully mature neurons, suggests that these cells may have a unique role in the processing of the dentate gyrus circuit[10, 11]. By considering the dentate gyrus's theorized role in hippocampal processing, we are able to make specific predictions about how immature neurons may be affecting hippocampus-dependent learning and memory formation[12].

Notably, new neurons are not integrating into a cell layer thought to be involved in memory storage, but rather into a hippocampal structure with the theoretically straightforward role of pattern separation. While there are many hypotheses about how the hippocampal circuit functions in memory formation, storage, and retrieval, the idea that the dentate gyrus provides distinct codes to the network via the granule cells' mossy fibers has been one of the least controversial[13-15]. This function appears computationally inevitable due to its highly divergent input structure (~200,000 entorhinal cortex cells project to >1 million dentate gyrus granule cells in rat) and the sparse, powerful mossy fiber projection to the CA3[16, 17]. Sparse activity in the dentate gyrus following exposure to spatial environments has been observed experimentally with both implanted electrodes[18] and immediate-early gene studies[19], with

the latter study suggesting that different sets of granule cells are activated in response to exposure to two distinct environments.

The necessity of sparse, orthogonal (distinct) inputs into the hippocampus has been well described computationally[20], and it has been suggested biologically that the powerful dentate gyrus projection drives how the CA3 responds to its entorhinal and recurrent inputs[21, 22]. Behaviorally, there is evidence that dentate gyrus-specific lesions can disrupt the acquisition of some spatial memories, despite the remaining direct entorhinal to CA3 input[23, 24]. However, neurogenesis knock-downs are most likely not the equivalent of a full dentate gyrus lesion, and there is no reason to believe that neurogenesis is required for the dentate gyrus to produce sparse codes. Notably, the dentate gyrus was considered to be the hippocampus's source of sparse-code generation long before the existence of adult neurogenesis was widely recognized[20]. Consistent with the idea that neurogenesis may not be required for all dentate gyrus functions, experiments that repress neurogenesis have generally failed to show the same short-term acquisition deficits that dentate gyrus lesions show[25].

Effects of Neuron Addition on Dentate Gyrus Coding

Several recent studies have looked at the computational effects of neurogenesis within the biological context of the hippocampus[26, 27]. Mathematically, it is unclear what effect the continuous addition of neurons to a sparsification layer would have on the structure of codes produced by the dentate gyrus, but it has been suggested that the increased number of possible distinct codes would ultimately increase hippocampal memory capacity[27] or reduce interference between existing memories[26]. While these functions are plausible implications of replacing neurons in this circuit, the increasing evidence suggesting that these new neurons are not functionally identical to the existing granule cells during their development has additional implications for their function within the dentate gyrus[28](Fig. 1). Although it is still unknown

when or how these new neurons begin to influence CA3 pyramidal neurons, it is known that they form mossy fiber connections onto the CA3 early in development [29, 30] and exhibit action potentials within 3 weeks [31]. On the other hand, the new neurons' electrophysiological properties appear to remain different from mature granule cells even when they are over a month old[32]. Newborn granule cells have lower activation thresholds, higher resting potentials, and increased levels of LTP compared to fully developed granule cells [10, 11]. Furthermore, immediate early gene studies (an indicator of cellular activity) suggest that these cells are more responsive to novel environments (H. Makino, A. Tashiro, and FHG, *Soc. Neurosci. Abst.* 141.3, 2005).

What would this increased activity of new cells mean for the sparsification performed by the dentate gyrus? Immediately, the activity patterns of the dentate gyrus would become somewhat less sparse, as there would be several thousand new neurons responding to a wider range of inputs. Presumably, the function of the dentate gyrus is to provide not just sparse codes but codes that are orthogonal, or distinct, to the CA3[20]. However, the inclusion of neurogenesis might have the opposite effect on the separation of two events. If the newborn neurons are more likely to fire than mature cells, they would provide a disproportionately larger part of the dentate gyrus's sparse code. Two unique and novel events would have the same subset of neurons disproportionately represented in their sparse codes. The production of less sparse codes by the newborn neurons would result in more overlap in their outputs in response to different events. As a result, the overall overlap between codes for two distinct events would be substantially increased.

For example, when a novel event occurs, a sparse subset of neurons (referred to here as a "pattern") in the dentate gyrus will respond (Fig. 2a, 1st frame). These granule cells in turn project to a limited set of CA3 pyramidal neurons, which are connected to one another and form a new CA3 pattern. According to conventional hippocampal theory, a different novel event will

then induce a different pattern of dentate gyrus granule cells, which in turn will create a new pattern in the CA3 (Fig. 2a, 2nd frame). Now these two events are encoded in highly independent patterns by the recurrent network in the CA3 (Fig. 2a, 3rd frame). This creation of distinct patterns within the CA3 has long been the assumed role of the dentate gyrus[20].

The inclusion of more responsive new neurons would alter this mechanism. When the dentate gyrus responds to a novel event, young neurons are included in the sparse pattern that is generated (Fig. 2b, 1st frame). However, these same young neurons are likely to be included in the coding for the other events as well (Fig. 2b, 2nd frame). Since these new cells also have connections to the CA3, a subset of CA3 pyramidal cells exists that would be included in both events. As a result, the patterns generated within the CA3 are no longer independent; rather, they overlap because each event's pattern includes the pyramidal cells activated by the newborn neurons (Fig. 2b, 3rd frame).

This rough analysis would suggest that neurogenesis is detrimental to the proposed goal of separating similar inputs into distinct codes for the hippocampus. But this would only be the case transiently. It is important to consider that these newborn neurons make up a dynamic, constantly changing subset. The neurons mature out of this group, ultimately becoming less active and almost indistinguishable from the other granule cells[31]. New neurons are constantly being born, forming unique downstream connections, and passing through periods of higher activity compared to more mature cells. Eventually, those that survive are destined to become sparse coders themselves. Therefore, although newborn neurons may be included in encoding a novel event (Fig. 2c, 1st frame), those neurons will mature or die and a different set of newborn neurons (with different CA3 projections) will be involved in the encoding of different events that occur at later times (Fig. 2c, 2nd frame). As a result, two events that occur far apart in time will not form the same degree of overlap as two events that occur close in time (Fig. 2c, 3rd frame).

From these empirical observations and analytical conclusions, we are able to hypothesize that there are two functional populations of granule cells. One is the set of mature granule cells, creating highly sparse representations of entorhinal inputs and, in so doing, providing distinct codes to the downstream CA3. The other group is a constantly changing set of newborn neurons that respond to entorhinal inputs in a less discriminatory manner, thus adding a component of temporal similarity to the codes sent to the CA3. The CA3, driven by mossy fiber input, may not distinguish between these two populations. The implications of such a hypothetical binding are substantial. It is plausible that these similarities in dentate gyrus outputs would result in a parallel subset of recurrently connected pyramidal cells in the CA3, and this subset would in turn be included in the CA3 representations of the events that are occurring. As the subset of immature granule cells changes, so would the CA3 subset. Once such codes exist in the CA3, this effect would theoretically propagate to downstream regions – the CA1, subiculum and ultimately back to the deep layers of the entorhinal cortex. In this manner, time-associated patterns could become fully integrated into the hippocampal processing based on temporal information provided not by sensory information, but by the intrinsic rate of neurogenesis.

How long would this time-association occur within the sparse codes produced by the dentate gyrus? The maturation of newborn neurons takes approximately one to two months in rodents[33], but there are several phases of this maturation process[2]. The excitability of newborn neurons most likely decreases from the time spinogenesis begins (~16 days), which is around the same time GABA may begin to have an inhibitory influence on the neuron[31]. While there are indications that newborn neurons remain more responsive than fully mature cells for some time[10], the maturation of dendritic arborizations and reduced spine formation by one month of age[30] probably make the neurons respond more selectively. Because the subset of newborn neurons is constantly changing, the similarity in coding would be greatest for events occurring at about the same time, when each event would activate a similar newborn neuron

population (Fig. 2b). As days and weeks pass, the young neurons would mature or die and others would be born, changing the newborn neuron subset, thereby reducing the similarity between the two events. After several weeks, the population of young neurons would be completely different (Fig. 2c).

Structure of Temporal Information in Human Memory

What we have described here is a biological mechanism by which temporal associations can form in the outputs of the dentate gyrus. While the physical incorporation of such information into neural networks is a novel concept biologically, the existence of "time" in our memories has been debated for centuries. The actual manner in which long-term memories are associated has been a matter of contention psychologically for over a century and philosophically for much longer. Aristotle's *On Memory and Reminiscence,* for example, discusses the association of memories by time. However, while it has long been evident that human memories have a temporal component, it is not at all clear how this information is encoded.

Psychology research over recent decades has led to several distinct hypotheses of how time is associated with autobiographical memories[34]. Although these studies suggest that conventional dates are most likely not "tagged" onto most memories, there is increasing evidence that important events, or temporal landmarks, are either encoded with conventional dates[35] or have dates that are predictable, such as birthdays and holidays[36]. Furthermore, there is increasing evidence that proximal memories somehow remain associated with one another later in life[37, 38](Fig. 3). Consistent with this finding, it appears that priming with an unrelated but proximal dated memory rapidly improves our ability to date less important autobiographical memories. For example, an individual may not be able to provide a date for a memory but may be able to use nearby memories with known dates to approximate an answer[36].

Most psychological theories that have shown that time is encoded into autobiographical memory are based on interviews and diary studies and do not focus on the biological mechanism by which this encoding takes place. Although there is little biological understanding of how specific mnemonic associations are formed, neurological observations over the past century have indicated where in the brain long-term memories are physically formed and eventually stored in humans[39, 40], with the hippocampus being recognized as one of the critical structures in the establishment of long-term memories[41, 42]. It is our hypothesis that an overlap in dentate gyrus sparse codes initiates these temporal associations in the hippocampus during early stages of memory formation. This temporal association memory may be permanently coupled with the sensory information to contribute to the formation of memories that are temporally tied into the individual's autobiography.

The dentate gyrus is presumably only involved in the initial encoding of information. Little is known about how memories are stored long-term and retrieved beyond the observation that, at some point, these new memories become independent of the hippocampus[39, 40, 43]. How information storage occurs remains unclear, though there is evidence that prefrontal cortical regions are involved in the consolidation, storage and retrieval of long-term memories, and it is likely that combinations of the prefrontal and other cortical regions are involved in the ability to recall remote memories[39, 44]. If this temporal information is initially encoded by the hippocampus, as we are proposing, its long-term storage and eventual retrieval (as shown in Fig. 3) would most likely occur in other regions and no longer involve the newborn neurons that originally took part in its encoding.

Future Testing of Temporal Association Memory Hypothesis

The experimental observation of the process outlined here will most likely not be a trivial task. Temporal associations in memories have been described mostly in humans, but our

understanding of human neurogenesis is limited[5]. Human neurogenesis has only been definitively studied post-mortem, and there has been no rigorous quantitative analysis of neurogenesis levels in young adults. One possibility is to look to several neurological observations as an opportunity to test this hypothesis, as there is a large body of research suggesting that neurogenesis levels are altered in numerous animal models of human conditions. Neurogenesis has been shown to decrease substantially in animal models of aging, depression, and alcoholism[45-47] - conditions that have all been associated with memory loss in humans[48-50]. Although each of these conditions is associated with widespread neurological pathologies, with the role of neurogenesis in each being poorly defined, it would still be interesting to see if these conditions are also accompanied by a degraded ability to remember temporal associations in memories formed during putatively low-neurogenesis periods. Not all neurological conditions exhibit a decrease in hippocampal neurogenesis – for example, some seizures and neurodegenerative disorders are accompanied by increased levels of neurogenesis[2]. It may also be interesting to monitor whether temporal association memory is enhanced in any such conditions that do not lead to general cognitive impairments.

Likewise, because neurogenesis has been characterized primarily in rats and mice, it would be beneficial to find an appropriate behavioral task that tests temporal associations in these animals. Testing rodents on complex memory tasks is difficult for numerous reasons[40], and to our knowledge temporal associations between tasks have never been studied at this level. It may be possible to design a behavioral paradigm that relies on temporal associations between two hippocampus-dependent tasks in which the role of neurogenesis can be fully ascertained in conditions that either amplify or knock down neurogenesis.

It should also be emphasized that the hypothesis presented here focuses exclusively on newborn granule cells at the specific developmental time between their initial involvement in the network and full maturation. Determining when newborn neurons begin to communicate to the

CA3 will be interesting, as it appears that their axons reach CA3 when the GABAergic inputs are excitatory[30] – though there are indications that GABA-driven cells may not exhibit action potentials[31]. Fully characterizing the duration of maturation and whether it is behaviorally modulated will help us further understand the impact of developing new neurons. The long-term survival of adult-born granule cells suggests an additional long-term function for these cells, since once the excitability of newborn neurons returns to the level of "mature" granule cells, the time coding function would presumably be complete. Therefore, it is likely that the long-term impact of these cells is quite different from that during their early period of increased excitability. Although this later function remains unclear, we feel that this early property of neurogenesis is a possible explanation of one of the long-standing problems facing our understanding of memory – how do we remember when things happened?

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Chapter III, in full, is a reprint of the article "Potential Role for Adult Neurogenesis in the Encoding of Time in New Memories," Aimone, James B; Wiles, Janet; and Gage, Fred H.; *Nature Neuroscience*, June 2006. The dissertation author was the primary investigator and author of this paper.

Figures

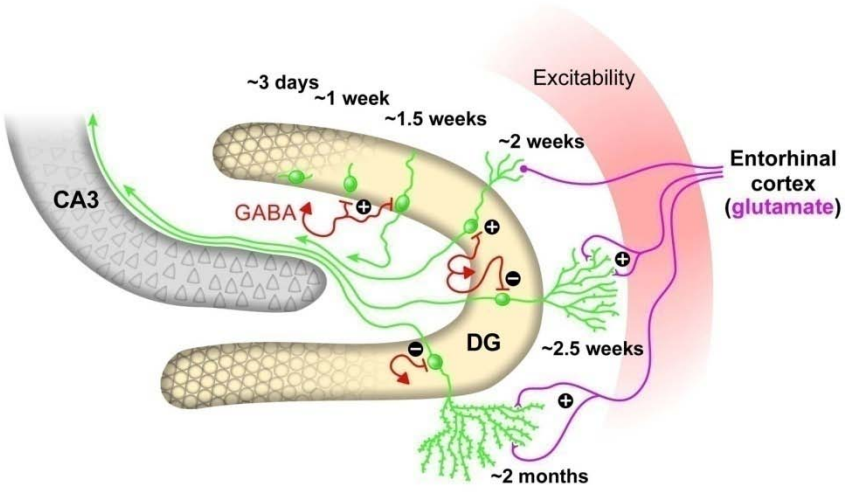

Figure III-1: Growth and maturation of adult newborn granule cells.
At 3 days, newborn cells have few projections and are migrating to their final location. At 1 week, early non-oriented dendrites appear, coupled with the onset of somatic GABA inputs (red neurons), and by 1 ½ weeks the primary apical dendrite extends to the molecular layer. 2 week-old neurons have aspiny, developed dendritic arborizations with extensive GABA input. By 2 ½ weeks, spines have begun to appear, indicating the onset of entorhinal input, though not at the densities seen in fully mature cells. By 2 months, neurons have arborizations and electrophysiology similar to mature neurons. GABA is excitatory in immature neurons but becomes inhibitory around the time the excitatory glutamatergic synapses are established. The bar labeled "excitability" indicates the time period when immature neurons have a distinct physiology, such as more depolarized resting potentials, and demonstrate increased levels of LTP.

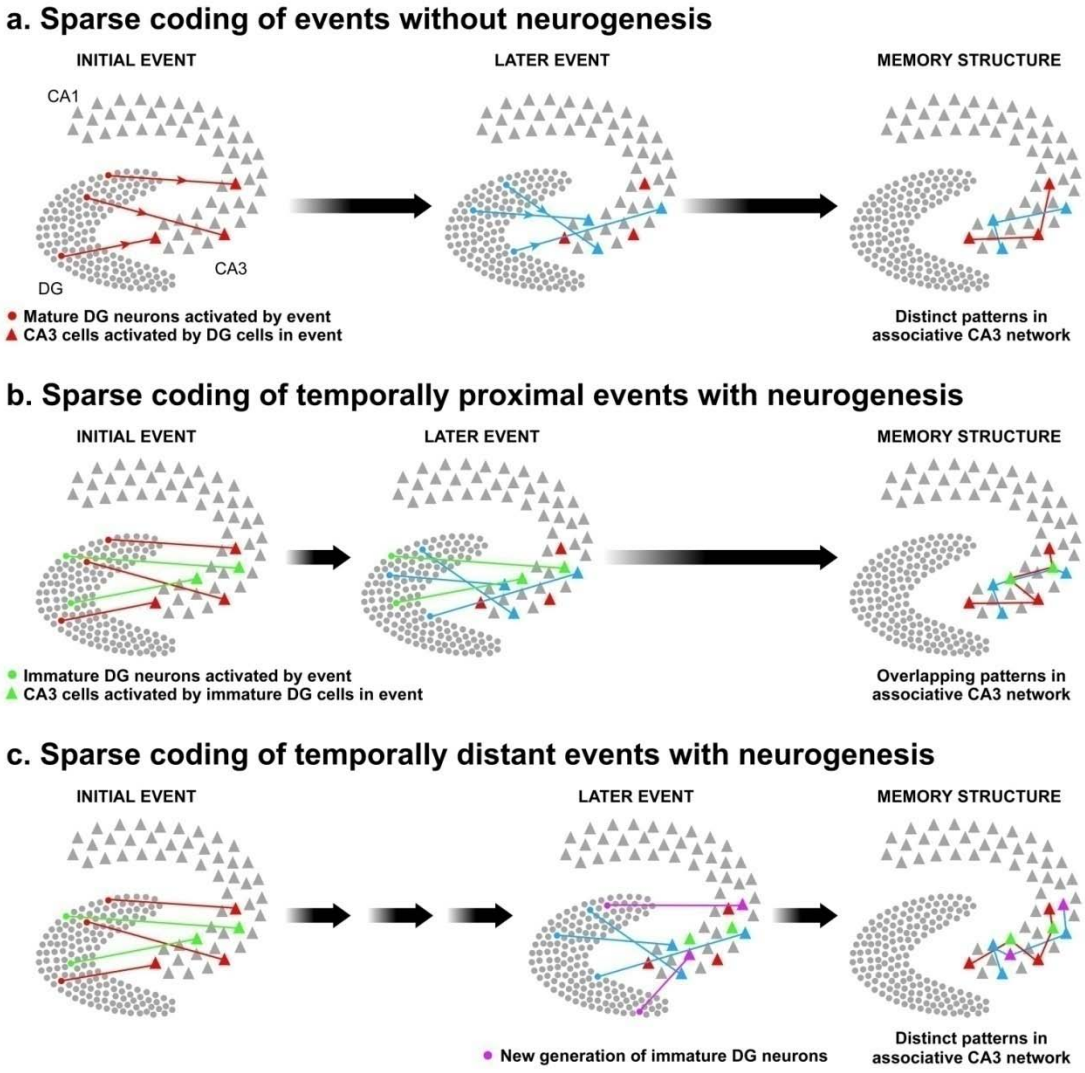

Figure III-2: Schematic showing how newborn granule cells may encode temporal memories in the hippocampus.
(a) Without neurogenesis, two distinct events would activate separate sparse populations of dentate gyrus granule cells (red and blue). These patterns would result in two highly distinct CA3 patterns (red and blue). (b) With neurogenesis, two events that occur at about the same time (for example, within a week of each other) would activate the same population of newborn neurons (green) in addition to the distinct patterns (red and blue) formed within the mature granule cell population. As a result, the CA3 patterns for the two memories, while different, now partially overlap. (c) Events that are temporally remote would encounter different populations of newborn neurons (green and purple). As a result, the memories formed within the CA3 no longer overlap.

Figure III-3: Cartoon example of how temporal associations may exist in long-term human memories.
The reactivation of an old memory – such as hearing a hit song again years later – can induce the recollection of other memories that were formed at the same time. According to this hypothesis, these memories would have been originally encoded in part by the same set of young neurons, although this recall would most likely be hippocampus-independent. Some memories may be general to the time of life – a summer internship, for example. Others may be repeated events that also occurred during that time period, such as visiting a relative. Finally, meaningful personal events may be recalled, such as meeting someone important for the first time.

References

1. Aimone, J.B., Wiles, J., and Gage, F.H., *Potential role for adult neurogenesis in the encoding of time in new memories.* Nat Neurosci, 2006. **9**(6): p. 723-7.

2. Ming, G.L. and Song, H., *Adult neurogenesis in the mammalian central nervous system.* Annual Review of Neuroscience, 2005. **28**: p. 223-50.

3. Gage, F.H., *Neurogenesis in the adult brain.* Journal of Neuroscience, 2002. **22**(3): p. 612-3.

4. Altman, J. and Bayer, S.A., *Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods.* Journal of Comparative Neurology, 1990. **301**(3): p. 365-81.

5. Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., and Gage, F.H., *Neurogenesis in the adult human hippocampus.* Nature Medicine, 1998. **4**(11): p. 1313-7.

6. van Praag, H., Kempermann, G., and Gage, F.H., *Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus.* Nature Neuroscience, 1999. **2**(3): p. 266-70.

7. Gould, E., Beylin, A., Tanapat, P., Reeves, A., and Shors, T.J., *Learning enhances adult neurogenesis in the hippocampal formation.* Nature Neuroscience, 1999. **2**(3): p. 260-5.

8. Kempermann, G., Kuhn, H.G., and Gage, F.H., *More hippocampal neurons in adult mice living in an enriched environment.* Nature, 1997. **386**(6624): p. 493-5.

9. Leuner, B., Gould, E., and Shors, T.J., *Is there a link between adult neurogenesis and learning?* Hippocampus, 2006. **16**(3): p. 216-24.

10. Schmidt-Hieber, C., Jonas, P., and Bischofberger, J., *Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus.* Nature, 2004. **429**(6988): p. 184-7.

11. van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., and Gage, F.H., *Functional neurogenesis in the adult hippocampus.* Nature, 2002. **415**(6875): p. 1030-4.

12. Kempermann, G., Wiskott, L., and Gage, F.H., *Functional significance of adult neurogenesis.* Current Opinion in Neurobiology, 2004. **14**(2): p. 186-91.

13. McNaughton, B.L. and Morris, R.G., *Hippocampal synaptic enhancement and information storage within a distributed memory system.* Trends in Neurosciences, 1987. **10**(10): p. 408-415.

14. Treves, A. and Rolls, E.T., *Computational analysis of the role of the hippocampus in memory.* Hippocampus, 1994. **4**(3): p. 374-91.

15. Hasselmo, M.E., Wyble, B.P., and Wallenstein, G.V., *Encoding and retrieval of episodic memories: role of cholinergic and GABAergic modulation in the hippocampus.* Hippocampus, 1996. **6**(6): p. 693-708.

16. Henze, D.A., Wittner, L., and Buzsaki, G., *Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo.* Nature Neuroscience, 2002. **5**(8): p. 790-5.

17. Amaral, D.G., Ishizuka, N., and Claiborne, B., *Neurons, numbers and the hippocampal network.* Prog Brain Res, 1990. **83**: p. 1-11.

18. Jung, M.W. and McNaughton, B.L., *Spatial selectivity of unit activity in the hippocampal granular layer.* Hippocampus, 1993. **3**(2): p. 165-82.

19. Chawla, M.K., Guzowski, J.F., Ramirez-Amaya, V., Lipa, P., Hoffman, K.L., Marriott, L.K., Worley, P.F., McNaughton, B.L., and Barnes, C.A., *Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience.* Hippocampus, 2005. **15**(5): p. 579-86.

20. Treves, A. and Rolls, E.T., *Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network.* Hippocampus, 1992. **2**(2): p. 189-99.

21. Kobayashi, K. and Poo, M.M., *Spike train timing-dependent associative modification of hippocampal CA3 recurrent synapses by mossy fibers.* Neuron, 2004. **41**(3): p. 445-54.

22. McMahon, D.B. and Barrionuevo, G., *Short- and long-term plasticity of the perforant path synapse in hippocampal area CA3.* Journal of Neurophysiology, 2002. **88**(1): p. 528-33.

23. Kesner, R.P., Lee, I., and Gilbert, P., *A behavioral assessment of hippocampal function based on a subregional analysis.* Reviews in the Neurosciences, 2004. **15**(5): p. 333-51.

24. Lee, I. and Kesner, R.P., *Encoding versus retrieval of spatial memory: double dissociation between the dentate gyrus and the perforant path inputs into CA3 in the dorsal hippocampus.* Hippocampus, 2004. **14**(1): p. 66-76.

25. Shors, T.J., Townsend, D.A., Zhao, M., Kozorovitskiy, Y., and Gould, E., *Neurogenesis may relate to some but not all types of hippocampal-dependent learning.* Hippocampus, 2002. **12**(5): p. 578-84.

26. Wiskott, L., Rasch, M., and Kempermann, G., *What is the functional role of adult neurogenesis in the hippocampus?* Cognitive Sciences EPrint Archive, 2005. **CogPrints**(4012): p. http://cogprints.org/4012/.

27. Becker, S., *A computational principle for hippocampal learning and neurogenesis.* Hippocampus, 2005. **15**(6): p. 722-38.

28. Schinder, A.F. and Gage, F.H., *A hypothesis about the role of adult neurogenesis in hippocampal function.* Physiology (Bethesda), 2004. **19**: p. 253-61.

29. Hastings, N.B. and Gould, E., *Rapid extension of axons into the CA3 region by adult-generated granule cells.* Journal of Comparative Neurology, 1999. **413**(1): p. 146-54.

30. Zhao, C., Teng, E.M., Summers, R.G., Ming, G.L., and Gage, F.H., *Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus.* Journal of Neuroscience, 2005. **in press**.

31. Esposito, M.S., Piatti, V.C., Laplagne, D.A., Morgenstern, N.A., Ferrari, C.C., Pitossi, F.J., and Schinder, A.F., *Neuronal Differentiation in the Adult Hippocampus Recapitulates Embryonic Development.* Journal of Neuroscience, 2005. **25**(44): p. 10074-10086.

32. Song, H., Kempermann, G., Wadiche, L.O., Zhao, C., Schinder, A.F., and Bischofberger, J., *New neurons in the adult mammalian brain: synaptogenesis and functional integration.* Journal of Neuroscience, 2005. **25**(45): p. 10366-8.

33. Kempermann, G., Jessberger, S., Steiner, B., and Kronenberg, G., *Milestones of neuronal development in the adult hippocampus.* Trends in Neurosciences, 2004. **27**(8): p. 447-52.

34. Friedman, W.J., *Memory for the time of past events.* Psychological Bulletin 1993. **113**(1): p. 44-66.

35. Loftus, E.F. and Marburger, W., *Since the eruption of Mt. St. Helens, has anyone beaten you up? Improving the accuracy of retrospective reports with landmark events.* Memory and Cognition, 1983. **11**: p. 114-120.

36. Shum, M.S., *The Role of Temporal Landmarks in Autobiographical Memory Process.* Psychological Bulletin 1998. **124**(3): p. 423-442.

37. Brown, N.R. and Schopflocher, D., *Event Cueing, Event Clusters, and the Temporal Distribution of Autobiographical Memories.* Applied Cognitive Psychology, 1998. **12**: p. 305-319.

38. Burt, C.D.B., Kemp, S., and Conway, M.A., *Themes, events, and episodes in autobiographical memory.* Memory and Cognition, 2003. **31**(2): p. 317-325.

39. Frankland, P.W. and Bontempi, B., *The organization of recent and remote memories.* Nat Rev Neurosci, 2005. **6**(2): p. 119-30.

40. Squire, L.R., Stark, C.E., and Clark, R.E., *The medial temporal lobe.* Annual Review of Neuroscience, 2004. **27**: p. 279-306.

41. Zola-Morgan, S., Squire, L.R., and Amaral, D.G., *Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus.* Journal of Neuroscience, 1986. **6**(10): p. 2950-67.

42. Marr, D., *Simple memory: a theory for archicortex.* Philos Trans R Soc Lond B Biol Sci, 1971. **262**(841): p. 23-81.

43. Bayley, P.J., Gold, J.J., Hopkins, R.O., and Squire, L.R., *The neuroanatomy of remote memory.* Neuron, 2005. **46**(5): p. 799-810.

44. Wiltgen, B.J., Brown, R.A., Talton, L.E., and Silva, A.J., *New circuits for old memories: the role of the neocortex in consolidation.* Neuron, 2004. **44**(1): p. 101-8.

45. Kuhn, H.G., Dickinson-Anson, H., and Gage, F.H., *Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation.* Journal of Neuroscience, 1996. **16**(6): p. 2027-33.

46. Nixon, K. and Crews, F.T., *Binge ethanol exposure decreases neurogenesis in adult rat hippocampus.* Journal of Neurochemistry, 2002. **83**(5): p. 1087-93.

47. Malberg, J.E., Eisch, A.J., Nestler, E.J., and Duman, R.S., *Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus.* Journal of Neuroscience, 2000. **20**(24): p. 9104-10.

48. White, A.M., Matthews, D.B., and Best, P.J., *Ethanol, memory, and hippocampal function: a review of recent findings.* Hippocampus, 2000. **10**(1): p. 88-93.

49. Austin, M.P., Mitchell, P., and Goodwin, G.M., *Cognitive deficits in depression: possible implications for functional neuropathology.* British Journal of Psychiatry, 2001. **178**: p. 200-6.

50. Erickson, C.A. and Barnes, C.A., *The neurobiology of memory changes in normal aging.* Experimental Gerontology, 2003. **38**(1-2): p. 61-9.

CHAPTER IV: METHODOLOGY: COMPUTATIONAL MODELING OF ADULT NEUROGENESIS

Overview and Objectives of Modeling

This chapter will describe in detail the computational model used to obtain the results described in subsequent chapters. The computational details here were described in the Supplemental Text of Aimone et al., 2009 [1]. Computational models can exist at very different levels of detail. As such, the complexity of the model is often dictated by the questions being asked and the type of answers desired. In the design of the computational model described here, there were several important project constraints that motivated how to approach the modeling.

Objective #1: Biological Realism

The primary constraint in designing the model was to ensure that the model was representative of the dentate gyrus and the neurogenesis process in particular. There are several reasons to emphasize this realism. First, it was important for the model to make biologically testable predictions. Second, it was important that the model design and results of the model could be communicated effectively to neuroscientists, as it is that community that would choose whether to follow up on the results experimentally. Finally, a well-designed biologically realistic model can be updated in response to changing scientific literature, whereas this may be more difficult in models that generalize from the literature.

It is important to observe that none of these constraints mandate a complex modeling approach. For instance, simple models can produce biologically testable predictions and often reveal fundamental features of the system, as well as being easier describe to non-experts. Furthermore, because most models are abstractions of a real system, the biological realism of all models is at some level limited. However, the more abstract a model is, the less confident one is

that the results emerged from the biology in the model as opposed to the simplifications involved in its generation.

Objective #2: Minimize assumptions about neurogenesis function

Adult neurogenesis is an unusual process in that there are no widely accepted theories for its function. Scientists have considered that new neurons may have roles in memory formation and spatial processing, but there is little consensus for the actual mechanism. While there have been several computational models that were designed to examine specific hypotheses for neurogenesis function [2-4], there has not been a substantial movement to validate their conclusions biologically.

The drawback to building a model to test a particular hypothesis is that a bias is built into the work. Because models can exist in a wide variety of forms, it is often not unexpected that the hypothesis will be supported. Such results demonstrate computational sufficiency (i.e., neurogenesis can help avoid catastrophic interference), but it is practically impossible to show necessity. Furthermore, the sufficiency argument only applies to the actual model used, not the system that has been modeled. In essence, claiming that a "model designed to do X in fact does X" may be interesting from an academic perspective, but it is not necessarily a strong indicator of the underlying function.

While it is important that a priori notions about function not affect model design, it is also important to minimize their influence on testing of the model. To some extent, it is impossible to avoid such bias, because there must be some focus of study. However, it is possible to commit to examining the types of functions already studied in the larger system. For example, with DG neurogenesis one can begin a study with pattern separation, which is widely accepted as the function of DG. While such an approach may limit the results of the study to pattern separation effects, the broader literature gives reason to believe that such effects may exist.

Objective #3: Make model computationally tractable

A more practical consideration when determining the model scope is that it is important to limit the complexity of the model to what can be simulated in a reasonable amount of time and for a reasonable cost. Many computational models have been designed to capture all biological details, only to be too resource intensive for simulation [5]. While increasing computer power partially alleviates this problem, there are still practical limits to what can be simulated and analyzed under standard conditions.

Similarly, there is natural belief that increasing the complexity of a model increases the precision of the results. This is often not the case. As complexity increases, the number of approximations necessary grows. Even if there is a strong amount of confidence with such approximations, when there are many assumptions errors can accumulate quickly.

Along these lines, the availability of biological data is a limiting factor in choosing the level at which to model. While there is substantial anatomical and structural data available for many neurons in the DG that can be incorporated into a sophisticated model [5-8], comparable data does not exist for the neurogenesis system. Since neurogenesis is the feature of interest, it is only feasible to model at the level of precision offered by anatomical and physiology studies on adult-born neurons [9-17].

Level of detail in model

Given the above goals in the design of this model, the decision was made to model neurogenesis in the DG at a relatively sophisticated level, but at a step less detailed than other models [6, 18]. Neurons were simulated at broad time steps (25ms), because data at the conductance level is not available for immature neurons and the time scale of neurogenesis (weeks and months) is many orders of magnitude greater than that used in spiking models.

Anatomically, the model incorporates many of the neuron populations present in the DG, as in the case with other DG models [5, 6]. However, because the networks were simulated over

longer time windows, size was a limiting factor in the computational feasibility of the design. Therefore, the model used on the order of 2,000 neurons, about 100-fold reduction from the mouse DG. At this size, several neuronal populations (i.e., axo-axonic neurons) were fractionally represented, and therefore removed from the model.

Future work will investigate the scalability of the model, in both architecture size and temporal resolution, as computational resources become available.

General Structure of Model Simulations

Building the model

Prior to simulation, the model is initially generated with a population of immature GC neurons and full populations of all other cell layers. All connections that are independent of the GC layer were initialized at onset, while all connections involving the GC layer are formed during simulation.

1> Load basic model parameters

2> Initialize all neuron layers

3> Initialize non-GC connections

"Growing" the model

The model was then simulated with a specific input structure for many events to permit the original neurons GC layer to mature and further GC neurons to be born (neurogenesis). This is referred to as "growing" the model.

A full run through the model during training takes the following form:

1> Load model

2> Determine how inputs will look in experiment

3> Advance through time – each full loop through model considered an "event"

1. Update all neurons' physiology properties based on age and connectivity
2. Calculate inputs for event (or time-vector of inputs)
3. Compute neuronal activity
    1. Neuronal activity was calculated for a series discrete time "steps"
4. Synaptic Learning
5. Mature immature neurons
6. Add new neurons
7. Cell death

4> Export grown model & activity history

Experiments

The model was tested within different environments at many different locations. During testing, there was no neurogenesis, maturation, learning or cell death (a "static" model) so there is no interaction between different test trials. A full run through the model during testing takes the following form:

1> Load model

2> Determine how inputs will look in experiment

3> Simulate model at different locations

- a. Update all neurons' physiology properties based on age and connectivity
- b. Calculate inputs for event at current position
- c. Compute neuronal activity
    1. Neuronal activity was calculated for a series discrete time "steps"

4> Export grown model & activity history

Table I shows the general model parameters used in the study.

Table IV-1: General Model Parameters

Parameter	*Value*
Evt_{Day}	10 events
Evt_{Week}	70 events
t_{step}	25 ms
t_{event} (training)	10 s
t_{event} (testing)	500 ms
Range	40%

Evt_{Day} is the number of simulated events in each day
Evt_{Week} is the number of simulated events in each week
t_{step} is the length of each discrete time-interval for which activity is calculated
t_{event} (training) is the length of time that each event is simulated during training
t_{event} (testing) is the number of time that each event is simulated during testing
Range is the septotemporal extent of the model used in these experiments

Simulation details

Simulations and all subsequent analysis was performed using MATLAB 7.4 running on a Linux platform and were performed on a cluster of four Dell Precision 490n machines (2 x Dual Core Xeon 5130 2Ghz; 16GB RAM), for a total of 16 independent processors using the MATLAB Distributed Computing Engine.

The model contains involves considerable usage of random variables. Random numbers were either generated from a uniform distribution, using the `rand()` function in MATLAB, or from a Gaussian distribution, using the `randn()` function in MATLAB, which returns a random value from a normal distribution with mean 0 and standard deviation of 1. To attain a random value,η, from a different Gaussian distribution (mean = μ, standard deviation = σ), the following equation was used

$$\eta(\mu,\sigma) = \mu + \sigma \times randn() \quad \text{(IV-1)}$$

The random seed was initialized to a unique value (current date/time) prior to all simulations.

Statistics

For each of the simulation runs described below, eight different model networks were generated and simulated independently. While the initialization parameters were the same for each network, the environments used and their growth differed across runs, resulting in considerably different networks at the point at which they were examined.

Error bars plotted in the data represent the **standard deviation** across the different model networks.

Model Architecture and Layout

Neuron classes and parameters

The DG model consists of six classes of neurons, referred to as "layers" (Figure IV-1). Each layer contains many neurons, with the cell numbers selected in order to reflect the underlying biological system (see Chapter II). Each neuron in the model was defined by a set of physical and physiology parameters and has a set of dynamic variables used to calculate activity within events. For non-neurogenic cell layers, many of the physiology parameters were uniform across like neurons, but because neurogenesis introduces heterogeneity, the physiology parameters were tracked separately for each neuron within the GC layer. The following sections will describe the type of neuron parameters, and then summarize how these parameters were determined for each neuron type.

It is important to observe that including only six layers of neurons is a substantial simplification of the actual biological system. Nearly a dozen different interneuron types have been identified in the DG, however the classifications of these classes often overlap. For example, GABAergic neurons in the hilus have been divided into at least somatostatin-positive, NPY-positive, "HIPP", and "HICAP" in varying classification schemes. Due to the limited ability to separate these neuron types, these interneurons are combined into the generic "hilar

interneurons" group in the model. Likewise, there are at least two different populations of basket cell interneurons, located at the base of the GCL (PV and CCK positive neurons). Although these two types of basket cells may have a complex network interaction, particularly in response to acetylcholine, they are combined into a single neuron layer in the model. Other cell types (MOPP interneurons, axo-axonic interneurons, etc) were left out of the model because they have relatively low numbers in the network compared to other neuron types and have not been as extensively studied, particularly physiologically. This is not to say that these neurons are not important. Rather, their functions may be integral to understanding DG function, particularly when modulatory neurotransmitters such as ACh are considered.

Neuron physical parameters

The following physical parameters were determined for each individual neuron and were stable within events, but may be dynamic over longer periods of time due to maturation.

χ_x is the relative dorsal-ventral axis position ($0 \leq \chi_x \leq 1$)

χ_y is the relative transverse axis position ($0 \leq \chi_y \leq 1$)

χ_y is the within layer depth ($0 \leq \chi_z \leq 1$)

δ_x is the spatial radius (dorsal-ventral axis) of the dendritic arborization ($0 \leq \delta_x \leq 0.2$)

δ_y is the spatial radius (transverse axis) of the dendritic arborization ($0 \leq \delta_y \leq 0.2$)

δ_z is the dendritic length ($0.1 \leq \delta_z \leq 3$)

For non-neurogenic neuron layers, the neurons were uniformly distributed along the dorsal-ventral axis (χ_x), and have randomly distributed in the χ_y and χ_z axes. All non-neurogenic neurons have fully developed dendritic arborizations: $\delta = \delta_{MAX}$.

For the neurogenic layer (the GC layer), when the model is initialized a bulk population of immature neurons were provided. As with other cell layers, the neurons were uniformly distributed along the dorsal-ventral axis (χ_x), and have randomly distributed in the χ_y and χ_z axes. However, the immature GC neurons have no dendritic arborization: $\delta = \delta_{MIN}$. The maturation

process used to grow the dendrites is described below. Subsequent neurons added to the layer were provided with random χ_x and χ_y locations, though they were biased to the inner GC layer (χ_z<mean(χ_z)) and also have minimum dendrites.

The neurogenic layer also tracks several other physical parameters over time:

Age is the age of the neuron (weeks)

R_{mem} is the membrane resistance (G-Ohms; estimated from number of synapses)

Vol is the approximate volume of the neuron (estimated from size of neuron)

These parameters were not directly used in calculating activity, but were important for calculating maturation-dependent physiology parameters (see below).

Neuron physiology and activity parameters

The following physiology parameters are user-defined values for individual neurons in the model. These parameters were static within an event, but may be dynamic over longer periods of time due to maturation. Their use will be described in subsequent sections:

$V_{Threshold}$ is the voltage (relative to rest) above which the neuron fires

dF/dV is the change in firing rate for each mV above threshold

F_{max} is the maximum firing rate for the neuron

F_{min} is the minimum firing rate for the neuron (neurons that burst)

E^*_{GABA} is a maturation dependent parameter that represent the neuron's relative sensitivity to GABA, relative to glutamate

$E^*_{Glutamate}$ is the parameter that represent the neuron's relative sensitivity to glutamate and is set to be equal to 1.

τ is the membrane time constant of the neuron

Age_{fire} is the age at which the immature firing rate is estimated

For all non-neurogenic cell layers, these neurons were initialized to values in Table IV-2, and they remain constant throughout the study. For the neurogenic cell layer (GC only), most of these parameters were initialized at immature values and slowly approach their mature levels.

The following variables for individual neurons were dynamic within an event and were used to determine the activity of the neurons:

V is the voltage (relative to rest) of neuron i

f is the firing of neuron in the previous time step

P_{Fire} is the potential for that neuron to fire in that time step

κ is a tracking variable that distributes spiking according to the firing rate.

Summary of neuron layers

Table IV-2 shows details regarding the numbers of neurons in each cell layer, compared to the numbers observed anatomically [5, 19], and also provides the neurogenesis rate used in the model for GC.

Table IV-2: Neuron layer numbers and neurogenesis rates

Cell Layer	*Cell Number (N_{layer})*	*Actual Cell Number (approximate)*	*Neurogenesis rate (cells/day)*	*Death rate (k_{death}) (for inactive neurons only)*
lEC	200	100,000	--	--
mEC	200	100,000	--	--
BC	120	10,000	--	--
MC	220	30,000	--	--
HI	220	30,000	--	--
GC	800 (start) ~1600 (test)	1,000,000	10/day (~15%/month)	.0015 (~1.5%/day)

Table IV-3 shows the physiology parameters utilized in the model for each neuron layer. These parameters were based on biological recordings from several sources that have investigated the physiological properties of granule cells, mossy cells, and interneurons [6, 20-22].

Table IV-3: Neuron activity parameters

Cell Layer	$V_{Threshold}$ *(mV)*	*dF/dV (Hz/mV)*	F_{max} *(Hz)*	F_{min} *(Hz)*	E^*_{GABA}	τ *(ms)*	Age_{fire} *(weeks)*
lEC	25	.008	20	0	-1	20	--
mEC	25	.008	20	0	-1	20	--
BC	12	.043	230	0	-1	10	--
MC	20	.048	50	0	-1	30	--
HI	15	.088	69	0	-1	30	--
GC – immature	20		30	0	2	~160	4
GC – mature	35	.083	72	20	-1	40	--

Connection types and parameters

There are 11 different connections in the model. The DG, as with most hippocampal areas, does not have a full connection matrix. Rather, there is a substantial degree of topological specificity that determines neuron connectivity. Most connections are sparse globally, with any single neuron being relatively unlikely to synapse upon a random neuron in its target layer. However, locally the probability of synapses can be much higher.

As was mentioned above in describing the selection of neuron types for inclusion in the model, there was a necessary degree of filtering the number of connection types in the model. Major connection pathways were included; however minor pathways and those that have only been described qualitatively were not included. For instance, a very recent study (Larimer and Strowbridge, 2008) was the first to systematically describe connections between hilar neurons (specifically, mossy cells and hilar interneurons). Although such connections had long been suspected, there was at the time no direct evidence to justify their inclusion in the model as described here.

Connection parameters

Like neuron types, different connections in the model were described by a set of static parameters and a set of experience-dependent variables. Each synapse type has general

parameters describing the structure of the connection within the network. Connections were made using a normal distribution around a target zone.

μ_{syn} (Target) – The average dorsal-ventral location of the synapse relative to source neuron's soma.

σ_{syn} (Range) – The spatial variance of the dorsal-ventral synapse location

$\rho_{syn,local}$ (Density) – The density of synapses at the center of synapse distribution

$\rho_{syn,ideal}$ (Ideal Density) – The relative density of synapses in the whole network

w_{max} – The maximum synaptic strength for the connection

$w_{max,immature}$ – The maximum synaptic strength for the connection onto an immature neuron

$k_{synapse}$ – The rate at which synapses mature (independent from neuron maturation rates)

k_{comp} is the rate that synaptic competition winners are determined

$\delta_{z,syn}$ – The size of an immature neuron's dendrite required for that synapse to be formed

$\delta_{x,max}$ is the spatial width (dorsal-ventral axis) of the dendritic arborization of a fully mature neuron

$\delta_{y,max}$ is the spatial width (transverse axis) of the dendritic arborization of a fully mature neuron

age_{ref} is the reference age (weeks) approximating when non-spiny synapses are first present

k_{conn} is the approximate number of weeks required for connection to fully develop (weeks)

At runtime, the parameters μ_{syn} and σ_{syn} are adjusted for the **Range** over which the model is simulated. As the model is only simulating a thick slice of the dentate gyrus, rather than the whole structure, the parameters must be rescaled accordingly. The scaling is as follows:

$$\mu_{syn} = \mu_{syn}^{0} \Big/ Range \qquad \text{(IV.2)}$$

$$\sigma_{syn} = \sigma_{syn}^{0} \Big/ Range \qquad \text{(IV.3)}$$

Range is a number between 0 and 1, and set to equal 0.4 in these simulations. In the case of the long-range MC to GC projection , the scaling of μ_{syn} and σ_{syn} is also constrained by the additional equalities ($-0.50 \leq \mu_{syn} \leq 0.50$) and ($\sigma_{syn} \leq 0.30$) to ensure that the source neuron

Variable parameters

For spiny synapses (those that are capable of learning), the strength of the synapse, w_{ij}, was determined by both a fixed and a variable component that were specific to each synapse and change over time. These parameters were initialized for each synapse between neurons *i* and *j* as follows:

$$\widetilde{w}_{ij}^{fixed} = 0.5 \times w_{ij} \qquad \text{(IV.4)}$$

$$\widetilde{w}_{ij}^{\text{var}iable} = 0.5 \times w_{ij} \qquad \text{(IV.5)}$$

$$\widetilde{w}_{ij}^{lost} = 0.5 \times \left(1 - w_{ij}\right) \qquad \text{(IV.6)}$$

where:

w_{ij} is the strength of the connection from neuron *i* to neuron *j*'

$\widetilde{w}_{ij}^{\text{var}iable}$ is the plastic component of existing synaptic strength

$\widetilde{w}_{ij}^{fixed}$ sets the lower limit of strength below which the synapses may not shrink

$\widetilde{w}_{ij}^{lost}$ sets the upper limit of strength above which the synapse cannot grow

The network was also run in a local region of the DG, rather than over the full longitudinal axis, in order to better view the orthogonalization by the DG. The pattern separation

ability of the DG is thought to be effective even in local areas where most neurons have similar connections, and only the subtle differences lead to activity. To adjust the network for wider/narrower ranges of the hippocampus, the 'range' parameter was scaled accordingly. In addition, connection strengths were modified to account for the different number of resulting synapses (broader network = sparser projections).

Summary of connection types

Table IV-4 outlines the parameters used for each connection type in the model. In some cases, as with the lateral perforant path (lEC to GC) and medial perforant path (MC to GC) synapses, the relative strengths of synapses has been discussed in previous studies[23]. Other synapses have not been as well described physiologically, however other aspects of connectivity have been well characterized [5, 19, 22, 24-26]. Figure IV-2 shows the topographical structure of the connections of a fully developed model.

Table IV-4: Connection parameters

Connection	μ^0_{syn} Target	σ^0_{syn} Range	$\rho_{syn,local}$ Density	$\rho_{syn,ideal}$	w_{max} (mV)	w_{max}- immature
Granule Cell Afferents						
lEC to GC	0	0.14	75%	0.105	1.7	1.7
mEC to GC	0	0.14	75%	0.105	2.2	2.2
MC to GC	+/-0.3	0.15	50%	0.15	2.8	2.8
BC to GC	0	0.2	50%	0.1	2.2	-1.0
HI to GC	0	0.1	100%	0.1	3.0	-3.0
Basket Cell Afferents						
lEC to BC	0	0.14	75%	0.105	2.4	--
mEC to BC	0	0.14	75%	0.105	3.2	--
MC to BC	+/-0.3	0.15	50%	0.15	1.7	--
GC to BC	0	0.05	100%	0.05	32.7	2.2
Hilar Neuron Afferents						
GC to MC	0	0.05	100%	0.05	5.6	2.2
GC to HI	0	0.05	100%	0.05	5.6	2.2

Table IV-5: Connection parameters (continued)

Connection	$k_{synapse}$ (%/event)	k_{comp}	$\delta_{z,syn}$	age_{ref} (weeks)	k_{conn} (weeks)
Granule Cell Afferents					
lEC to GC	0.05	0.1	3	3	2.5
mEC to GC	0.05	0.1	2.5	3	2.25
MC to GC	0.05	0.1	2	3	4
BC to GC	--	--	0	1	4
HI to GC	--	--	3	2	2.5
Basket Cell Afferents					
lEC to BC	--	--	--	--	--
mEC to BC	--	--	--	--	--
MC to BC	--	--	--	--	--
GC to BC	--	--	1.5	2	2
Hilar Neuron Afferents					
GC to MC	--	--	1.5	2	2
GC to HI	--	--	1.5	2	2

Synapse Initialization

Synapses were created according to the following equations. Note: because the network is initialized with only immature granule cells, synapses to and from the GC layer were created during the maturation process and not during initialization. The following equations describe

how synapses were chosen at both times. Figure IV-2 shows the topographical distribution of connections to neurons.

For each neuron in the source layer, the number of synapses that the neuron connects to, $N_{synapses}$, is determined by:

$$N_{synapses} = N_{target} \times \rho_{syn,ideal} \qquad \text{(IV.7)}$$

where:

N_{target} is the number of neurons in the target cell layer

$\rho_{syn,ideal}$ is the ideal density of that projection

Once the number of synapses required for the source neuron was determined, a set of non-repeating target (or source) neurons were selected by the following equation which was repeated until $N_{synapses}$ were found for each neuron

$$tar = N_{target} \times \eta\left(\mu_{syn}, \sigma_{syn}\right) \qquad \text{(IV.8)}$$

where:

$\eta(\mu_{syn},\sigma_{syn})$ represents a random number from a Gaussian distribution of standard deviation σ_{syn} around the target zone μ_{syn}.

Importantly, only one synapse was permitted between two neurons, if a synapse already existed with the selected neuron equation (IV.8) was repeated.

Each cell layer was treated as a "circle" in order to eliminate boundary effects in the number of synapses each neuron receives. That is, if a projection field of a neuron extended beyond the edge of the cell layer, the target (or source) neuron was selected from the opposite end of the network, as follows:

$$if\,(tar > N_{target}) \rightarrow tar = tar - N_{target} \qquad \text{(IV.9)}$$

$$if\,(tar < 1) \rightarrow tar = N_{target} + tar \qquad \text{(IV.10)}$$

This simplification reduces errors associated with simulating only a "slice" of hippocampus, but precludes the ability to make any conclusions about trans-laminar behavior that may emerge. This approximation will not be needed in larger scale models that extend the full septotemporal length of the hippocampus.

The initial synaptic strength, $w_{i,tar}$, of each synapse from the source neuron *i* onto its target neuron, *tar,* was calculated by the following equations:

$$if\begin{cases} spine & w_{i,tar} = 0.1 \le \eta(0.5,0.25) \le 0.9 \\ non-spine & w_{i,tar} = 1 \end{cases} \qquad \text{(IV.11)}$$

where:

spine indicates that the synapse type utilizes dendritic spines (lEC to GC, mEC to GC, MC to GC)

non-spine indicates that the synapses is not spiny

$\eta(0.5, 0.25)$ represents a random number from a Gaussian distribution of standard deviation 0.25 around a mean of 0.5. This value is constrained by an upper limit of 0.9 and a lower limit of 0.1.

Model Function

Motivation

Neurons in neural networks can be simulated at many different degrees of complexity. While there is considerable interest in developing highly sophisticated biologically-motivated neuron models, such as Hodgkin-Huxley models, this level of detail is accompanied by several significant drawbacks. The primary drawback is simulation time, which can be considerable, however a second drawback is the necessity to assign biological values to the many parameters. Although these values have been determined experimentally for many systems, the conductances of different ion channels have not been thoroughly characterized for all the different neuron

classes in the DG, nor have they been studied during the development of new neurons. There are other faster approaches to modeling at millisecond resolution, such as the Izhikevich model [27], which approximate the appropriate dynamical equations based on action potential profiles. The Izhikevich approach allows the investigation of dynamics of large neuron populations at high resolution, however it also requires detailed biological observations at different stages (in this case spike trains).

The other extreme consists of firing rate models that essentially sum inputs and estimate a firing rate for the target cell. While these models are quick to simulate and can provide significant insight about the relationship of different neuron populations, they tend to average out the effects of network dynamics by assuming that the system always exists at steady state.

For this model, it was necessary to determine a level of neuron simulation that was both computationally feasible and biologically-derived, yet with sufficient resolution to reveal interesting behaviors. Of particular concern was the time-scale of the experiments. Most conductance based models are typically designed for simulations of systems for at most several seconds [6] and look at behaviors on the order of milliseconds. This is in contrast to neurogenesis, which likely requires simulation over behavioral time scales (many seconds) and investigating model responses over many "months."

Mathematical description

The model used in this study is referred to as a *digitized firing rate* model (DFR; Figure IV-3). Its name derives from its mechanism of estimating the ideal firing rate for a neuron given its synaptic inputs, and then calculating the number of spikes that would occur within the simulation time window. As a result, the model is a hybrid of a spiking model and a firing rate model, thus allowing simulation at intermediate time scales (~25 ms time steps).

There are three steps to the DFR model:

1. Sum synaptic inputs to neurons

$$V_i(t) = e^{-t_{step}/\tau_i} \times V_i(t-1) + \sum_{j=1}^{J} E^*_{Glutamate,i} \times f_j(t-1) \times w_{\max} \times w_{ji} + \sum_{k=1}^{K} E^*_{GABA,i} \times f_k(t-1) \times w_{\max} \times w_{ki} \quad \text{(IV.12)}$$

where for each neuron *i*:

'j=1…J' are Glutamatergic neurons

'k=1…K' are GABAergic neurons.

τ_i is the membrane time constant of neuron *i*

$E^*_{Glutamate,i}$ and $E^*_{GABA,i}$ are maturation dependent parameters that represent the neuron's sensitivity to glutamate and GABA, respectively.

$f_j(t\text{-}1)/f_k(t\text{-}1)$ is the firing of neuron *j*/*k* in the previous time step

w_{max} is the maximum strength for that synapse type

$w_{j,i}$ ($w_{k,i}$) is the relative strength of the connection from neuron *j*(*k*) to neuron *i*

2. Estimate short-term firing rate & spiking

Whether a neuron fires or not (*f(t)*) was computed by the following equations:

$$if\left(V(t) > \Delta V_{threshold}\right) \longrightarrow P_{Fire} = \text{minimum}\begin{pmatrix} F_{Max} \times t_{step}, \\ F_{Min} \times t_{step} + \kappa(t-1) + \left(V(t) - \Delta V_{threshold}\right) \times \left(dF/dV\right) \end{pmatrix} \quad \text{(IV.13)}$$

$$if\left(V(t) \le \Delta V_{threshold}\right) \longrightarrow P_{Fire} = \kappa(t-1) \quad \text{(IV.14)}$$

$$f(t) = \text{round}(P_{Fire}) \quad \text{(IV.14)}$$

$$\kappa(t) = P_{Fire} - f(t) \quad \text{(IV.15)}$$

where for each neuron *i*:

P_{Fire} is the potential for that neuron to fire in that time step

$\Delta V_{Threshold}$ is the voltage (relative to rest) above which the neuron fires

F_{Max} is the maximum firing rate for the neuron

F_{Min} is the minimum firing rate for the neuron

κ is a tracking variable that distributes spiking according to the firing rate.

dF/dV is the change in firing rate for each mV above threshold

When $P^{Fire}>0.50$, the neuron spikes ($f(t)=1$; IV.14), and the tracking variable κ is lowered (IV.15), thereby reducing the likelihood of a spike in the next timestep. In the event that $0<P^{Fire}<0.5$, then neuron does not spike ($f(t)=0$), but the κ of the neuron persists until the next time step, making a spike then more probable.

In the model, the κ term was randomized within a very narrow range ($\eta(0.025,0.05)$) at the beginning of each event to account for variations in the initial state of the neurons.

3. Reset neurons and track parameters

$$if\,(V(t) < -10mV) \longrightarrow V(t) = -10mV \qquad \text{(IV-16)}$$

$$if\,(V(t) > \Delta V_{threshold}) \longrightarrow V(t) = \Delta V_{threshold} \qquad \text{(IV-17)}$$

Theta oscillations in model

Activity in the model was assumed to be occurring during periods known to exhibit theta rhythm. Theta is believed to be an oscillating inhibitory influence on the network, though the actual mechanism by which it occurs is unclear. Theta was implemented by including an 8Hz oscillating dampening effect on the voltage neurons carry over from one time step to another. This has the effect of gradually "resetting" the network every 125ms.

$$V_i(t) = V_i(t) \times (1 - \text{maximum}(\text{cosine}(\theta_0 + (t \times t_{step})/125ms), 0)) \quad \text{(IV-18)}$$

The phase of theta (θ_0) is uniformly random at the beginning of each event.

Commentary on other types of neuron models

Ideally, the level that a system is modeled at should be sufficient for capturing the dynamics and behavior necessary to address the specific question asked. All modeling approaches have limitations - the DFR model used here does not permit the observation of network dynamics at short time scales (within 25 ms), and the investigation of other dynamics requiring high temporal resolution, such as network oscillations, is difficult. However the experiments described here focus on the activities of neurons at long time scales (~seconds). It cannot be ruled out that a network behavior missed in this model may have substantial effects at larger time scales, but most likely the resolution of the model is sufficient for capturing the long-term effects of neurogenesis dynamics.

Neurogenesis and Maturation

Overview

The addition of new neurons to the network is the focus of this study, so the model was designed to best reflect what is known about the maturation process.

New neurons were born into the network randomly and in a raw form. Initially, they have no synapses and very unique physiological properties. Over time, the neurons matured by gradually increasing in size, which in turn permitted the gradual addition of new synapses. A schematic of this growth process is shown in Figure IV-4.

Addition of new neurons

The model ran at a user-defined neurogenesis rate (New neurons / day). After each activity event, there was a random chance that a new neuron will be added. The new neuron was

simply added to the existing layer in a random location, and the sizes of the connection matrices were adjusted accordingly, however with no initial connections. The physiology of the new cells was initialized at levels observed in new neurons (Table IV-3).

General maturation of immature neurons

After each activity event, neurons which are less than 10 weeks of age were considered immature neurons and part of the maturation process. Furthermore, there was an activity dependent aspect to the maturation, as new neurons can only mature if they were effectively being integrated into the network.

During maturation, the following happens to immature neurons:

1. *Age of neuron increases* – the age of each neuron, Age, is measured in weeks and is updated after each experienced event:

$$Age_i = Age_i + {}^{1}\!/\!_{Evt_{Week}} \qquad \text{(IV.19)}$$

where i are all neurons that are less than ten weeks old.

2. *Neurons grow in size (activity dependent)* – the relative size of granule cells was tracked in the model, and used during synapse formation. One parameter tracked how far into the molecular layer the primary dendrite reaches (δ_z), and the other two parameters determined the transverse (δ_y) and longitudinal breadth (δ_x) of the dendritic arborization.

For all neurons i that were depolarized during the previous event, the size of the neuron's apical dendrite grew at a fractional rate:

$$\delta_{z,i} = \delta_{z,i} + {}^{1.5}\!/\!_{Evt_{Week}} \qquad \text{(IV.20)}$$

The dendrite's arborization (x,y spread) only grew after the apical dendrite reached the molecular layer ($\delta_{z,i}$>2)

$$\delta_{x,i} = \delta_{x,i} + {0.2}/{Evt_{Week}} \quad \text{(IV.21)}$$

$$\delta_{y,i} = \delta_{y,i} + {0.2}/{Evt_{Week}} \quad \text{(IV.22)}$$

(in the model, the full z-extent is arbitrarily 3, and the full x/y radii are 0.2: 20% of the longitudinal axis)

Maturation of synaptic connectivity

3. *Addition of synapses (size dependent)* – Immature neurons became capable of forming synapses at different times in their development. The probability of forming a new synapse was related to both the length (δ_z) and width (δ_x) of the dendritic arborization. The probability that a particular type of synapse will be generated onto or from an immature neuron, *i*, is given by the following equations:

$$N_{syn,ideal} = \rho_{syn,ideal} \times N_{target/source} \quad \text{(IV.23)}$$

$$if\,(spine) \rightarrow N^{*}_{syn,i} = N_{syn,ideal} \times \frac{\delta_{x,i}}{\delta_{x,\max}} \quad \text{(IV.24)}$$

$$if\,(non-spine) \rightarrow N^{*}_{syn,i} = N_{syn,ideal} \quad \text{(IV.25)}$$

$$if\,(\delta_z > \delta_{z,syn}) \rightarrow P_{syn} = \frac{\left(N_{syn,i} - N^{*}_{syn,i}\right)}{\left(0.5 \times Evt_{Week} \times k_{conn}\right)} \quad \text{(IV.26)}$$

where:

$N_{target/source}$ is the total number of source / target neurons for the connection

$\rho_{syn,ideal}$ is the ideal density for that connection

spine indicates that the post-synaptic structure of the synapse is a spine. Only lEC, mEC and MC inputs onto GC are classified as spiny.

non-spine indicates that the synapse does not use normal spines. All GC outputs and HI and BC synapses onto GCs are non-spiny

δ_x is the spatial width of the dendritic arborization of neuron *i*

$\delta_{x,max}$ is the spatial width of the dendritic arborization of a fully mature neuron

$N_{syn,i}$ is the current number of synapses for the neuron

$N^*_{syn,i}$ is the ideal number of synapses for the neuron

$N_{syn,ideal}$ is the ideal number of synapses for a fully mature neuron

δ_z is the dendritic length of neuron *i*

$\delta_{z,syn}$ is the minimum dendritic length requirement for each synapses type

k_{conn} is the rate of synapse formation

Evt_{Week} is the number of simulated events in each week

For instance basket cells, which target the soma, could synapse early in maturation, whereas EC inputs, which are at the distal ends of dendrites, required the neuron to be fully grown. For spine-based synapses, the extent of the dendritic arborization determines how many synapses are desired. For instance, if a fully mature neuron has 50 synapses, then one with 50% of the "volume" will have a target number of 25 synapses. If the neuron has less than the target number of synapses, it may gain a new synapse, the probability of which scales with the drive. For example, if the neuron has 20 synapses with an ideal of 25, then it will have a strong probability of gaining a new synapse.

When a new synapse is to be formed, an appropriate neuron in the target/source layer is selected. This selection of the partner neuron is dependent on two factors: the topography of the projection is taken into account (would an axon/dendrite of the partner neuron be nearby the new neuron?) and that there is not already a connection between those two neurons. Once selected, a new synapse is formed between the two neurons and is initialized at a random strength. The equations used were the same as discussed in the connection setup description (Equations IV.2-

IV.11). The development of downstream efferent connections of GCs is shown in Figure IV-5, and the development of afferent connections to new GCs is shown in Figure IV-6.

4. Addition of Competitive Synapses: For spiny synapses, once a source neuron is selected, there is a possibility that the synapse formed will "compete" with an existing synapse[28]. The probability that this occurs is related to the relative density of the projection and the number of possible competitors. The set of possible competitors for a synapse from projecting neuron *j* is determined by:

$$\mathbf{S}_{\mathbf{PossComp}} = \left\{ \mathbf{Syn}_{\mathbf{j}} \neq \mathbf{Comp} \cup \left| \chi_y - \chi_{y,i} \right| \leq 0.4 \right\} \qquad \text{(IV.27)}$$

where:

Syn$_j$ represents all neurons that receive a synapse from neuron *j*,

Comp represents those synapses that are already competing (not allowed for second competition)

$|\chi_y - \chi_{y,i}|$ is the transverse distance (within slice) between the possible competitor and the immature neuron *i*

Essentially, possible competitors were restricted to those neurons already receiving a non-competitive input from the source neuron, and the immature neuron and possible competitor must be close enough to have overlapping dendritic arborizations.

Once the possible list of competitors is chosen, the probability that a competitive synapse is formed, P_{comp}, is given by:

$$\rho_{syn} = \frac{N_{Syn,Total}}{N_{Source} \times N_{Target}} \qquad \text{(IV.28)}$$

$$\rho_{syn,ratio} = \frac{\rho_{syn}}{\rho_{syn,ideal}} \qquad \text{(IV.29)}$$

$$P_{comp} = \frac{N_{PossComp}}{\left(\rho_{syn,ratio}\right)^2} \qquad \text{(IV.30)}$$

where:

$N_{Syn,Total}$ is the total number of synapses of that connection type

N_{Source} is the total number of source neurons for that connection

N_{Target} is the total number of target neurons for that connection

$\rho_{syn,ideal}$ is the ideal density for that connection

$N_{PossComp}$ is the number of neurons in the set available for competition (**$S_{PossComp}$**)

P_{comp} is then compared to a random number to determine if the new synapse is either competitive with an existing synapse or formed de novo.

$$Syn = \begin{Bmatrix} Comp & if\ P_{comp} > rand \\ deNovo & if\ P_{comp} < rand \end{Bmatrix} \quad \text{(IV.31)}$$

If the synapses is selected to be competitive, a random neuron from **$S_{PossComp}$** is selected to be its competitor.

5. Determining Winner of Competitive Synapses - The ultimate 'winner' of the competition is decided by comparing the relative activities of the two competing neurons, the overall activity level of the cell layer, and the strengths of the synapses. At any given time, only neurons whose activity is below a certain activity threshold are susceptible to losing a connection:

$$f_{thresh} = \mu_f - \sigma_f \quad \text{(IV.32)}$$

where:

f_{thresh} is the firing rate below which neurons may lose synapses

μ_f is the average firing rate for the cell layer

σ_f is the standard deviation of the cell layer's firing rate

If one neuron is below threshold, and the other is above this threshold, then the probability that it loses the synapse is given by:

$$P_{lose,1} = \left(\frac{1 - w_1}{k_{Comp}} \right) \times e^{-.5 \times \left(\frac{\tilde{f}_1 - \tilde{f}_2}{\sigma_f} \right)^2} \quad \text{(IV.33)}$$

where:

w_1 is the synaptic weight of the source neuron onto the low-firing neuron

k_{Comp} is the rate that synaptic competition winners are determined

$\tilde{f}_1$ is the time-weighted average firing rate of the low-firing neuron

$\tilde{f}_2$ is the time-weighted average firing rate of the high-firing neuron

If both neurons are firing below f_{thresh}, then the probability for each to lose is calculated by the following equation (same for both neurons):

$$P_{lose,1} = \left(\frac{1 - w_1}{k_{Comp}} \right) \times e^{-.5 \times \left(\frac{\tilde{f}_1 - \mu_f}{\sigma_f} \right)^2} \quad \text{(IV.34)}$$

It is possible for both neurons to lose at the same time, though this is rare. Finally, it is also possible that both synapses 'win' – in essence the synapse splits into two separate synapses. This can only occur if both synapses are very strong:

$$if\,(\min(w_1, w_2) > 0.5) \rightarrow P_{win,1,2} = \frac{w_1 \times w_2}{.25 \times k_{Comp}} \quad \text{(IV.35)}$$

where:

w_1 and w_2 are the synaptic weights onto both neurons

When two synapses cease to be competitive, the 'winning' synapses are simply reassigned to being non-competitive, whereas the losing synapses are removed from the network entirely. The relationship of synaptic competition to neuron age is shown in Figure IV-7.

6. Maturation of non-plastic synapses - Non-spiny synapses, including all inhibitory synapses and GC outputs, are all initialized with a fixed synaptic weight that does not change

over time. The age of the immature neuron does impose a bias on the synaptic strength that gradually disappears as the neuron matures. The realized strength of non-spiny synapses, *w*, is calculated by:

$$bias = \min\left(\frac{Age - Age_{ref} - 0.5 \times k_{conn}}{k_{conn}}, 1\right) \qquad \text{(IV.36)}$$

$$w = w_{immature} + (1 - bias) \times (w_{mature} - w_{immature}) \qquad \text{(IV.37)}$$

where:

bias is the degree that the synapse weight is adjusted due to the neuron's age

Age is the age of the GC

Age_{ref} is the age of the neuron when it begins to receive connections of that type

k_{conn} is the number of weeks that the synapse requires for maturation

w_{mature} is the strength of the fully mature synapse

$w_{immature}$ is the strength of the synapse when those synapses first appear on the newborn neurons.

Maturation of physiology parameters

Prior to any event being processed by the network, the physiology of each individual neuron is calculated from its age and physical parameters (i.e., size, # of synapses).

Mature granule cells have a standard physiology which is shown in Table III. New neurons, however, have distinct properties during their early maturation [13, 16, 17, 29, 30]. The properties that have been well described include membrane resistance (R_{mem}), capacitance (C_{mem}), resting potential (V_{rest}), firing rate (f_{max}), and response to GABA. Some of these electrical properties can be attributed in part to the physical dimensions of the neuron – for example, capacitance scales with neuron volume and resistance is inversely related to number of synapses.

From studies looking at this maturation process, the development of the other key properties to the model can be estimated as well for each neuron:

$$Vol = .12 + \max(.5, \delta_x) \times \max(.5, \delta_y) \times \delta_z \times \frac{N_{Connections}}{N_{Connections,Max}} \quad \text{(IV.38)}$$

$$R_{mem} = 4 - 3.8 * \tanh\left(\frac{N_{Connections,Max}}{2 \times (\max(0, N_{Connections,Max} - N_{Connections}) - 0.5)} \right) \quad \text{(IV.39)}$$

$$\tau = \frac{Vol \times R_{mem}}{.12} \times \tau_{mature} \quad \text{(IV.40)}$$

$$if\,(\tau > 4 \times \tau_{mature}) \rightarrow \tau = 4 \times \tau_{mature} \quad \text{(IV.41)}$$

$$E^*_{GABA} = E^*_{Glutamate} \times \min(1, Age - 2) \quad \text{(IV.42)}$$

$$\Delta V_{threshold} = \Delta V_{threshold,mature} - (1 - \frac{Vol}{Vol_{mature}}) \times (\Delta V_{threshold,mature} - \Delta V_{threshold,immature}) \quad \text{(IV.43)}$$

$$F_{Max} = t_{step} \times (F_{Max,mature} - (\frac{10 - Age}{10 - Age_{fire}}) * (F_{Max,mature} - F_{Max,immature})) \quad \text{(IV.44)}$$

$$if\,(N_{Spines} = 0) \longrightarrow F_{Max} = 0 \quad \text{(IV.45)}$$

$$F_{Min} = t_{step} \times (F_{Min,mature} - \min(1, (\frac{10 - Age}{10 - Age_{fire}}) * (F_{Min,mature} - F_{Min,mature}))) \quad \text{(IV.46)}$$

$$\left(dF / dV \right) = \frac{F_{Max}}{\left(e^{t_{step}/\tau} - 1 \right) \times \Delta V_{threshold}} \quad \text{(IV.47)}$$

where:

$N_{Connections}$ is the total number of synapses that the neuron makes

$N_{Connections,max}$ is the total number of synapses that a fully connected neuron would make

N_{Spines} is the total number of spiny synapses that the neuron makes (lEC, mEC, and MC afferents onto GCs)

Vol is the estimated volume (arbitrary units). Proportional to capacitance

R_{mem} is the estimated membrane resistance (GΩ)

τ is the estimated membrane time constant (ms)

E^*_{GABA} is the relative response to GABA

$\Delta V_{threshold}$ is the voltage (relative to rest) required for the neuron to reach threshold (mV)

F_{Max} is the maximum firing rate of the neuron

F_{Min} is the minimum firing rate of the neuron (i.e., any firing is bursting)

Age_{fire} is the age at which the immature neuron firing rate is estimated

dF/dV is the change in firing rate for each mV above threshold

Because the properties such as synapse number and volume are scaled arbitrarily, the values are computed in without units and then compared to the corresponding values for mature neurons with their known physiology correlates. The physiological maturation of neurons in the model is shown in Figure IV-8.

Synaptic Plasticity

The dentate gyrus is the site of significant synaptic plasticity, with substantial amounts of LTP having been shown in the perforant path input. In the model, synapse classes which are excitatory and utilize spines experience learning (EC to GC, MC to GC), whereas aspiny neurons and GABAergic synapses do not learn. The plasticity rule used in the model is shown in Figure IV-9.

Synaptic plasticity learning rule

A simple spike-timing covariance learning algorithm was used to train the network. This STDP learning was implemented after each event by filtering the input layer spike train with a STDP profile, making time before each spike positive and time after each spike negative. This filtered input signal was then compared to spike train of the downstream neuron, and the covariance of the two neurons was used to determine the direction of learning. [31]

$$\mathbf{stdp} = \{-0.1;-0.3;0.0;0.5;0.15\} \quad \text{(IV.48)}$$

$$\hat{f}_i(t) = stdp(1)\times f_i(t-2) + stdp(2)\times f_i(t-1) + stdp(3)\times f_i(t) + stdp(4)\times f_i(t+1) + stdp(5)\times f_i(t+2) \quad \text{(IV.49)}$$

$$Cov_{ij} = \frac{\left(\hat{\mathbf{f}}_\mathbf{i} - \mu_{\hat{f}_i}\right)\bullet \mathbf{f}_\mathbf{j}}{\sum_t \mathbf{f}_\mathbf{j}} \quad \text{(IV.50)}$$

$$\tilde{w}_{ij}^{variable} = w_{ij} - \tilde{w}_{ij}^{fixed} \quad \text{(IV.51)}$$

$$\tilde{w}_{ij}^{free} = 1 - w_{ij} - \tilde{w}_{ij}^{lost} \quad \text{(IV.52)}$$

$$\tilde{w}_{ij}^{possible} = \tilde{w}_{ij}^{free} + \tilde{w}_{ij}^{variable} \quad \text{(IV.53)}$$

$$\tilde{w}_{ij}^{variable} = \tilde{w}_{ij}^{variable} + Cov_{ij}\times\left(\tilde{w}_{ij}^{possible} - \tilde{w}_{ij}^{variable}\right) \quad \text{(IV.54)}$$

$$w_{ij} = \tilde{w}_{ij}^{variable} + \tilde{w}_{ij}^{fixed} \quad \text{(IV.55)}$$

where:

$\mathbf{f_i}$ is the spike train of the source neuron

$\mathbf{f_j}$ is the spike train of the target neuron

$\hat{\mathbf{f}}_\mathbf{i}$ is the STDP filtered signal from the source neuron

stdp is the spike-timing dependent plasticity filter used

Cov_{ij} is the covariance between the filtered source neuron trace and the spike train of the target neuron

$\widetilde{w}_{ij}^{\text{var}iable}$ is the plastic component of existing synaptic strength

$\widetilde{w}_{ij}^{free}$ represents the potential range into which the synapse can grow

$\widetilde{w}_{ij}^{possible}$ is the total potential of the synapse for plasticity

$\widetilde{w}_{ij}^{fixed}$ sets the lower limit of strength below which the synapses may not shrink

$\widetilde{w}_{ij}^{lost}$ sets the upper limit of strength above which the synapse cannot grow

As dendritic spines mature in the model, their relative level of plasticity decreases. This is accomplished by transferring a portion of the variable strength to fixed strength, and a portion of free strength to lost strength.

$$\widetilde{w}_{ij}^{fixed} = \widetilde{w}_{ij}^{fixed} + k_{synapse} \times \widetilde{w}_{ij}^{\text{var}iable} \qquad \text{(IV.56)}$$

$$\widetilde{w}_{ij}^{\text{var}iable} = \left(1 - k_{synapse}\right) \times \widetilde{w}_{ij}^{\text{var}iable} \qquad \text{(IV.57)}$$

$$\widetilde{w}_{ij}^{lost} = \widetilde{w}_{ij}^{lost} + 0.4 \times k_{synapse} \times \widetilde{w}_{ij}^{free} \qquad \text{(IV.58)}$$

$$\widetilde{w}_{ij}^{free} = \left(1 - 0.4 \times k_{synapse}\right) \times \widetilde{w}_{ij}^{free} \qquad \text{(IV.59)}$$

Plasticity in immature neurons

Immature neurons have been shown to have a significantly different response to LTP. In particular, the potentiation seen in 4 to 6 week old neurons is considerably higher than that seen before and afterwards [9, 32]. This increased ability for learning is not simulated directly in the model, rather the increased number of younger, more plastic synapses in immature neurons leads to a profile of potential LTP that heavily biases younger neurons.

Cell Death

Cell death in the model is limited to the GC layer. This cell death is activity dependent – if a cell fires substantially less than the average activity in the network, there is a small probability that the neuron may die [33]. There is no enforced rate of death, if no cells qualify for dying, then no cells will die. Mature cells can die, but do so rarely.

$$f_{thresh} = \max(\mu_f - 2 \times \sigma_f, 0.25 \times \mu_f) \quad \text{(IV.60)}$$

$$if \left\{ \begin{array}{c} Age < 10 \\ or \\ N_{Spines} < \left(\mu_{Spines} - \sigma_{Spines}\right) \end{array} and \left(\tilde{f} < f_{thresh}\right) \right\} \rightarrow P_{Die} = k_{death} \quad \text{(IV-61)}$$

where:

μ_f is the average firing rate of the neuron layer

σ_f is the standard deviation of the firing rates of the neuron layer

f_{thresh} is the firing rate threshold below which a neuron may die

Age is the age of the neuron (in weeks)

N_{Spines} is the total number of spiny synapses onto the neuron (lEC, mEC, MC afferents onto GC)

μ_{Spines} is the average number of spiny synapses onto that type of neuron

σ_{Spines} is the standard deviation of the number of spiny synapses onto that type of neuron

$\tilde{f}$ is the filtered firing rate of the neuron

P_{Die} is the probability that the neuron may die

k_{death} is the rate at which neurons susceptible to death may die

When a cell dies, its connections are eliminated and the neuron is noted as dead in the network, which precludes the possibility of future growth and activity in the network. The relationship between cell death and neuron age in the model is shown in Figure IV-10.

Entorhinal Cortex Simulation

The model is controlled during simulations and testing by modifying the two entorhinal cortex layers. Medial entorhinal cortex (mEC) neurons show a distinct "grid cell" behavior during spatial exploration [34-36]. The model implemented properties of the mEC's spatial response, including the relationship of grid size to dorsal-ventral position and the fixed relationship between different grid cells across environments. Lateral entorhinal cortext (lEC) responses are less well understood, but it is likely that they provide the hippocampus highly processed representations of contextual feature and object information – the "what" to the mEC grid cells' "where." The lEC input was constant at different spatial locations but varied considerably across different contexts. The different control of the two inputs means that switching environments changed the spatial response of the grid cells and activated a different set of lEC neurons, whereas changing position within a single environment only affected the mEC neurons' response (Figure IV-11).

lEC neurons were chosen to depolarize at different levels for each environment.

$$V_{lEC} = \left(\frac{abs(\eta(0,1)) \times abs(\eta(0,1))}{2} \right) \times {df}/{dV} + V_{thresh} \quad \text{(IV.62)}$$

where:

V_{lEC} is the depolarization of a lEC neuron for that environment

V_{thresh} is the depolarization required to fire

df/dV is the increase in firing rate per unit of increased depolarization

$\eta(0,1)$ is a random number selected from a Gaussian distribution with a mean of zero and a standard deviation of one. The absolute value was taken to make this variable positive.

mEC inputs were generated using a previously described method [37].

Each mEC neuron was assigned three parameters: grid size/frequency (λ), grid orientation (θ), and spatial offset (φ). Grid size varied with the dorsal-ventral location of the neuron (χ_x), while orientation and offset were random. As with biological observations, the orientation and offset varied between environments, but the inter-neuronal relationships (φ_1- φ_2 & θ_1-θ_2) remained constant. Therefore, the generation of a new environment involved the random selection of an environmental orientation (θ_{env}) and offset (φ_{env}).

The calculation of an mEC neuron's relative response, G, for a spatial location (x,y) is given by:

$$k_1 = \frac{4\pi\lambda}{\sqrt{6}} \times \left(\begin{array}{r} \left(\cos(\theta + \frac{\pi}{12}) + \sin(\theta + \frac{\pi}{12}) \right) \times \left(x - \varphi_x \right) + \\ \left(\cos(\theta + \frac{\pi}{12}) - \sin(\theta + \frac{\pi}{12}) \right) \times \left(y - \varphi_y \right) \end{array} \right) \quad \text{(IV.63)}$$

$$k_2 = \frac{4\pi\lambda}{\sqrt{6}} \times \left(\begin{array}{r} \left(\cos(\theta + \frac{5\pi}{12}) + \sin(\theta + \frac{5\pi}{12}) \right) \times \left(x - \varphi_x \right) + \\ \left(\cos(\theta + \frac{5\pi}{12}) - \sin(\theta + \frac{5\pi}{12}) \right) \times \left(y - \varphi_y \right) \end{array} \right) \quad \text{(IV.64)}$$

$$k_3 = \frac{4\pi\lambda}{\sqrt{6}} \times \left(\begin{array}{r} \left(\cos(\theta + \frac{3\pi}{4}) + \sin(\theta + \frac{3\pi}{4}) \right) \times \left(x - \varphi_x \right) + \\ \left(\cos(\theta + \frac{3\pi}{4}) - \sin(\theta + \frac{3\pi}{4}) \right) \times \left(y - \varphi_y \right) \end{array} \right) \quad \text{(IV.65)}$$

$$G = \frac{2}{3}\left(\frac{k_1 + k_2 + k_3}{3} + .5 \right) \quad \text{(IV.66)}$$

As with lEC neurons, mEC neurons also have an space-independent environmental bias that this spatial gain is added to.

$$V_{mEC} = G \times abs\left(.75 + \frac{abs(\eta(0,1))}{5} \right) \times {df}/{dV} + V_{thresh} \quad \text{(IV.67)}$$

Basic Description of Experimental Design

Input Structure and Experimental Design

During training and growth, each "event" experienced in the model involves the network 'moving' along a path within that context for twenty seconds. The mEC neurons fire according to the spatial location at each instant, whereas the lEC neurons fire at rates determined by the environment's context.

During the testing phase, the network is successively placed in static locations within that environment for either 500ms. During this time, mEC and lEC firing rates remain constant. These trials completely tile the environment and the responses of the model are recorded for each location.

The training and testing paradigms are shown schematically in Figure IV-12.

Growth phase of experiment

After the model is initialized with , the model then "grows" in a series of environments (Figure IV-13). Each environments had a separately calculated lEC activity vector, as well as a random mEC activity vector and shifted grid loci (θ_{env} and φ_{env}).

Each environment is used for a total of 40 days, which allows considerable growth of the GC layer as well as experience-dependent maturation to the environments. Each day consists of 10 separate training events that consist of the animal moving along a random path for 10 seconds.

Description of initialized networks

Each of the studies described in the following chapters has the same basic experimental design. The GC layer was initialized with a large number of immature neurons, and these, as well as all later newborn neurons, matured and developed connections according to the maturation process described above. Initially the GC layer had twice the number of input EC neurons, 800 GC compared to 400 EC neurons (including both the mEC and lEC layers), but after full growth the GC layer had approximately five times the total number of EC neurons (Figure IV-14). This ratio corresponds to the ratio observed in the developed rat DG (200,000 EC neurons to 1 million GC [19]). New neurons were born at a rate of 10 per day - though not all survived (Figure IV-10). At the time of testing, the model GC layer grew at roughly 10% per month, similar to what has been estimated in young rats (~6% [38]).

After initialization, the input layers provided highly structured inputs representing different "environments" for the equivalent of 120 days, during which time each network grew by generating new neurons and integrating them in the circuit in an activity-dependent manner (Figure 1D). At 120 days, the network was duplicated, with one network continuing to grow with neurogenesis ("NG" network) while the other network ceased to have new neurons born ("No NG" network). These two networks were presented with a fourth environment for 40 days before experimentation.

Figure IV-15 shows sample responses from a subset of neurons from each of the input layers (mEC and lEC) and the GC layers.

Chapter VII, in part, includes material that was published in the article "Computational Influence of Adult Neurogenesis on Memory Encoding," Aimone, James B; Wiles, Janet; and Gage, Fred H.; *Neuron*, January 2009. The dissertation author was the primary investigator and author of this paper.

Figures

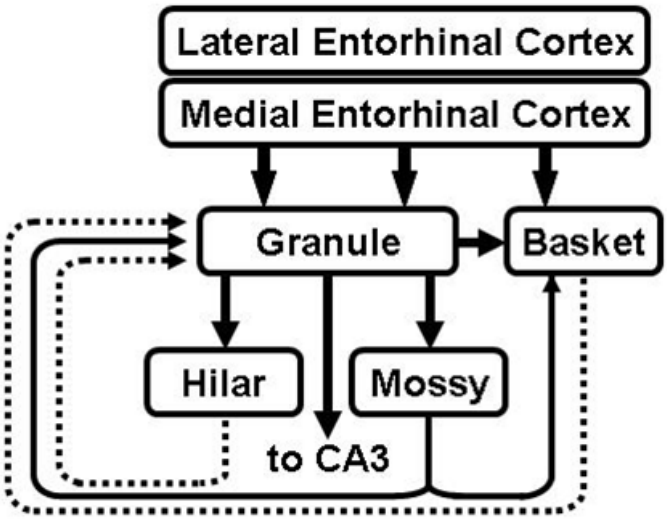

Figure IV-1: Network Architecture
Simplified block diagram of network architecture. The lateral entorhinal cortex and medial entorhinal cortex are the controlled input layers, the granule cells are the neurogenic, excitatory principal neurons of the DG, the basket cells and hilar cells are inhibitory interneurons, and the mossy cells are excitatory interneurons. Solid lines refer to excitation, dotted lines to inhibition.

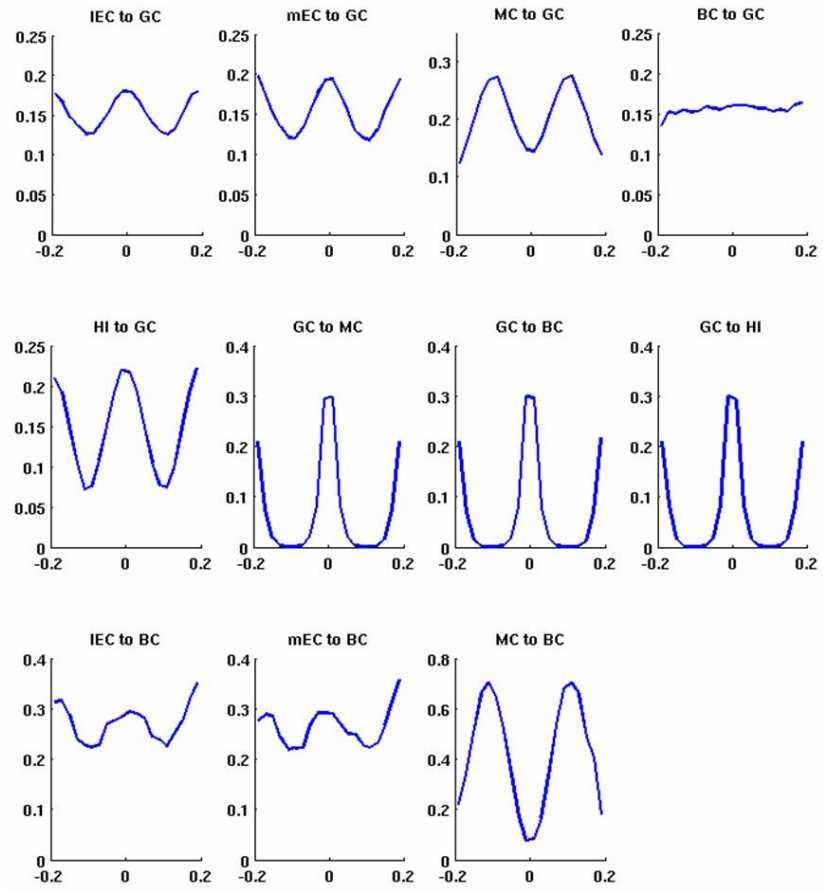

Figure IV-2: Connection architecture
Probability that two neurons were connected given as a function of relative distance. The x-axis of each panel refers to the difference between two neuron's location along septo-temporal axis. The y-axis of each panel represents to the ratio of existing synapses to potential synapse sites. The increase of connection densities for long distances in some of the panels is due to the ringed layer structure - neurons on the edges of the network layers were permitted to project to the opposite edge.

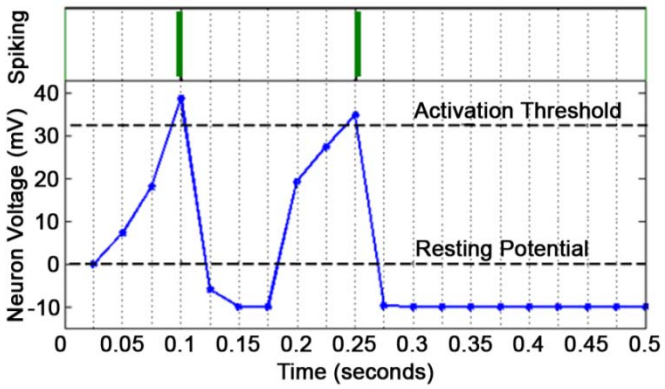

Figure IV-3: Schematic of Firing Calculation in Model
How neuron activity was calculated in the model using a digitized firing rate model. The voltage (blue) was calculated for each 25 ms time step. If the voltage surpasses an activation threshold, the firing rate of the neuron was calculated which may lead to a spike at that time.

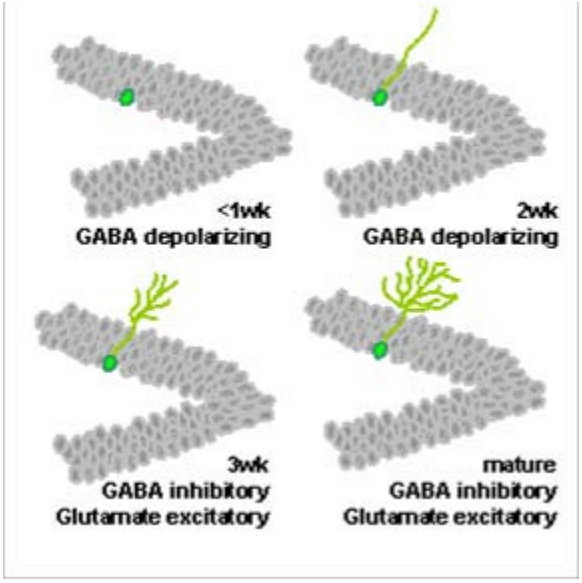

Figure IV-4: Schematic of Neurogenesis Maturation Process
Sketch of newborn granule cell (GC) maturation process implemented in model. Maturation was implemented continuously, with new neurons passing through several stages of physiology and structural maturation.

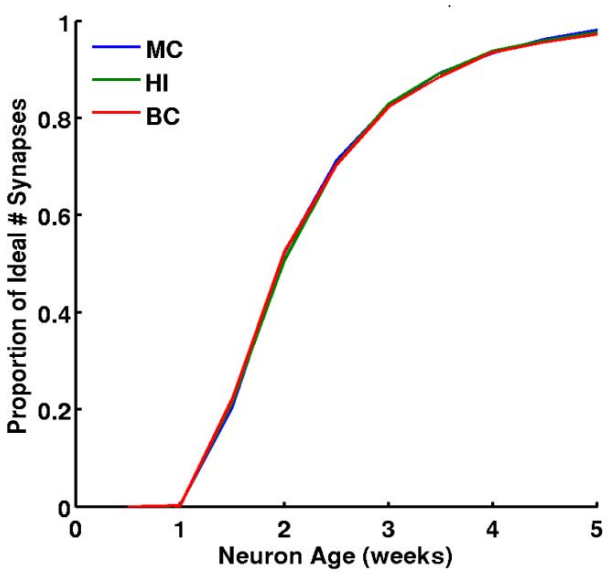

Figure IV-5: Synapse Maturation – GC Efferents
Timeline of axonal connectivity development for maturing GCs in model.

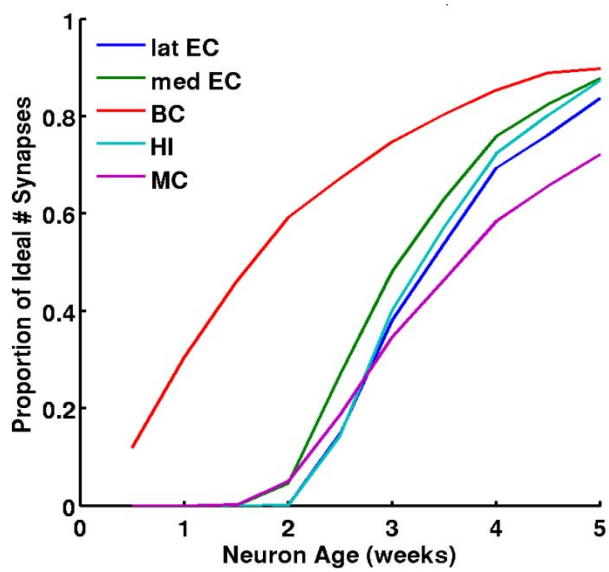

Figure IV-6: Synapse Maturation – GC Afferents
Timeline of dendritic connectivity development for maturing GCs in model.

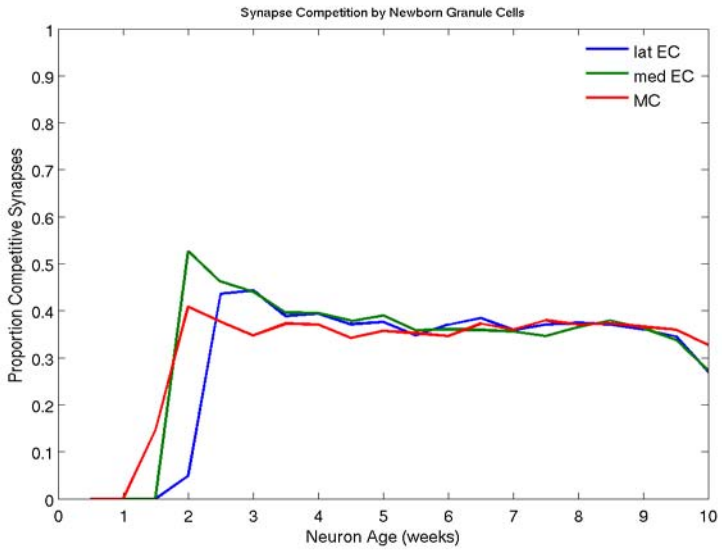

Figure IV-7: Synapse Maturation – Excitatory Synapse Competition
Proportion of synapses of each type that were competitive for different maturation ages.

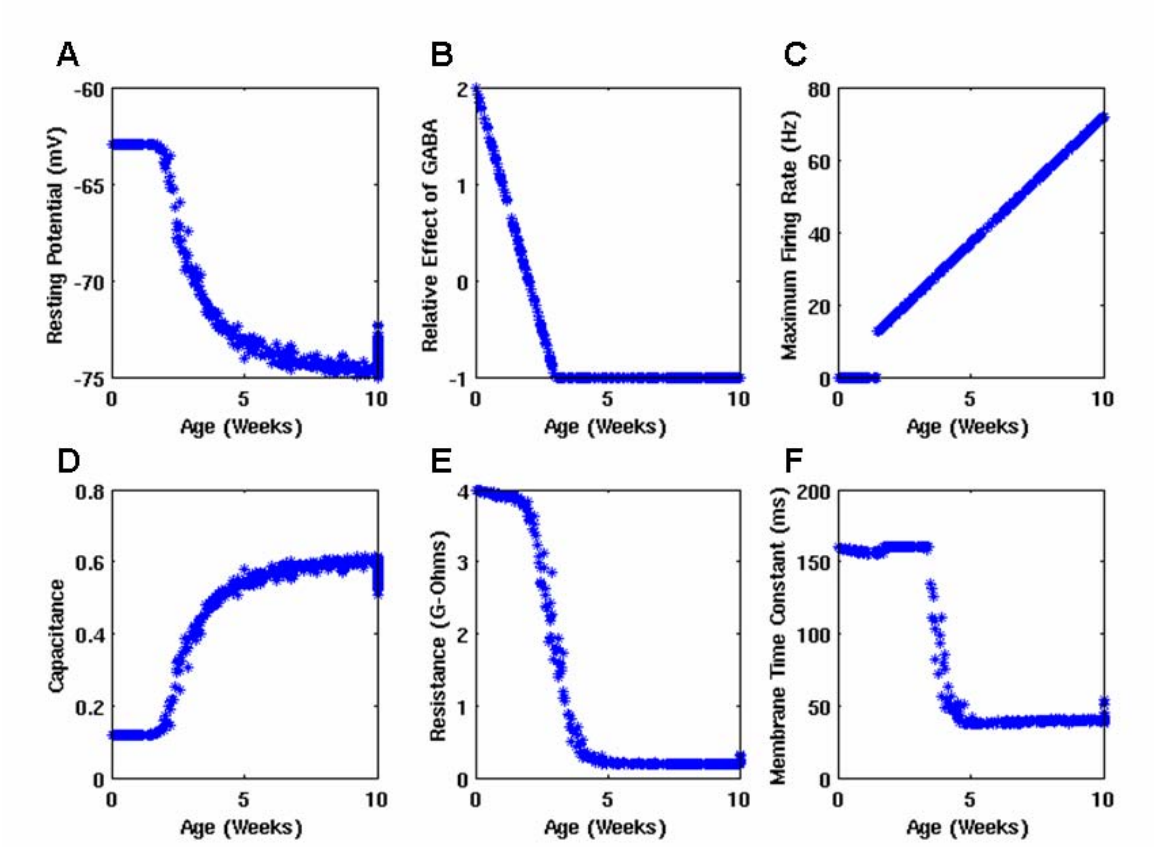

Figure IV-8: Physiological Maturation of Granule Cells in Model
(A) Resting potential decreased as volume increases (Equation IV.43). (B) Relative effect of GABA on immature neurons was age dependent (Eqn. IV.42). (C) Maximum firing rate of neuron increased with age. Neurons must receive glutametergic synapses to fire (Eqn. IV.44). (D) Membrane capacitance was proportional to the volume of the neuron. The size of the neuron (and the volume) increased with age and the growth of connections (Eqn. IV.38). (E) Membrane resistance decreased as number of connections increases (Eqn. IV.39). (F) The membrane time constant τ was a function of resistance, volume, and the mature time constant (Eqn. IV.40). The maximum τ was set at 4 times the time constant of mature neurons.

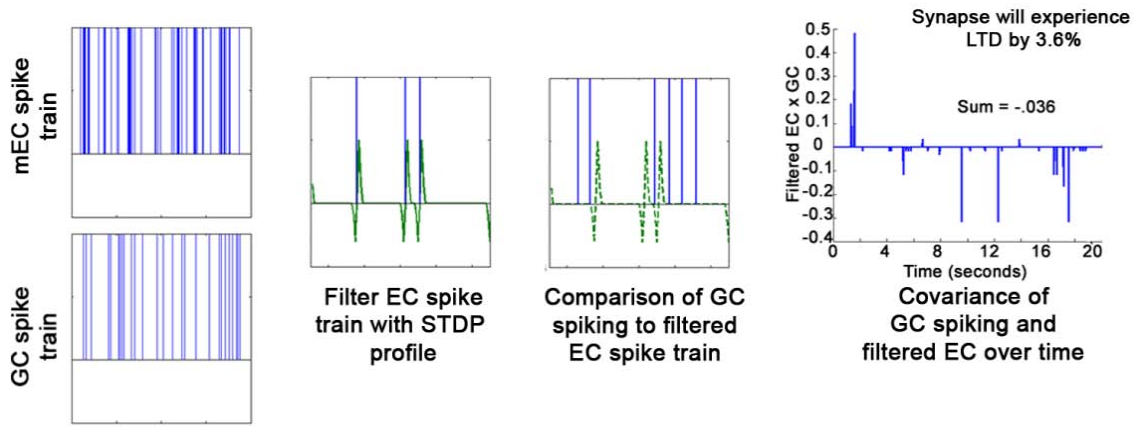

Figure IV-9: Schematic of Synaptic Learning
For each synapse, the spiking of the pre-synaptic neuron was filtered with a STDP curve and compared to the spike-train of the post-synaptic neuron. The temporal covariance of the pre- and post-synaptic neurons was used to determine the amount the synapse learns.

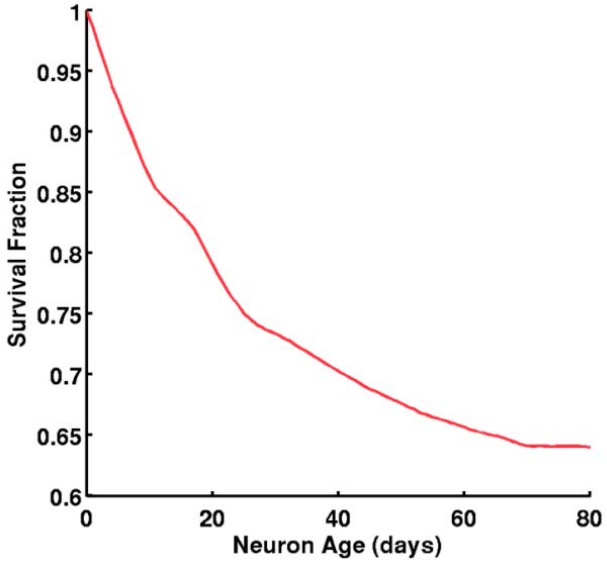

Figure IV-10: Cell Death in Model
Proportion of newly born GCs that survived to given age in model under standard maturation conditions.

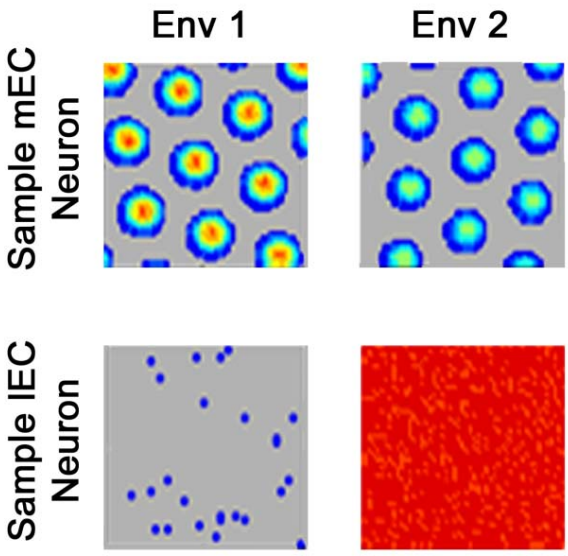

Figure IV-11: EC input structure
Sample input neuron activity in different environments (Env). Medial entorhinal cortex (mEC) neurons (top) had a spatial response, lateral EC (lEC) neurons (bottom) fired at equal rates at all spatial locations.

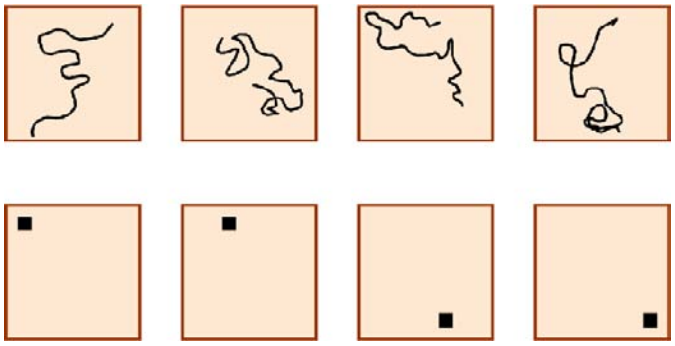

Figure IV-12: Illustration of Environment Structure during Training and Testing
During training (top), model "explored" random paths within an environment. During testing (bottom), network activity was measured in a series of spatial locations that tile the environment.

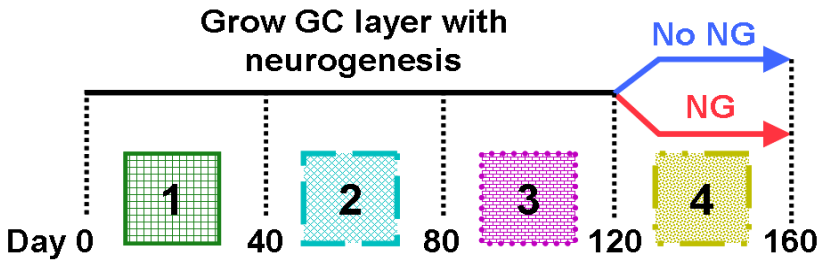

Figure IV-13: Timeline of Model Initialization and Growth
For all studies, the model was initialized on day 0 with only immature GCs, and the networks were grown, with neurogenesis, until day 120. Environments were changed every 40 days. Neurogenesis was stopped in No NG networks after day 120, and continued in NG networks. Testing began on day 160.

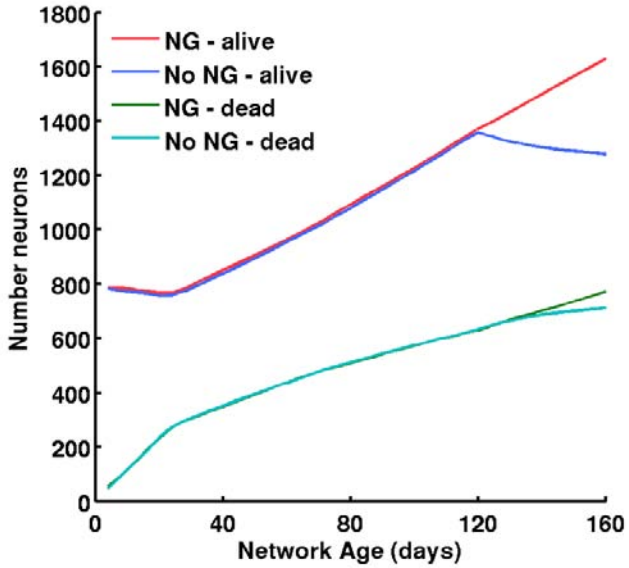

Figure IV-14: Growth of the GC layer and cell death
Networks were initialized with 800 immature GCs and proceed to grow in multiple environments for 120 days. On day 120, NG and No NG networks were separated, and only the NG network continued to grow with neurogenesis. Shown in green and light blue is the cumulative number of dead cells in the model over time.

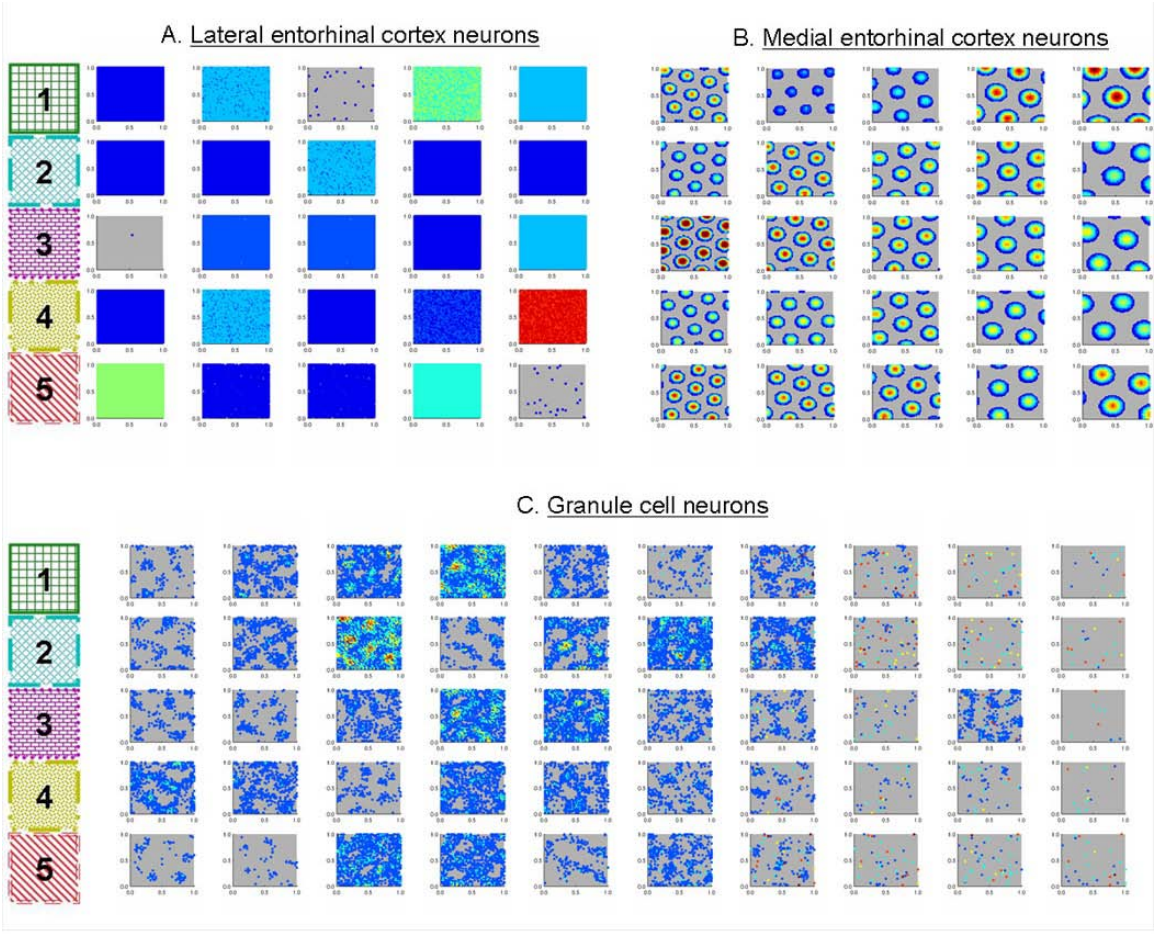

Figure IV-15: Neuron behavior in model
The spatial responses of several sample neurons from the lEC, mEC, and GC neuron layers in response to four familiar environments (1-4) and one novel environment (5). Each column represents the same neuron's responses in different environments. Note the spatial properties of mEC neurons (top right) and the lack of spatial responses in lEC neurons (which differ in firing rates between environments). Grey = no firing, blue = light firing, red = heavy firing.

References

1. Aimone, J.B., Wiles, J., and Gage, F.H., *Computational influence of adult neurogenesis on memory encoding.* Neuron, 2009. **61**(2): p. 187-202.

2. Butz, M., Teuchert-Noodt, G., Grafen, K., and van Ooyen, A., *Inverse relationship between adult hippocampal cell proliferation and synaptic rewiring in the dentate gyrus.* Hippocampus, 2008. **18**(9): p. 879-98.

3. Wiskott, L., Rasch, M.J., and Kempermann, G., *A functional hypothesis for adult hippocampal neurogenesis: avoidance of catastrophic interference in the dentate gyrus.* Hippocampus, 2006. **16**(3): p. 329-43.

4. Butz, M., Lehmann, K., Dammasch, I.E., and Teuchert-Noodt, G., *A theoretical network model to analyse neurogenesis and synaptogenesis in the dentate gyrus.* Neural Netw, 2006. **19**(10): p. 1490-505.

5. Patton, P.E. and McNaughton, B., *Connection matrix of the hippocampal formation: I. The dentate gyrus.* Hippocampus, 1995. **5**(4): p. 245-86.

6. Santhakumar, V., Aradi, I., and Soltesz, I., *Role of mossy fiber sprouting and mossy cell loss in hyperexcitability: a network model of the dentate gyrus incorporating cell types and axonal topography.* J Neurophysiol, 2005. **93**(1): p. 437-53.

7. Samsonovich, A.V. and Ascoli, G.A., *Statistical morphological analysis of hippocampal principal neurons indicates cell-specific repulsion of dendrites from their own cell.* J Neurosci Res, 2003. **71**(2): p. 173-87.

8. Ascoli, G.A. and Atkeson, J.C., *Incorporating anatomically realistic cellular-level connectivity in neural network models of the rat hippocampus.* Biosystems, 2005. **79**(1-3): p. 173-81.

9. Ge, S., Yang, C.H., Hsu, K.S., Ming, G.L., and Song, H., *A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain.* Neuron, 2007. **54**(4): p. 559-66.

10. Zhao, C., Teng, E.M., Summers, R.G., Jr., Ming, G.L., and Gage, F.H., *Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus.* J Neurosci, 2006. **26**(1): p. 3-11.

11. Piatti, V.C., Esposito, M.S., and Schinder, A.F., *The timing of neuronal development in adult hippocampal neurogenesis.* Neuroscientist, 2006. **12**(6): p. 463-8.

12. Laplagne, D.A., Esposito, M.S., Piatti, V.C., Morgenstern, N.A., Zhao, C., van Praag, H., Gage, F.H., and Schinder, A.F., *Functional convergence of neurons generated in the developing and adult hippocampus.* PLoS Biol, 2006. **4**(12): p. e409.

13. Ge, S., Goh, E.L., Sailor, K.A., Kitabatake, Y., Ming, G.L., and Song, H., *GABA regulates synaptic integration of newly generated neurons in the adult brain.* Nature, 2006. **439**(7076): p. 589-93.

14. Overstreet Wadiche, L., Bromberg, D.A., Bensen, A.L., and Westbrook, G.L., *GABAergic signaling to newborn neurons in dentate gyrus.* J Neurophysiol, 2005. **94**(6): p. 4528-32.

15. Esposito, M.S., Piatti, V.C., Laplagne, D.A., Morgenstern, N.A., Ferrari, C.C., Pitossi, F.J., and Schinder, A.F., *Neuronal differentiation in the adult hippocampus recapitulates embryonic development.* J Neurosci, 2005. **25**(44): p. 10074-86.

16. Ambrogini, P., Lattanzi, D., Ciuffoli, S., Agostini, D., Bertini, L., Stocchi, V., Santi, S., and Cuppini, R., *Morpho-functional characterization of neuronal cells at different stages of maturation in granule cell layer of adult rat dentate gyrus.* Brain Res, 2004. **1017**(1-2): p. 21-31.

17. van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., and Gage, F.H., *Functional neurogenesis in the adult hippocampus.* Nature, 2002. **415**(6875): p. 1030-4.

18. Samsonovich, A.V. and Ascoli, G.A., *A simple neural network model of the hippocampus suggesting its pathfinding role in episodic memory retrieval.* Learn Mem, 2005. **12**(2): p. 193-208.

19. Amaral, D.G., Scharfman, H.E., and Lavenex, P., *The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies).* Prog Brain Res, 2007. **163**: p. 3-22.

20. Ratzliff, A.H., Howard, A.L., Santhakumar, V., Osapay, I., and Soltesz, I., *Rapid deletion of mossy cells does not result in a hyperexcitable dentate gyrus: implications for epileptogenesis.* J Neurosci, 2004. **24**(9): p. 2259-69.

21. Frazier, C.J., Strowbridge, B.W., and Papke, R.L., *Nicotinic receptors on local circuit neurons in dentate gyrus: a potential role in regulation of granule cell excitability.* J Neurophysiol, 2003. **89**(6): p. 3018-28.

22. Lubke, J., Frotscher, M., and Spruston, N., *Specialized electrophysiological properties of anatomically identified neurons in the hilar region of the rat fascia dentata.* J Neurophysiol, 1998. **79**(3): p. 1518-34.

23. McNaughton, B.L. and Barnes, C.A., *Physiological identification and analysis of dentate granule cell responses to stimulation of the medial and lateral perforant pathways in the rat.* J Comp Neurol, 1977. **175**(4): p. 439-54.

24. Han, Z.S., Buhl, E.H., Lorinczi, Z., and Somogyi, P., *A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurons in the dentate gyrus of the rat hippocampus.* Eur J Neurosci, 1993. **5**(5): p. 395-410.

25. Freund, T.F., *Interneuron Diversity series: Rhythm and mood in perisomatic inhibition.* Trends Neurosci, 2003. **26**(9): p. 489-95.

26. Freund, T.F. and Buzsaki, G., *Interneurons of the hippocampus.* Hippocampus, 1996. **6**(4): p. 347-470.

27. Izhikevich, E.M., *Simple model of spiking neurons.* IEEE Trans Neural Netw, 2003. **14**(6): p. 1569-72.

28. Toni, N., Teng, E.M., Bushong, E.A., Aimone, J.B., Zhao, C., Consiglio, A., van Praag, H., Martone, M.E., Ellisman, M.H., and Gage, F.H., *Synapse formation on neurons born in the adult hippocampus.* Nat Neurosci, 2007. **10**(6): p. 727-34.

29. Esposito, M.S., Piatti, V.C., Laplagne, D.A., Morgenstern, N.A., Ferrari, C.C., Pitossi, F.J., and Schinder, A.F., *Neuronal Differentiation in the Adult Hippocampus Recapitulates Embryonic Development.* Journal of Neuroscience, 2005. **25**(44): p. 10074-10086.

30. Overstreet-Wadiche, L.S. and Westbrook, G.L., *Functional maturation of adult-generated granule cells.* Hippocampus, 2006. **16**(3): p. 208-15.

31. Lin, Y.W., Yang, H.W., Wang, H.J., Gong, C.L., Chiu, T.H., and Min, M.Y., *Spike-timing-dependent plasticity at resting and conditioned lateral perforant path synapses on granule cells in the dentate gyrus: different roles of N-methyl-D-aspartate and group I metabotropic glutamate receptors.* Eur J Neurosci, 2006. **23**(9): p. 2362-74.

32. Schmidt-Hieber, C., Jonas, P., and Bischofberger, J., *Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus.* Nature, 2004. **429**(6988): p. 184-7.

33. Tashiro, A., Sandler, V.M., Toni, N., Zhao, C., and Gage, F.H., *NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus.* Nature, 2006. **442**(7105): p. 929-33.

34. Fyhn, M., Hafting, T., Witter, M.P., Moser, E.I., and Moser, M.B., *Grid cells in mice.* Hippocampus, 2008. **18**(12): p. 1230-8.

35. Hafting, T., Fyhn, M., Molden, S., Moser, M.B., and Moser, E.I., *Microstructure of a spatial map in the entorhinal cortex.* Nature, 2005. **436**(7052): p. 801-6.

36. Fyhn, M., Molden, S., Witter, M.P., Moser, E.I., and Moser, M.B., *Spatial representation in the entorhinal cortex.* Science, 2004. **305**(5688): p. 1258-64.

37. Solstad, T., Moser, E.I., and Einevoll, G.T., *From grid cells to place cells: a mathematical model.* Hippocampus, 2006. **16**(12): p. 1026-31.

38. Cameron, H.A. and McKay, R.D., *Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus.* J Comp Neurol, 2001. **435**(4): p. 406-17.

CHAPTER V: STUDY 1: PATTERN INTEGRATION AND TEMPORAL CODING

Introduction

The presumed pattern separation function of the DG has been examined biologically at several different levels [1, 2]. Behaviorally, rats whose DG has been lesioned with colchicine demonstrate an impaired ability to perform certain behavioral tasks in which spatial separation is the experimental variable [3]. Mice conditionally lacking the NR1 receptor in the DG (thus having no learning in GC neurons) show behavioral deficits in a fear conditioning task that requires the mice to distinguish between two different contexts[4]. Physiologically, the in vivo activation of DG neurons is consistent with a pattern separation role[5-7], but the nature of this separation is unclear [8, 9].

The main drawback to both behavioral and physiological studies investigating pattern separation is that quantifying separation requires an ability to measure input similarity. Computationally, the pattern separation hypothesized for the DG involves the separation of input signals from the EC [10]. In most biological experiments, the similarity between different inputs to the hippocampus is assumed to be dependent on the extent that two test events or environments are different [9]. However, with the exception of studies on mEC grid cells, the degree to which EC inputs change based on behavioral inputs is not clear. This presents a dilemma in experimentally assessing the DG's ability to pattern separate; without knowing the extent to which a contextual or behavioral shift changes the network's input, there is no proper reference to assess DG function.

In contrast to the difficulty of measuring similarity in biological contexts, the quantification of pattern separation is straightforward in a computational modeling domain. A

simple but effective quantification of separation is to measure the similarity between network inputs and compare them to the similarity of network outputs [10].

Experimental Procedures

The experiments performed on the computational model that are described in this and subsequent chapters have the same initial design. Networks were initialized with fully connected layers of non-neurogenic neurons and 400 immature neurons. After initialization, the networks were grown in a series of three separate environments for 40 days each. On day 120, the networks were replicated and one network continued to grow in a fourth environment with neurogenesis (NG) and the other grew without neurogenesis (No NG). Both networks retained full synaptic plasticity, neuron maturation, and cell death. After day 160, the behavior of the NG and No NG networks was then examined in various tasks. During the test phase of the model, the networks had no plasticity (synaptic plasticity, maturation, neurogenesis, and cell death were turned off).

Pattern separation was tested in the network by generating test environments that spanned a large range of input similarities. These test environments were generated by creating a random environment (Env 1) and then calculating the most orthogonal lEC representation possible with the same activity distribution for a second environment (Env 2). This was performed by ranking all the lEC neurons' activities in the environment (most active to least active), and inverting those activities for the lEC neurons in the second environment; i.e., the most depolarized neuron in Env 1 is the least depolarized in Env 2, the 2^{nd} most depolarized in Env 1 is the 2^{nd} least in Env 2 and so on until the least depolarized in Env 1 is the most depolarized in Env 2. After generation of these two distinct environments, a series of intermediate environments were also generated. These environments were weighted combinations of activities from the original two environments. For example, an environment that is 80% Env 1 vs. 20% Env 2 would have 80%

of its lEC neurons responding at Env1 rates and 20% responding at Env 2 rates. Grid cell positions and activities were unchanged between networks, so different mEC activities were obtained by varying the spatial location used in testing.

The networks were then tested in 400 different spatial locations within that environment. These locations uniformly tiled the environment, ensuring that many distinct combinations of grid cells were activated. The network was exposed for 500 milliseconds (ms) (20 time steps in the model). The activities of the EC and GC neurons were summed up over the duration of this experimental period. After exposure to each of the test environments, the responses to different spatial locations and different environments were compared to one another using the normalized dot product (described below) to compute similarity. The DG similarities from events with comparable EC similarity were averaged together to determine the DG output similarity for a given input similarity.

Normalized dot product

The normalized dot product (NDP) was used in this study to measure pattern separation. NDP was chosen over other similarity measures such as correlation and distance for several reasons. First, NDP is a bounded parameter due to its normalization. This is in contrast to distance and covariance, whose scales are determined by the dimensionality and scale of the vectors being compared. Since the model uses layers of different (and continuously changing) sizes and different activity levels, a measure such as distance would be difficult to compare across layers. Secondly, unlike correlation, the mean response of each neuron is not subtracted from the signal. Therefore, NDP is independent of zeros.

NDP, which is also known as cosine similarity, is the dot product of two vectors normalized by the mean of each vector (norm)

$$NDP(\mathbf{x_i}, \mathbf{x_j}) = \frac{\mathbf{x_i} \bullet \mathbf{x_j}}{\|\mathbf{x_i}\| \times \|\mathbf{x_j}\|} \quad \text{(V-1)}$$

Where:

$\mathbf{x_i}$ is the 'i'th vector of neuron responses

$\mathbf{x_j}$ is the 'j'th vector of neuron responses

$\|\mathbf{x_i}\|$ is the norm of the 'i'th ('j'th) neuron response vector. The norm corresponds to the length of the vector from zero (square root of the sum of the vector values squared)

NDP does have limitations in analyzing network activities. One limitation arises from its normalization. For instance, consider the following vectors **a**, **b**, and **c**:

a=[0, 0, 0, 0, 0, 1, 0]

b=[1, 1, 1, 1, 1, 0, 0]

c=[0, 0, 0, 0, 0, 0, 1]

The NDP between any two of these vectors is 0. However, by other similarity or distance measures, **a** and **c** would be substantially closer to each other than to **b**. Which measure is most appropriate depends on the function and requirements of the structure receiving the information, which is unclear in the case of the CA3. Therefore, while NDP is used exclusively for measuring similarity in the following studies, it is important to consider that other similarity measures may have different responses.

Temporal separation experiment

In the second part of the study, networks continued to grow in between testing days. For ten separate days, the model was grown in a fifth environment (distinct from the test

environment). At the end of each model day, the system was tested in the same environments used in the pattern separation study. As in the previous study, during the testing of the model, there was no plasticity of any kind. Therefore, although the response of the networks to test environment was measured multiple times, the environment was always "novel" to the network.

The responses of the networks to the test environments were collected each day, and then the similarities of the networks' inputs and outputs were tested across different days. The neuron responses in each location/environment combination were compared to the responses of every other location/environment combination from different days.

Responses separated by the same amount were pooled together (e.g., days 2 and 4 were two day apart, as were days 5 and 7). As with the study above, for each number of days apart, the output similarities between events with comparable EC similarities were averaged together, giving an estimate of separation by the DG network. This allowed pattern separation to be measured for events separated by a prescribed number of days. By this definition, the study in the first experiment concerned pattern separation for event separated by zero days.

Pattern Separation Results

After the NG and No NG networks were grown, pattern separation was tested in the model by measuring the response of the network to multiple locations in a series of test environments. These environments were designed to test a range of input similarities in both the lEC layer, which conveys environmental context information, and the mEC layer, which contains information about spatial location. Based on previous studies (O'Reilly and McClelland, 1994), the DG's outputs were expected to be substantially less similar than the inputs.

The networks' responses to these test environments confirmed that both the NG and No NG models were capable of strong pattern separation (Figure V-2). For highly similar EC inputs (similar spatial and contextual inputs), the NG and No NG networks performed comparably at

pattern separation, both providing outputs that were significantly more distinct than the inputs. However, when the EC inputs were dissimilar (Figure V-2, left), the separation of outputs was different for NG and No NG networks. No NG networks were still effective at pattern separation, but the degree of separation was reduced because the inputs were already quite distinct. NG networks, on the other hand, were significantly worse at separating inputs than the No NG networks (NG vs. No NG; $p<0.01$; Figure V-2). This effect in the NG networks actually appeared to result in outputs that were more similar than the inputs for very separated EC inputs. These effects of input similarity on network separation were independent of which EC layer was providing the similarity that was separated by the DG (Figure V-3). The effect of neurogenesis was most pronounced when comparing two events for which the encoding by both EC layers was very distinct.

To determine which neurons in the network were contributing to the effect of NG on pattern separation, immature neurons were removed from the analysis of the NG networks' responses. Without the inclusion of young neurons (<6 weeks old) in the similarity calculation, the NG networks were then equally effective at pattern separation (Figure V-4). This suggests that the young neurons in the network were responsible for affecting the population pattern separation capability of the DG. The degree to which the similarity of NG networks differed from that of the No NG networks was dependent on the rate of neurogenesis: as would be expected, the greater the neurogenesis rate, the larger the effect on pattern separation (Figure V-5).

Temporal Separation Results

As described in Chapter III, the dynamics of the neurogenesis process may have an effect on this added similarity contributed by immature neurons during memory encoding. Because the immature neuron population is continuously changing in time, one possibility is that this

similarity is temporally dependent: while events close in time may utilize the same immature neurons, events encoded farther apart in time will encounter distinct sets of immature neurons, increasing the separation between events encoded by the DG.

To examine whether this proposed effect actually occurs in the model, the networks' responses to the test environments were measured on different days, with the networks continuing to grow in a separate environment in between test sessions (Figure V-6). After the growth phase on each day, the network was tested in each of the previous test environments at the 400 distinct locations used in the previous study. The separation of input and output responses was then calculated, but now with time between events as a controlled variable. The NG networks' ability to separate EC inputs was dependent on the time elapsed between events (Figure V-7, Figure V-8). If two events occurred within a short time of one another, the pattern separation was similar to what was observed in the previous pattern separation study (Figure V-3). However, if events were far apart in time, the separation was considerably stronger. No NG networks lacked this time dependence, with events occurring far apart in time separated similarly to events occurring at the same time (Figure V-9).

The time dependence of the separation of events with a given input similarity was tracked as time elapsed between event presentations (Figure V-10). As observed above, for each level of input similarity inspected, events close in time (less than two days) were encoded considerably more similarly in NG networks than events occurring farther apart in time (interaction of neurogenesis and time; $p<0.05$). The No NG networks did not reveal any contribution of time to output similarity. Importantly, this change in pattern separation was not a direct effect of network learning, since there was no plasticity during the test phase when the networks were exposed to the test environments.

There was an effect of input similarity on the temporal separation of information. Immature neurons did not contribute much similarity to events whose inputs were very similar

(80% input similarity, Figure V-10, top), as these events were strongly separated by mature cells in the network. However, over time, the changing of the immature neuron population is able to make pattern separation stronger in NG networks as opposed to No NG networks. This long-term contribution to pattern separation by new neurons was reduced when inputs were moderately similar (50%, Figure V-10, middle), and separation was essentially the same for NG and No NG networks during the encoding of highly dissimilar events separated in time (10%, Figure V-10, bottom). The effect of input similarity for events close in time was reversed, with immature neurons having a greater effect on pattern separation for highly dissimilar inputs, and less of an effect for highly similar inputs.

Discussion

Pattern separation

These results suggest that new neurons have several significant effects on the pattern separation properties of the DG network. While the DG model is quite effective at pattern separation, the presence of immature neurons increases the similarity between information outputted by the GC layer. The effect of young neurons is most prominent when comparing responses to information that is already very separated. The effect of young neurons on pattern separation can be referred to as *pattern integration* (Figure V-11).

Young neurons appear to be disrupting pattern separation by virtue of their increased activity in the network. The physiological properties of immature neurons may make them more responsive, in particular when they are either depolarized by GABA or only weakly inhibited by it. This lower threshold for responsiveness will cause the neurons to fire to a wider range of events. This is in contrast to the older GC population, which is highly inhibited by GABA and generally kept quiet in the network [1, 7]. Only inputs very specific to their synaptic complement will activate mature neurons, making them potent pattern separation devices. Within a system

where most neurons are tightly tuned and only responsive to a small fraction of inputs, the young neurons' indiscriminate responses will have a potent effect on the overall population response similarity.

From a functional point of view, this pattern integration may help to form associations in the CA3 during memory formation. This pattern integration effect is different from the pattern completion function that has been proposed for downstream hippocampal areas [9, 11, 12]. Pattern completion produces the same output from related but different inputs, allowing the reconstruction of a memory from a partial cue, whereas pattern integration, as described here, limits the amount of separation of very distinct inputs. The CA3 network is believed to have both pattern completion and pattern separation functions [11]. The CA3 "completes" input patterns when the information is similar to a previously stored memory, but when the input is distinct from previous information, it is believed to encode it more distinctly [9, 12]. The presence of a DG network upstream that is separating inputs, but ensuring that information is not overly separated (via pattern integration), may interact with this CA3 encoding to provide an interesting combined effect on memory encoding (Figure V-12).

Temporal separation

The pattern integration function contributed by immature neurons may have several important roles in cognition and memory formation (see full discussion in Chapter IX). However, a decrease of pattern separation can be accomplished by other, more simpler, mechanisms. Importantly, the neurons providing this function are not static. Over time, the population of immature neurons adding similarity to the network is continuously changing. Events occurring at different times (many days apart), would be expected to activate the population of immature neurons at those times, providing an additional mechanism of separation between events (Figure V-13).

The results of this study investigating how pattern separation changes as time passes between events confirms that new neurons provide an interesting temporal dynamic to the system. While events encoded at the same time see a strong pattern integration effect, the effect diminishes substantially as time passes between events. After three to four days pass between events, the effect of neurogenesis is essentially negated. Beyond that period, the neurogenic DG is sometimes actually better at separating events than the non-neurogenic networks. This increased separation is due to the fact that all GC neurons are performing pattern separation: old neurons separating by context, young neurons separating by time.

Chapter V, in part, includes material that was published in the article "Computational Influence of Adult Neurogenesis on Memory Encoding," Aimone, James B; Wiles, Janet; and Gage, Fred H.; *Neuron*, January 2009. The dissertation author was the primary investigator and author of this paper.

Figures

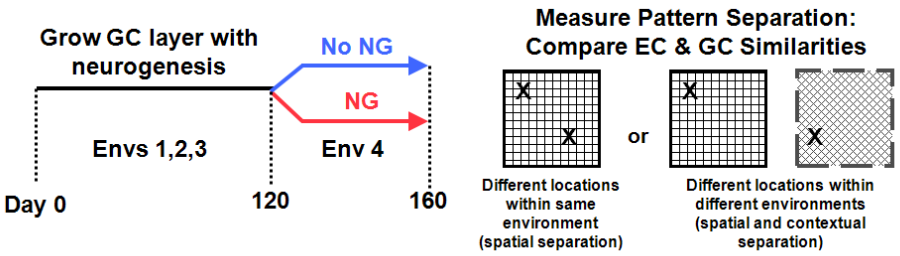

Figure V-1: Schematic of Pattern Separation Experiment
After initialization, networks were grown in a series of three environments for 40 days each. On Day 120, NG and No NG networks were generated and grown in a 4th environment for 40 days. On Day 160, pattern separation was examined by measuring the networks' responses to multiple spatial locations in different environments.

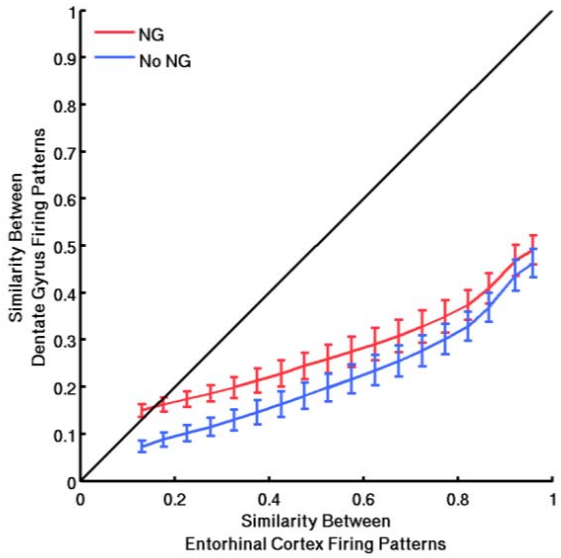

Figure V-2: Pattern Separation in NG and No NG Networks
Effect of EC similarity (x-axis) on the similarity between DG outputs (y-axis). In networks with neurogenesis (NG, red), very low input similarity resulted in relatively higher DG similarity, an effect referred to as pattern integration. Pattern integration did not occur in non-neurogenic networks (No NG,blue). Similarity was measured by the normalized dot product (NDP). The difference between NG and No NG networks was significant ($p<0.01$). Error bars represent standard deviation in this and all following figures.

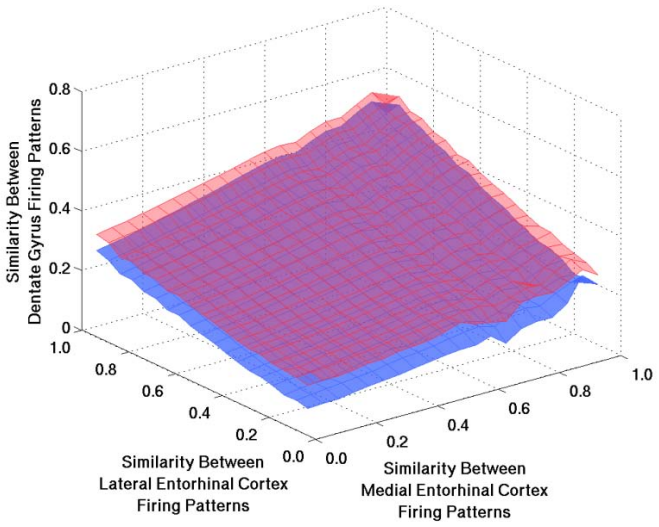

Figure V-3: Pattern Separation of Different EC Layers
The decrease in pattern separation with neurogenesis occurred with both spatial (medial EC) and contextual (lateral EC) inputs. NG separation is red (overlayed on top), No NG separation is blue (bottom).

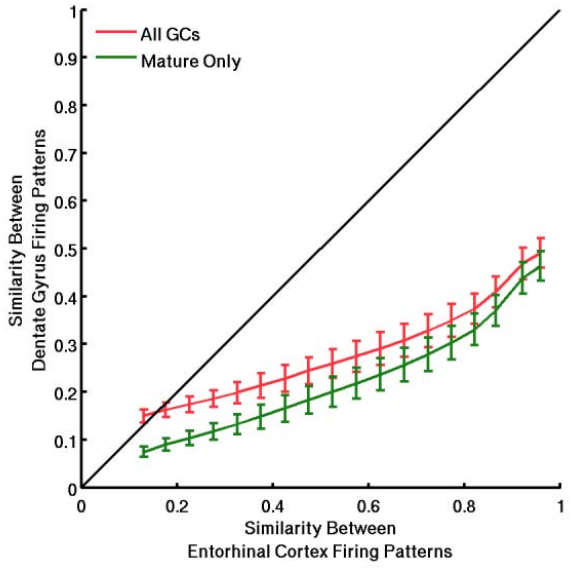

Figure V-4: Pattern Separation by Mature Neurons in NG Networks
The full population of GC showed the pattern integration effect at low levels of input similarity (red), whereas the mature neurons (>6 weeks of age, green) remained very effective at pattern separation.

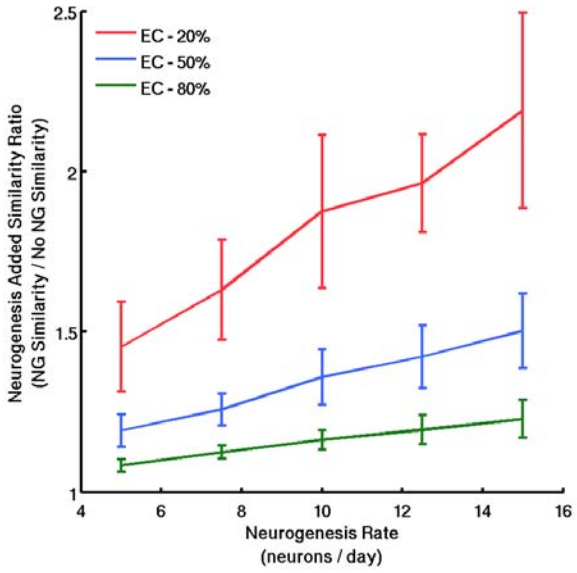

Figure V-5: Effect of Neurogenesis Rate on Pattern Integration
Pattern integration was measured for different rates of new neurons integrating into the network.

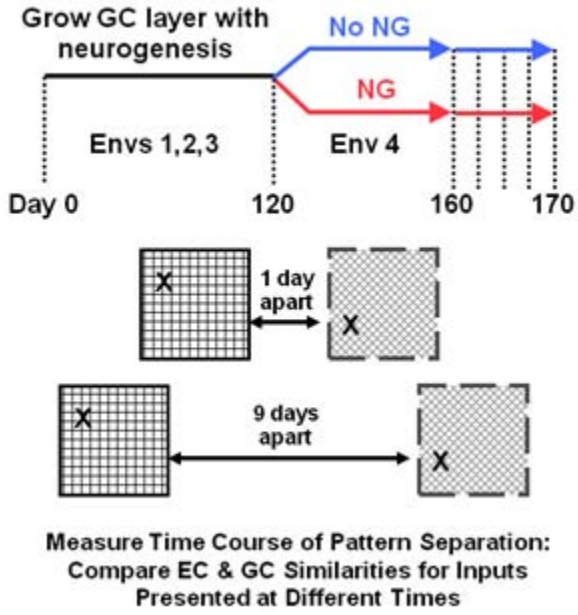

Figure V-6: Schematic of Temporal Separation Study
The model continued to grow with maturation, neurogenesis and cell death between testing sessions, at which time the response of the model was measured at different environments and spatial locations.

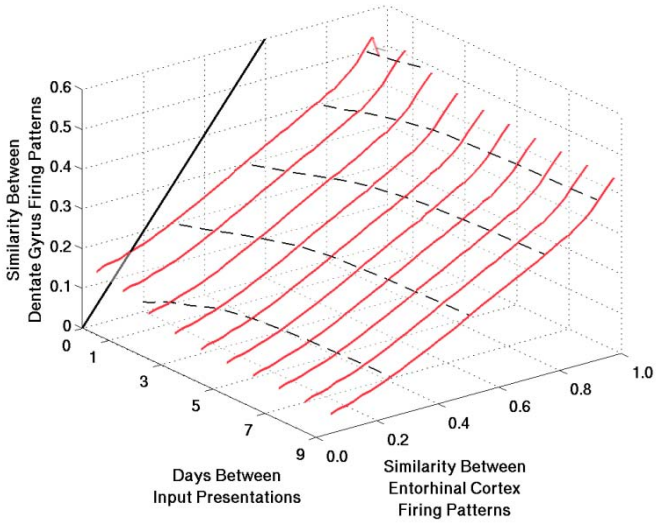

Figure V-7: Effect of Time on Pattern Separation Curves in NG Networks
Pattern separation got stronger as more time elapses between inputs to the network. Dashed lines represent DG similarity contours for NDPs of 0.1, 0.2, 0.3, 0.4, and 0.5 from bottom to top.

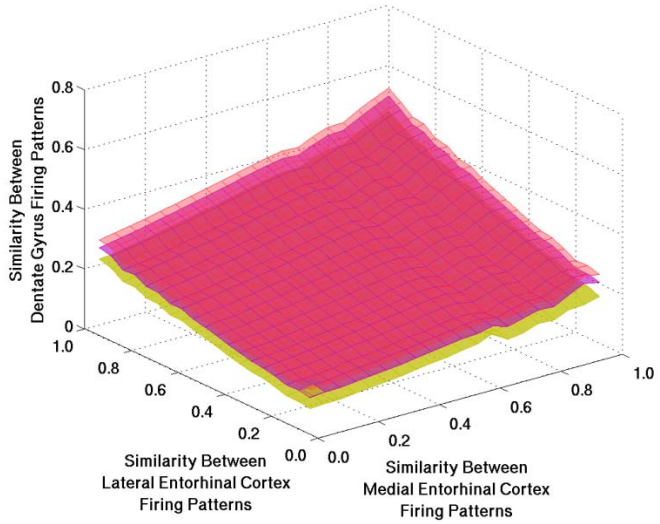

Figure V-8: Effect of Time on Pattern Separation in NG Networks
Pattern separation for inputs varying with both mEC and lEC inputs in NG networks for events separated by 1 (red), 3 (purple), and 9 (yellow) days.

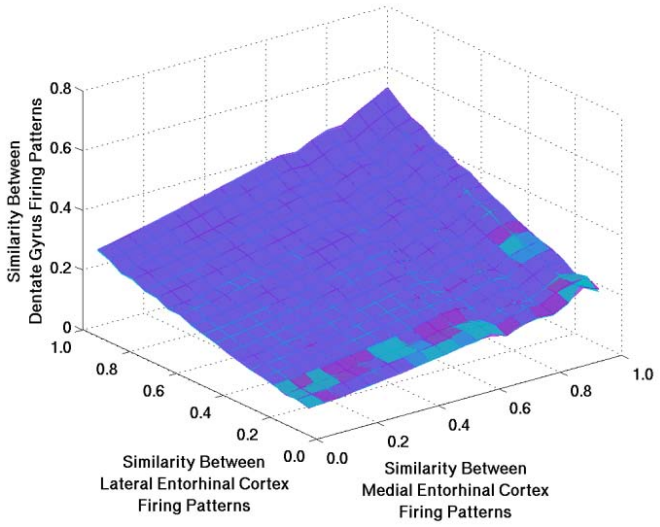

Figure V-9: Effect of Time on Pattern Separation in No NG Networks
Pattern separation for inputs varying with both mEC and lEC inputs in No NG networks for events separated by 1, 3, and 9 days. The three profiles are overlapping, and thus not discernable.

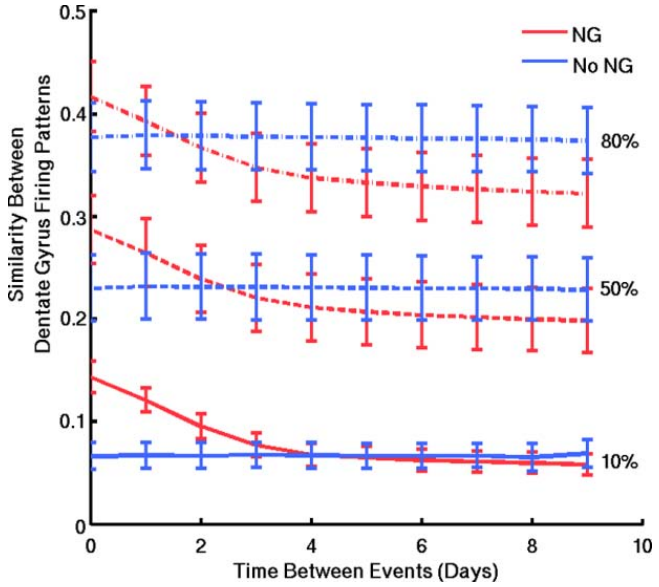

Figure V-10: Effect of Time on Separation of Events of Specific Input Separation
Effect of time between events on pattern separation of inputs that are 80% (top), 50% (middle), and 10% (bottom) similar. Note how DG similarities between events separated in time were lower than those tested on the same day. Both the decrease in similarity over time and the interaction between time and NG/No NG groups were significant for each of the input similarity groups ($p<0.01$).

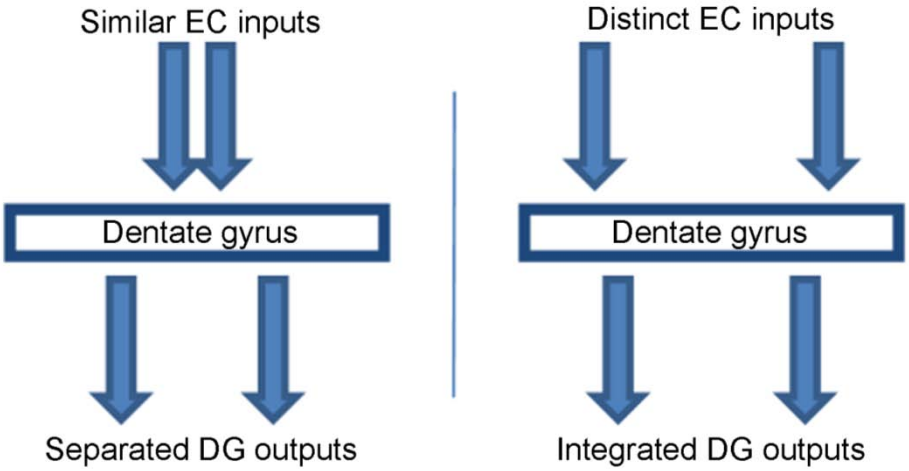

Figure V-11: Cartoon Schematic of Pattern Integration Function
In neurogenic network, events are separated by the DG when the inputs are highly similar, while events that are dissimilar gain associations. In No NG networks (not shown), the distinct EC inputs would also be separated.

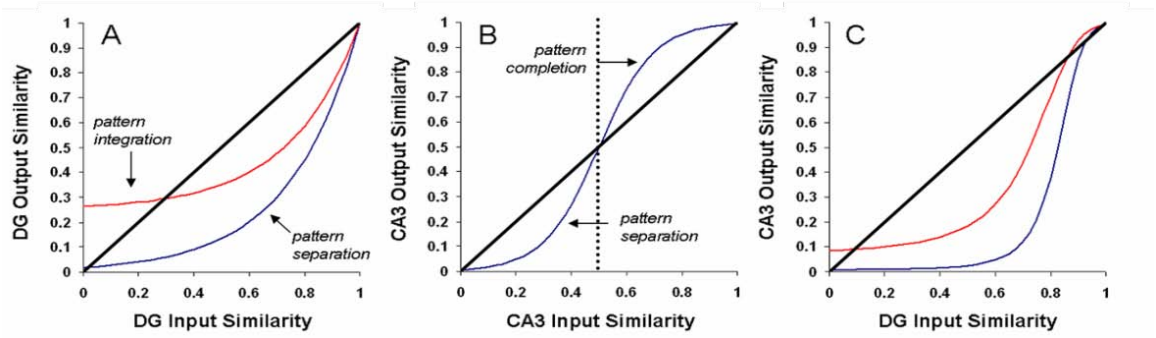

Figure V-12: Potential Interaction of Pattern Separation, Integration, and Completion
The effects of neurogenesis in the DG will likely affect the pattern completion and separation functions in the CA3. When the outputs of the DG (left panel) are used as the inputs to the CA3 (middle panel), a complex interaction arises (right panel), suggesting that CA3 outputs are never completely separated, but only completed for very similar inputs.

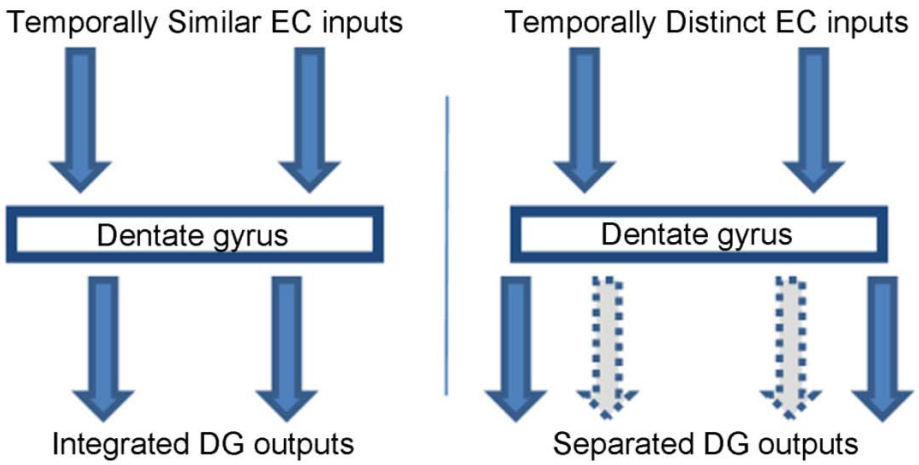

Figure V-13: Cartoon Schematic of Temporal Separation Function
While distinct events that are close in time can be pattern integrated by a NG network (left), events that are distinct in time are separated. This increase in separation is provided by the dynamics of neurogenesis in the network.

References

1. Treves, A., Tashiro, A., Witter, M.E., and Moser, E.I., *What is the mammalian dentate gyrus good for?* Neuroscience, 2008. **154**(4): p. 1155-1172.

2. Kesner, R.P., *A behavioral analysis of dentate gyrus function.* Prog Brain Res, 2007. **163**: p. 567-76.

3. Gilbert, P.E., Kesner, R.P., and Lee, I., *Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1.* Hippocampus, 2001. **11**(6): p. 626-36.

4. McHugh, T.J., Jones, M.W., Quinn, J.J., Balthasar, N., Coppari, R., Elmquist, J.K., Lowell, B.B., Fanselow, M.S., Wilson, M.A., and Tonegawa, S., *Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network.* Science, 2007. **317**(5834): p. 94-9.

5. Chawla, M.K., Guzowski, J.F., Ramirez-Amaya, V., Lipa, P., Hoffman, K.L., Marriott, L.K., Worley, P.F., McNaughton, B.L., and Barnes, C.A., *Sparse, environmentally selective expression of Arc RNA in the upper blade of the rodent fascia dentata by brief spatial experience.* Hippocampus, 2005. **15**(5): p. 579-86.

6. Henze, D.A., Wittner, L., and Buzsaki, G., *Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo.* Nat Neurosci, 2002. **5**(8): p. 790-5.

7. Jung, M.W. and McNaughton, B.L., *Spatial selectivity of unit activity in the hippocampal granular layer.* Hippocampus, 1993. **3**(2): p. 165-82.

8. Leutgeb, J.K. and Moser, E.I., *Enigmas of the dentate gyrus.* Neuron, 2007. **55**(2): p. 176-8.

9. Leutgeb, J.K., Leutgeb, S., Moser, M.B., and Moser, E.I., *Pattern separation in the dentate gyrus and CA3 of the hippocampus.* Science, 2007. **315**(5814): p. 961-6.

10. O'Reilly, R.C. and McClelland, J.L., *Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off.* Hippocampus, 1994. **4**(6): p. 661-82.

11. Leutgeb, S., Leutgeb, J.K., Moser, M.B., and Moser, E.I., *Place cells, spatial maps and the population code for memory.* Curr Opin Neurobiol, 2005. **15**(6): p. 738-46.

12. Leutgeb, S., Leutgeb, J.K., Barnes, C.A., Moser, E.I., McNaughton, B.L., and Moser, M.B., *Independent codes for spatial and episodic memory in hippocampal neuronal ensembles.* Science, 2005. **309**(5734): p. 619-23.

CHAPTER VI: STUDY 2: LONG-TERM FUNCTION OF NEW NEURONS

Section I: Long-Term Specialization of New Neurons

The functions for neurogenesis described in Chapter V derive from the possibility that immature neurons are more active in the DG network than mature neurons and that this heterogeneity is dynamic in that the population of broadly tuned neurons is continuously changing. If the role of neurogenesis only included pattern integration and temporal separation, the only requirement for the network would be a population that turns over constantly. For these functions, it would not matter if adult-born neurons survived in the network. This is not the case biologically, however. While there is considerable cell death in the network, a significant fraction of new neurons survive indefinitely [1].

The survival of new neurons does not appear random; rather there is considerable evidence that experience is critical to survival. Exposure to an enriched environment is one of the most potent survival cues [2], with neurons exposed between the first and second weeks after differentiation receiving the strongest survival boost [3]. This "critical period" for survival is temporally consistent with a survival dependence on NMDA activation via the NR1 receptor [4]. Coupled with the additional observation that immature neurons have increased synaptic plasticity relative to mature neurons [5, 6], it is reasonable to suspect that immature neurons are maturing to encode the environments that animals experience during maturation.

Consistent with this idea, several studies using immediate early genes (IEGs), such as c-fos and Zif, suggest that immature neurons are more likely to respond to an environment or task that it had been previously exposed to while maturing. After demonstrating that enrichment boosts the survival of 1-2 week old neurons, Tashiro and colleagues showed that those neurons are more likely to respond to that environment upon re-exposure than to other environments [3].

A similar study investigating when new neurons are used in spatial learning suggested that six week old neurons preferentially incorporate into those circuits involved in water maze learning [7].

These IEG studies provide good evidence that new neurons are incorporating information during their maturation stage for use later in life. However, IEGs have limitations as a functional tool. First, their relationship to neuronal activity is not fully understood, though their presence is also believed to be correlated to activity associated with plasticity, such as LTP [8]. However, whether non-plasticity-inducing activity shows IEG activation is unclear, further leaving unclear whether a c-fos negative neuron is negative because it did not fire, or because it did not experience synaptic plasticity within that particular environment. This is particularly important in the case of the DG, where the relationship between synaptic plasticity and DG function is unknown [9, 10]. Second, the translational response of IEGs can really only be investigated in one environment at a time. Finally, it is unclear whether immature neurons are capable of expressing IEGs and under what conditions [11].

This computational model of DG provides an alternative method to view the long-term responses of adult-born neurons. The following section describes an approach to investigate what new neurons respond to long after their maturation. The model permits the observation of all neurons in the network in response to multiple different environments at different times, providing a complement to IEG studies and suggesting what future biological experimental designs should look for.

Experimental Design for Measuring Long-Term Function

To investigate the long-term functional role of new neurons in the DG, model networks were initialized and grown to day 160 in four separate environments, as described in Chapter V. Briefly, networks were initialized with 400 immature GC and proceeded to mature in four

separate environments that are defined by distinct lEC response vectors and mEC grid arrangements. At day 120, after the third environment, each network was replicated, with one network continuing to grow with neurogenesis ('NG' network), and the other without further neurogenesis ('No NG' network).

After the networks had developed to day 160, the system was then tested in its response to each of the familiar environments (FEs) as well as a novel environment (NE). The model was simulated in 400 separate locations in each environment that uniformly tiled the virtual space. Firing rates for mEC neurons at the coordinates for each location was determined by calculating grid cell activity for that location. lEC neurons each fired at the specific firing rate for that environment. The system remained static in that location for 20 time steps (equivalent to 500 ms). The firing rate for each GC in that spatial location was determined by summing its cumulative response over that 500 ms time interval.

After testing in all five locations (four FEs and one NE) at day 160, the networks then continued to develop within the NE with full plasticity (the No NG networks still had no neurogenesis) until day 200. At day 200, each network was tested in the four FE's as well as the NE (though this is now actually a familiar environment as well).

During this testing, all plasticity was halted (no neurogenesis, maturation, cell death, or synaptic plasticity).

Neuron Specialization Results

Specialization in neurogenic networks

The response of a typical NG network to the five test environments (four FEs and one NE) at day 160 is shown in Figure VI-1. Neurons are displayed in different columns and are ordered by age (the oldest neurons are on the left, the youngest on the right). The graph shows responses to different spatial locations within each environment (rows).

There are several notable features of this network's response. First, while neurons of all ages are activated by each of the environments, there are distinct groups of neurons visible that appear to preferentially respond to certain environments. These apparently specialized neurons are clustered by age – neurons of the same approximate age respond greatest to the same FE. Second, only the FEs have these specialized groups of neurons, whereas the network did not show any specialization to the NE, which had not been experienced prior to this point. In addition, the fourth FE, which was experienced immediately prior to testing, appears to be developing a specialized group of neurons, but this group is not as well-formed as those of the other FEs.

Plotting the activity of neurons by date of birth (with responses of neurons of similar ages averaged together), it is apparent that the GC that responded to a given FE were those that were maturing within that environment (Figure VI-2). For instance, the neurons with the highest response to environment 1 were those that were initialized at the onset of the simulation (shown at Day 0) and those neurons born within the first 20 days. Even though neurons born between day 20 and day 40 were born within environment 1, they actually specialized to environment 2. This suggests that neurons in the model do not specialize to the environment of birth, but rather the environment that they were maturing within while 2-3 weeks old. While environments presented to the network switched at days 40, 80, and 120, the switch in specialization occurs for neurons born around days 20, 60, 100, and 140.

In addition to the populations of specialized neurons visible in Figures VI-1 and VI-2, there is a population of immature neurons that shows increased activity in all environments. This group is labeled by an asterisk in each figure. This population is the same set of broadly tuned neurons whose activity causes the effects on pattern separation described in Chapter V. Their response is indicative of the indiscriminant activity of these very young neurons – while their

individual firing rates are lower than mature cells firing to their preferred environment, they fire generally to everything.

After the testing phase at day 160, the networks were then grown in the NE that was previously tested. Maturing in this environment meant that the NE went from being novel to being familiar. After 40 days (day 200), the networks were then tested again on the five environments. A sample response is shown in Figure VI-3 and the average response broken down by age is shown in Figure VI-4. By day 200, the response to the fourth FE is now as robust as the response to the other FEs. In addition, the NE is now developing a specialized population of neurons. This is particularly evident in Figure VI-4, where the NE response now shows a group of neurons dedicated specifically to that environment.

Importantly, the neurons that comprised the indiscriminant group at day 160 became many of the neurons specialized to the NE at day 200. This suggests that while immature neurons are firing broadly to many inputs, they are also acquiring the information necessary for specialization later.

Specialization in non-neurogenic networks

Looking at specialization in networks without neurogenesis shows that the development of networks is dependent on the presence of new neurons. When No NG networks were tested at Day 160 (40 days after neurogenesis was stopped), there are strong specialized responses to the first three FEs, just as in the NG networks (Figure VI-5; Figure VI-6). Importantly, neurogenesis was still active in these networks during the initial presentation of these environments. Although there was no neurogenesis during the networks' experience of the fourth FE (Env 4), a specialized population of neurons still appears to be developing. Figure VI-6 shows that the neurons developing some specialization to environment 4 were those that had been born immediately prior to the cessation of neurogenesis, so they were maturing during the presentation of that environment.

While mature neurons in the No NG network still showed specialization to FEs, there was a conspicuous lack of the indiscriminate population of GC seen in the NG networks. The lack of this population is consistent with the inability for No NG networks to pattern integrate. This missing population also appeared to be the same set of neurons in the Day 160 NG network that became specialized to the NE by Day 200. Indeed, inspection of the No NG response to the FEs and NE at day 200 shows that no set of neurons preferentially specialized to the NE (Figure VI-7; Figure VI-8).

Section II: Dimensional Analysis of Long-term Network Responses

Overview of dimensional analysis of network responses

The growth of groups of neurons that prefer previously experienced environments suggests that the network is increasing the dimensionality of its response in a specialized manner. The addition of new neurons into the network is by definition increasing the dimensionality of the network (each neuron corresponds to a dimension), but it is unclear from this qualitative perspective how independent these dimensions are. It is possible that new neuron dimensions are redundant to other neurons, either just mimicking the response of other neurons or some linear combination of them. The other possibility is that they are independent, responding at least partly in a way that is distinct from the other neurons in the GC layer.

Principal components analysis (PCA) is a type of dimensional analysis tool that can be used to quantify the independence of the dimensions represented by new neurons. PCA is an analytical tool that isolates combinations of firing patterns, or principal components (PCs), that differentiate between different locations and environments. For example, a subset of neurons that fire in unison when the network is presented with one environment but not to any other environments would form a principal component partly explaining the variance in the network's response to different environments. While the absolute dimensionality of a network is defined by

the number of neurons, overlaps in neuronal responses can reduce the realized dimensionality of the response, an effect that is revealed by a decrease in the number of principal components that are necessary to explain the network's behavior.

PCA was used to determine the extent that network growth in the NE would result in the creation of new dimensions that were orthogonal, or independent, from those the network used to encode the FEs. Several steps were necessary to measure this independence (Figure VI-10). First, the PCs for the cumulative response of the network in different spatial locations within all four FEs were computed. The power of each PC in describing the network's output to a given location can be determined by computing the dot product of the PC vector and the network's response at that location, a process known as "projection." The response to each environment (the four FEs + the NE) was then projected onto this set of PC vectors. Figure VI-11 shows an example where different locations within each environment are locally dispersed in the space described by the first three PCs; in this graph, the different environments occupy separate regimes of PC space.

The network's response to an environment can be then reconstructed from the projection onto the PCs and the PCs themselves. The projection of the network's response to the novel inputs onto the familiar basis set will be "complete" (i.e., the response that is reconstructed from the projection will be identical to the original response) if the PCs contain the necessary dimensionality to encode the information. The alternative is that the projection is incomplete and the reconstruction will fail to account for the entire original signal. The difference between the original response and the reconstruction (the "residual" response) is, by definition, orthogonal to the response captured by the existing PCs. By comparing the residual variance to the variance of the original response, it is possible to quantify the extent of dimensional independence for that response, that is, the percentage of variance explained by dimensions that were not used in other environments.

Methods for dimensional analysis

PCA was performed on the aggregate of the responses of the model to 400 locations within each of the four environments used during initial growth (**X**=[**A**, **B**, **C**, **D**]). The set of eigenvectors returned by PCA ($\mathbf{V_X}$) represented the orthonormal basis for these responses that best explained the variance of the data.

PCA of the **X** data matrix is performed by normalizing the response of each neuron to that neuron's average response

$$\mathbf{X} = \begin{bmatrix} \mathbf{x_1} \\ \vdots \\ \mathbf{x_m} \end{bmatrix} \quad \text{(VI-1)}$$

$$\mathbf{X^*} = \begin{bmatrix} \mathbf{x_1 - \mu_{x_1}} \\ \vdots \\ \mathbf{x_{m-\mu_{x_m}}} \end{bmatrix} \quad \text{(VI-2)}$$

where:

$\mathbf{x}_1 \ldots \mathbf{x_m}$ are the vectors of each neuron's responses to the familiar environments

$\mathbf{X}^*$ is the mean-corrected data matrix

Once mean corrected, the covariance of the data ($\mathbf{C_X}$) is then calculated

$$\mathbf{C_X} = \left(\frac{1}{n-1}\right)\mathbf{X^*X^{*T}} \quad \text{(VI-3)}$$

where:

n is the number of total number of locations measured

$\mathbf{X^{*T}}$ is the transpose of $\mathbf{X}^*$

The principal components of the data matrix **X** are determined by performing eigenvalue decomposition on the $\mathbf{C_X}$:

$$\mathbf{X^*X^{*T}} = \mathbf{V_X \lambda V_X^T} \quad \text{(VI-4)}$$

Where:

$\mathbf{V_X}$ is the matrix of eigenvectors of the network's response.

$\boldsymbol{\lambda}$ is the vector of eigenvalues of the decomposition, and represent the variance associated with each eigenvector

The response of the network during the encoding of a NE was then examined to determine whether the novel environment was indeed using the dimensions described by the FEs. The model was tested at different spatial locations in an environment that had not been used during training. The resulting output, **Y**, was then projected onto the basis determined by PCA of the trained environments. This projection, $\mathbf{Y_X}$, is given by

$$\mathbf{Y_X} = \left(\mathbf{Y} \times \mathbf{V_X}\right) \times \mathbf{V_X^T} \quad \text{(VI-5)}$$

where:

Y represents the responses of the network to the NE environment

$\mathbf{V_X}$ represents the basis of the responses to the FEs environments

$\mathbf{V_X}^T$ is the transposed matrix of the earlier basis $\mathbf{V_X}$.

The additional dimensionality present in the response to the novel environment (**Y**) is found by performing PCA on the difference between the original data and the **X**-compressed data ($\mathbf{Y}$-$\mathbf{Y_X}$). Since the remainder matrix ($\mathbf{Y}$-$\mathbf{Y_X}$) is by definition the data that is unaccounted for in the compressed data ($\mathbf{Y_X}$), the basis vectors found by PCA($\mathbf{Y_X}$) and PCA($\mathbf{Y}$-$\mathbf{Y_X}$) are orthogonal and therefore their contributions to explaining the variance can be summed. The contribution of each basis set to the overall data response, ρ_Y, was found by the following:

$$\rho_Y = \frac{\sum \boldsymbol{\lambda}_{\mathbf{Y}-\mathbf{Y_X}}}{\sum \boldsymbol{\lambda}_{\mathbf{Y}-\mathbf{Y_X}} + \sum \boldsymbol{\lambda}_{\mathbf{Y_X}}} \quad \text{(VI-6)}$$

where:

$\boldsymbol{\lambda_{Yx}}$ is the vector of the eigenvalues from the projection of $\mathbf{Y}$ onto $\mathbf{V_X}$

$\boldsymbol{\lambda_{Y\text{-}Yx}}$ is the vector of the eigenvalues of the response unexplained by the familiar environments.

Because the eigenvectors associated with these eigenvalues ($\mathbf{V_{Y\text{-}Yx}}$ and $\mathbf{V_X}$) are orthogonal, the associated eigenvalues ($\boldsymbol{\lambda_{Y\text{-}Yx}}$ and $\boldsymbol{\lambda_{Yx}}$) are independent and thus the relative contribution of each basis set can be determined.

Results

The development of neurons specific to the NE during training in that environment suggests that the network may also be creating new components specifically to encode the NE. The degree to which the network's response to the NE utilized unique dimensions was determined (as opposed to those dimensions used to encode the FEs) by projecting the response to the NE onto the set of components defined by the familiar responses (Figure VI-11-13). The dimensional independence of the network's response to the NE at day 160 (prior to training in the NE) was not found to be significantly different from zero (Figure VI-14). This result indicates that the entire response to the NEs already existed in the response of the network to previous environments, suggesting that aspects of new environments were essentially encoded as some combination of aspects of previous environments. FEs, on the other hand, retained some dimensions unique from all of the other contexts while sharing other dimensions.

The dependence of the NE on FEs changed when the network was subsequently grown in that context; the longer the network experienced that environment, the higher that environment's dimensional independence became (Figure VI-14). This effect was greatly attenuated if neurogenesis was stopped prior to training in this NE, with the dimensional independence of No

NG networks staying around zero. By day 200, the reconstruction of the NE from just the response to the four FEs is no longer accurate, with the residual response indicating that the reconstruction using the FE PCs fail to utilize neurons that matured to the NE (marked by an '*') while generally overestimating the involvement of neurons dedicated to other environments (Figure VI-15).

In addition to comparing the networks' responses to one environment to the responses to other environments, this analysis can be used to compare the response to earlier encoding. In this analysis, the past response is used to define the basis dimensionality, and the current response is compared to that (Figure VI-16). Growing in the NE increased the dimensionality of the FEs with respect to their previous encoding, but to a lesser extent than the increased dimensionality used to encode the NE (Figure VI-17). This suggests that the dimensional structure of the encoding of FEs continues to change after the network stops experiencing the environment because of neurogenesis. When the No NG networks experience a novel event, their encoding of the NE only creates minimal unique dimensionality, and the FE encoding is essentially restrained to the previous dimensionality (Figure VI-18).

These results suggest that the development of dedicated populations of neurons by neurogenesis results in the development of unique dimensions that are specifically created to encode new contexts. It remains to be seen if the generation of new dimensions for encoding is critical in the transition from novel to familiar.

Discussion

The development of these dedicated populations suggests that the continual growth of the DG is not simply the random addition of new dimensions, but rather a process by which young GC form dimensions specialized to environmental features experienced during maturation (Figure VI-18). Starting with the large population of GC maturing at birth, the DG appears to be growing

into a structure designed to process information in the context of what the network has experienced in the past. In such a network, new events will be encoded using the dimensions defined by previous events. Importantly, because there may be aspects of new events that are fundamentally novel (thus not being accounted for by existing GC), neurogenesis allows the DG to adapt by adding new dimensions.

Dimensional analysis revealed that the neurons integrating into the DG are forming dimensions that are independent from those dimensions that encode previous memories. This did not necessarily need to be the case – new neurons could integrate into the system into dimensions that are redundant to those provided by existing GC. If a neuron simply represented a more efficient combination of several mature neurons, it would not appear as increased dimensionality, but rather a combination of existing dimensions.

Chapter VI, in part, includes material that was published in the article "Computational Influence of Adult Neurogenesis on Memory Encoding," Aimone, James B; Wiles, Janet; and Gage, Fred H.; *Neuron*, January 2009. The dissertation author was the primary investigator and author of this paper.

Figures

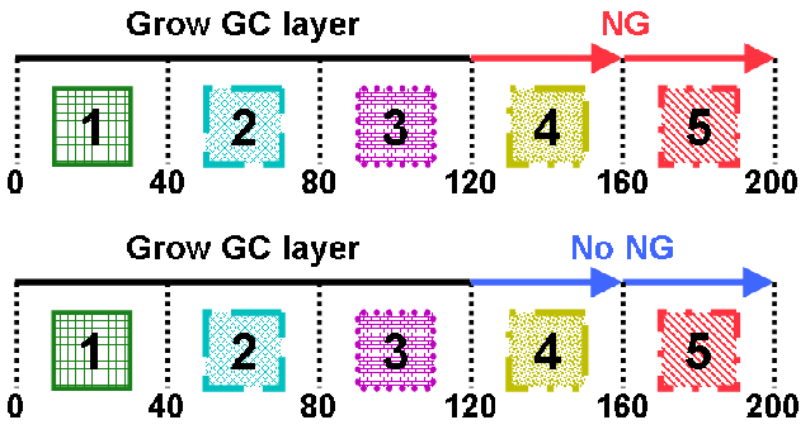

Figure VI-1: Schematic for Long-term Specialization Study
Networks were grown in multiple different environments, changing every 40 days. The response of each network to the familiar environments (Envs 1-4; FEs) and the novel environment (Env 5; NE) was tested on Days 160 and 200.

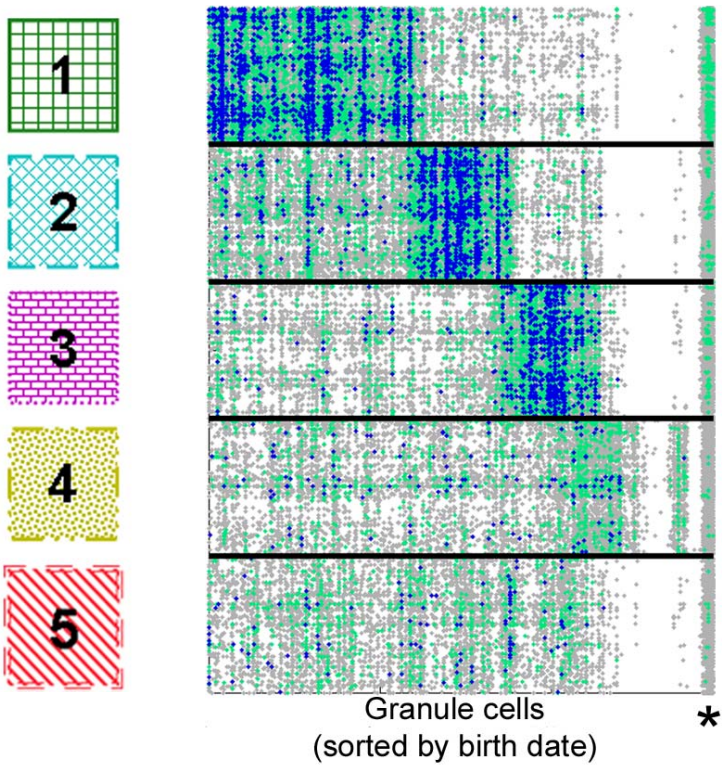

Figure VI-2: Response of an NG network to familiar environments on day 160.
Response shown for all GC neurons to 400 spatial locations in four familiar environments (FEs, Envs. 1-4) and on novel environment (NE, Env. 5). Gray:>2Hz; green:>4Hz; blue:>6Hz; firing at 2Hz and below not shown. Neurons are sorted on x-axis by age (oldest on left, youngest on right).

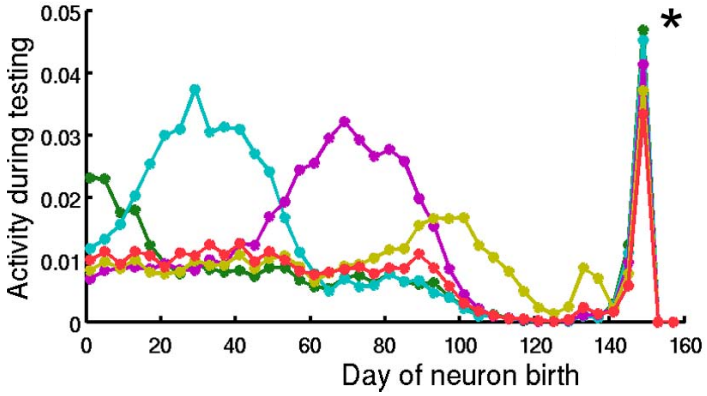

Figure VI-3: Response of NG network neurons by age on day 160.
Average firing rate of GCs born at different times in response to the four FEs and the NE in the NG network on day 160. The asterisk indicates the group of highly active neurons that responded indiscriminately to all environments.

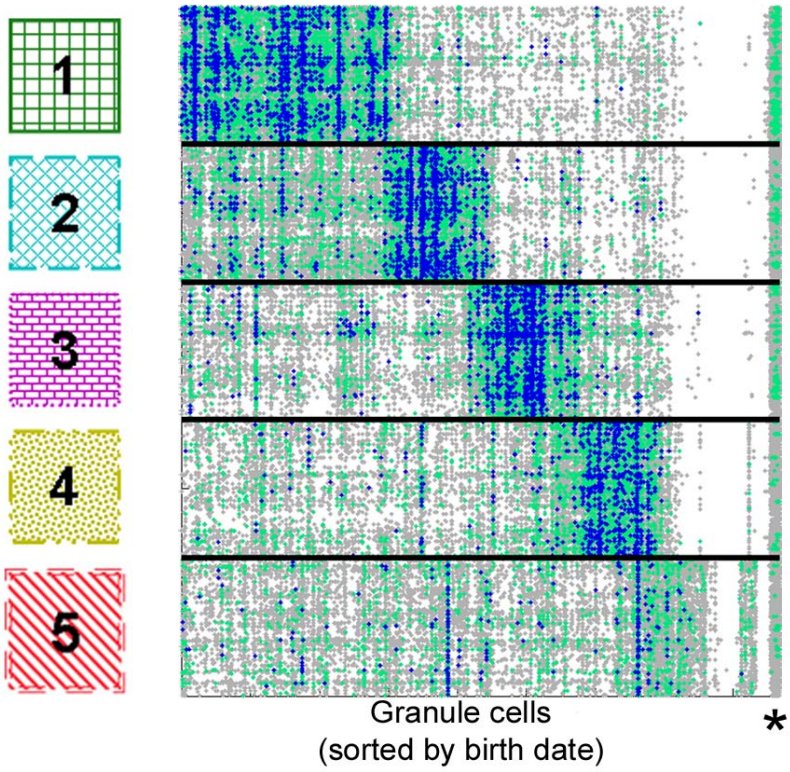

Figure VI-4: Response of an NG network to familiar environments on day 200.
Response shown for all GC neurons to 400 spatial locations in four familiar environments (FEs, Envs. 1-4) and on novel environment (NE, Env. 5). Gray:>2Hz; green:>4Hz; blue:>6Hz; firing at 2Hz and below not shown. Neurons are sorted on x-axis by age (oldest on left, youngest on right).

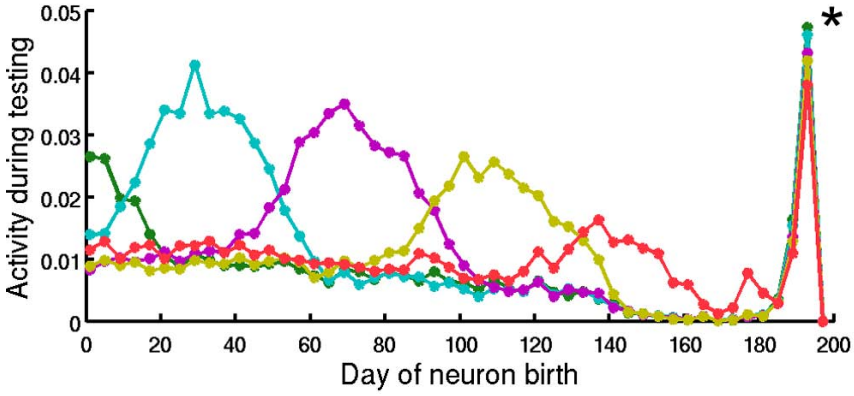

Figure VI-3: Response of NG network neurons by age on day 200.
Average firing rate of GCs born at different times in response to the four FEs and the NE in the NG network on day 200. The asterisk indicates the group of highly active neurons that responded indiscriminately to all environments.

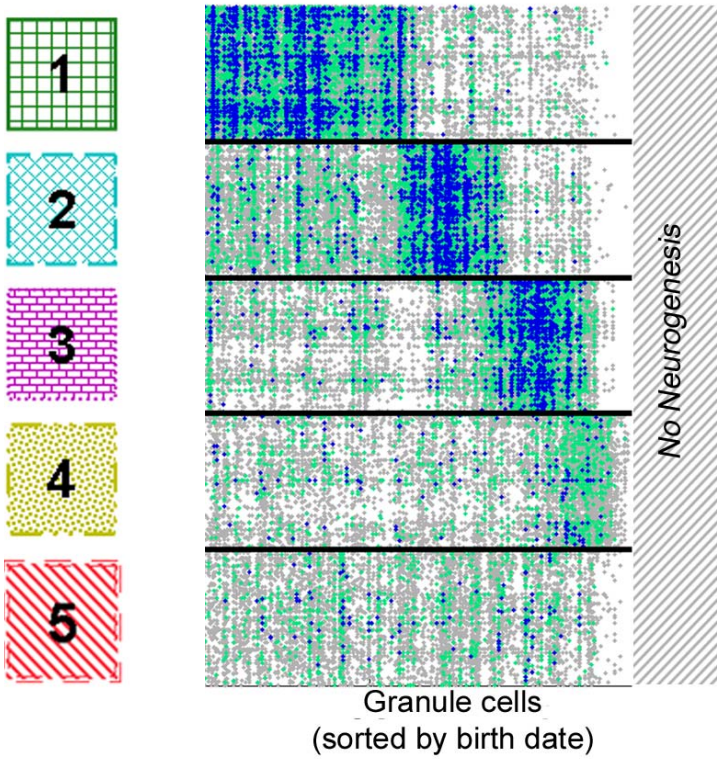

Figure VI-6: Response of a No NG network to familiar environments on day 160.
Response shown for all GC neurons to 400 spatial locations in four familiar environments (FEs, Envs. 1-4) and on novel environment (NE, Env. 5). Gray:>2Hz; green:>4Hz; blue:>6Hz; firing at 2Hz and below not shown. Neurons are sorted on x-axis by age (oldest on left, youngest on right).

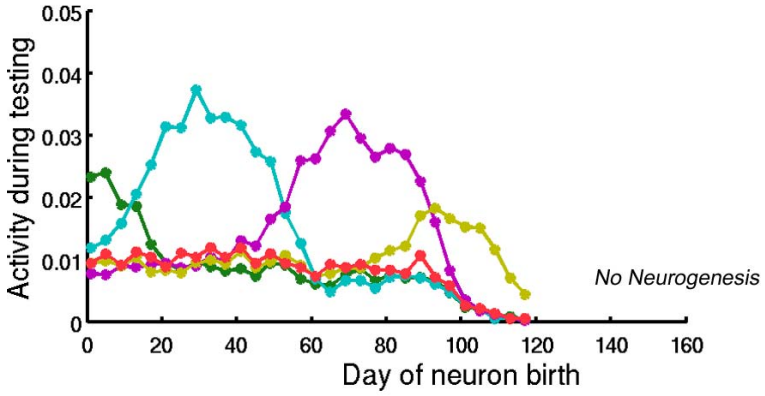

Figure VI-7: Response of No NG network neurons by age on day 160.
Average firing rate of GCs born at different times in response to the four FEs and the NE in the No NG network on day 160.

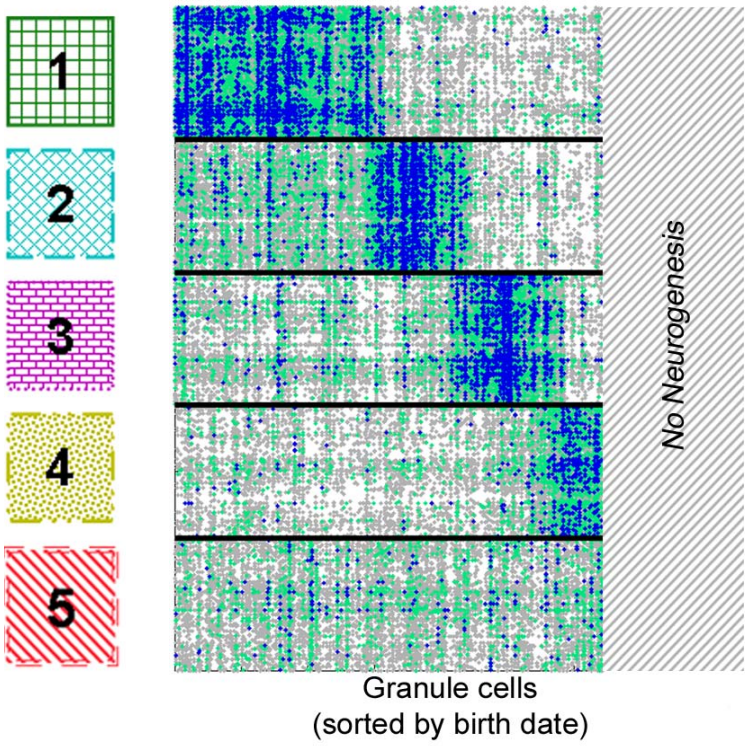

Figure VI-8: Response of a No NG network to familiar environments on day 200.
Response shown for all GC neurons to 400 spatial locations in four familiar environments (FEs, Envs. 1-4) and on once novel, now familiar environment (NE, Env. 5). Gray:>2Hz; green:>4Hz; blue:>6Hz; firing at 2Hz and below not shown. Neurons are sorted on x-axis by age (oldest on left, youngest on right).

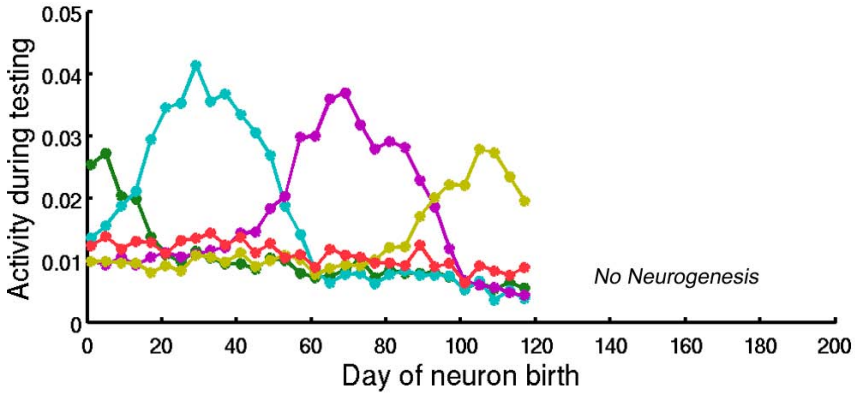

Figure VI-9: Response of No NG network neurons by age on day 200.
Average firing rate of GCs born at different times in response to the four FEs and the NE in the No NG network on day 200.

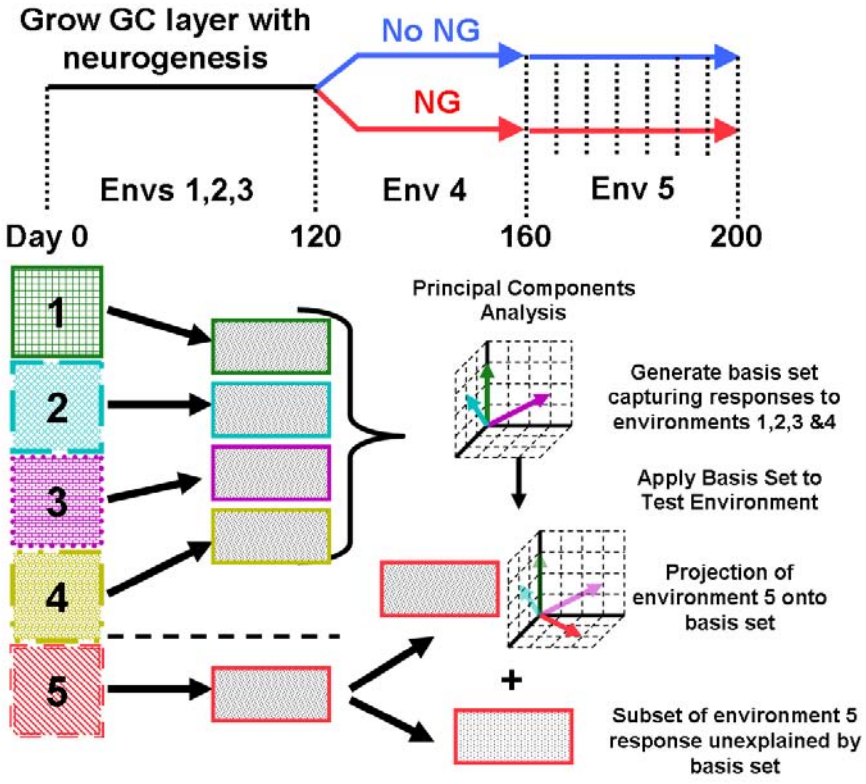

Figure VI-10: Schematic of dimensional analysis experiment
After network growth, responses to the four FEs were combined analyzed with principal components analysis (PCA). PCA provided a set of orthogonal dimensions ("basis set") that represent combinations of network neurons used in the encoding of information about the environments. The response to the NE, which was not included in the original PCA analysis, was projected into the FE basis set. This projection may not be complete, as a "residual" component of the NE response that is not encoded by any of the basis dimensions may remain. The size of this residual response was compared over time as the network grew in the NE.

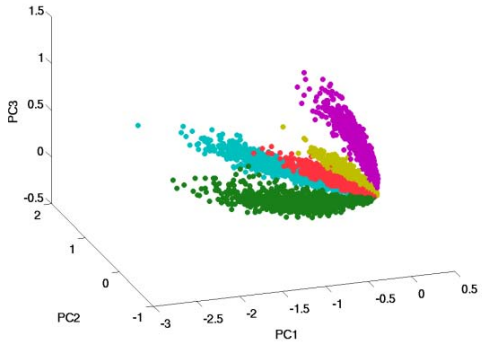

Figure VI-11: Sample of projection of responses onto PCA bases

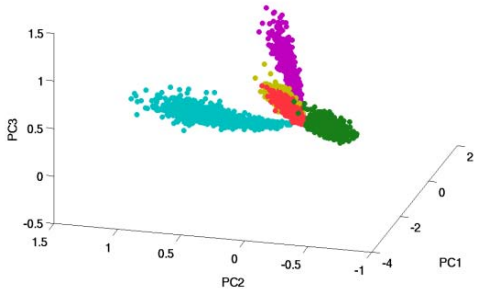

Figure VI-12: Sample of projection of responses onto PCA bases
Rotation of image shown in Figure VI-11

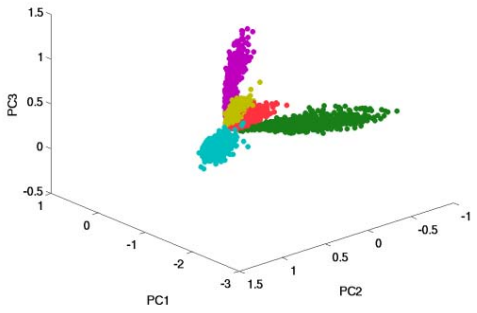

Figure VI-13: Sample of projection of responses onto PCA bases
Rotation of image shown in Figure VI-12

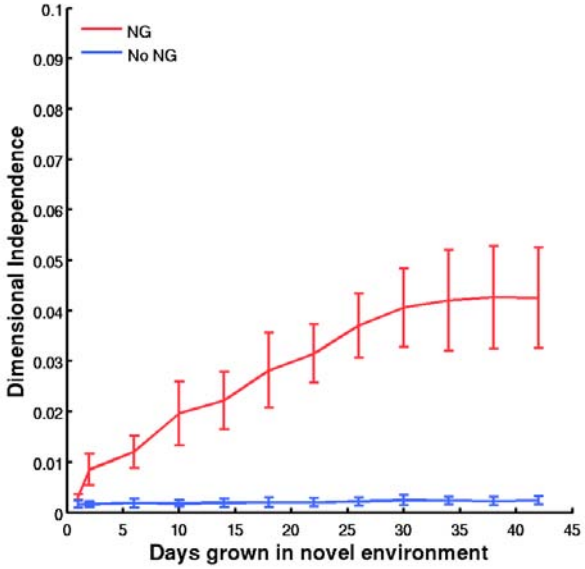

Figure VI-14: Extent of dimensional independence of NE from basis set determined by FE response
The network's response to the NE was compressed through the basis set calculated from the FE responses from the same day. The presence of neurogenesis enabled the networks to use dimensions for the encoding the NE that were unique from those dimensions used to encode the previously experienced FEs.

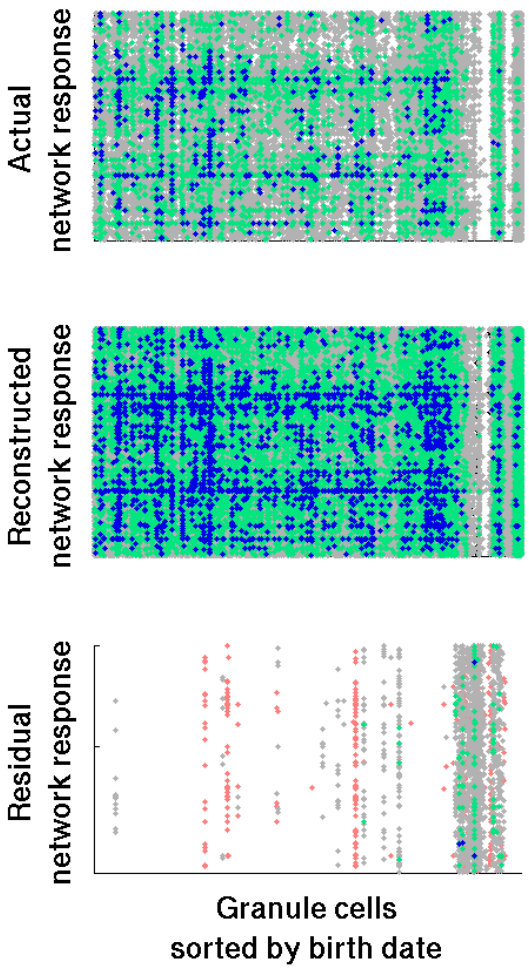

Figure VI-15: Reconstruction and residual of NE response using FE basis dimensions
The response of the network to the NE (top panel) was projected into the basis set determined by the response to the FE environments. The reconstruction of the NE response (middle panel) captured much of the structure of the original NE response, but appeared to over-estimate the response of many neurons in the system. The residual response (bottom panel) was determined by subtracting the reconstruction from the actual network response. The residual reveals that most of the error was in assigning function to the younger neurons in the network (far right).

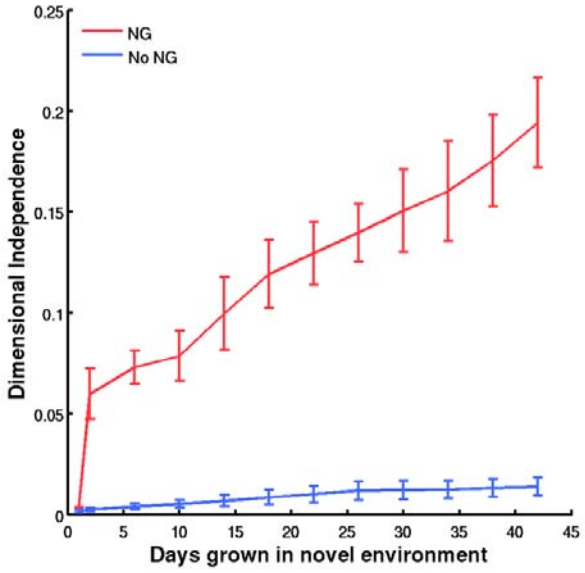

Figure VI-16: Extent of dimensional independence of NE from FE basis set determined on day 160

The network's response to the NE was compressed through the basis set calculated from the FE responses on day 160. The day 160 basis did not include neurons born after that time, causing the dimensional independence to grow considerably as new neurons are added to the system.

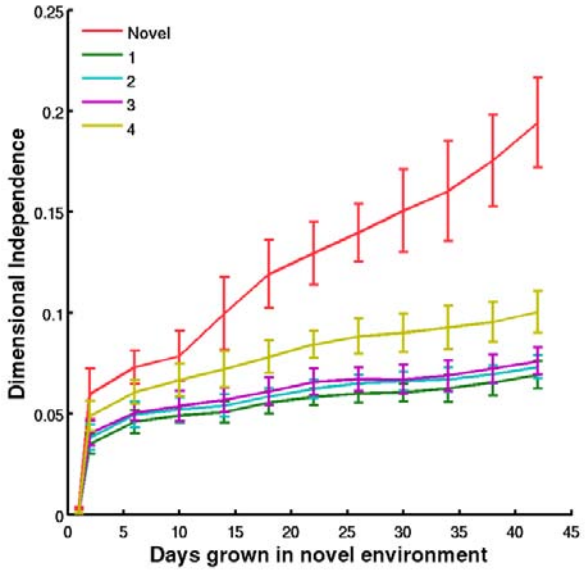

Figure VI-16: Extent of dimensional independence of NE and FEs from FE basis set determined on day 160
The network's response to all the environments, including the FEs, continued to become more dimensionally independent from the basis set determined on day 160, even for the FEs which were not experienced by the network after the basis set was calculated.

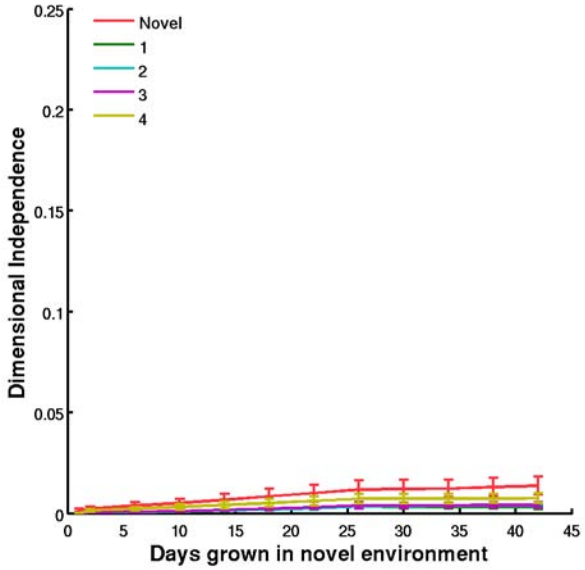

Figure VI-16: Extent of dimensional independence of NE and FEs from FE basis set determined on day 160 without neurogenesis
The network's response to all the environments only gained a limited amount of dimensional independence from the basis set determined on day 160 without the presence of new neurons.

References

1. Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., and Gage, F.H., *Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice.* Development, 2003. **130**(2): p. 391-9.

2. Kempermann, G., Kuhn, H.G., and Gage, F.H., *More hippocampal neurons in adult mice living in an enriched environment.* Nature, 1997. **386**(6624): p. 493-5.

3. Tashiro, A., Makino, H., and Gage, F.H., *Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage.* J Neurosci, 2007. **27**(12): p. 3252-9.

4. Tashiro, A., Sandler, V.M., Toni, N., Zhao, C., and Gage, F.H., *NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus.* Nature, 2006. **442**(7105): p. 929-33.

5. Ge, S., Yang, C.H., Hsu, K.S., Ming, G.L., and Song, H., *A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain.* Neuron, 2007. **54**(4): p. 559-66.

6. Schmidt-Hieber, C., Jonas, P., and Bischofberger, J., *Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus.* Nature, 2004. **429**(6988): p. 184-7.

7. Kee, N., Teixeira, C.M., Wang, A.H., and Frankland, P.W., *Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus.* Nature Neuroscience, 2007. **10**(3): p. 355-362.

8. Bramham, C.R., Worley, P.F., Moore, M.J., and Guzowski, J.F., *The immediate early gene arc/arg3.1: regulation, mechanisms, and function.* J Neurosci, 2008. **28**(46): p. 11760-7.

9. Niewoehner, B., Single, F.N., Hvalby, O., Jensen, V., Meyer zum Alten Borgloh, S., Seeburg, P.H., Rawlins, J.N., Sprengel, R., and Bannerman, D.M., *Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus.* Eur J Neurosci, 2007. **25**(3): p. 837-46.

10. McHugh, T.J., Jones, M.W., Quinn, J.J., Balthasar, N., Coppari, R., Elmquist, J.K., Lowell, B.B., Fanselow, M.S., Wilson, M.A., and Tonegawa, S., *Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network.* Science, 2007. **317**(5834): p. 94-9.

11. Jessberger, S. and Kempermann, G., *Adult-born hippocampal neurons mature into activity-dependent responsiveness.* Eur J Neurosci, 2003. **18**(10): p. 2707-12.

CHAPTER VII: STUDY 3: EFFECT OF NEUROGENESIS MODULATION ON DG FUNCTION

Introduction

One of the most distinguishing features of adult neurogenesis is its extensive regulation by several different intrinsic and extrinsic factors. The proliferation and survival of new neurons are modulated by many different factors. This regulation can either be positive or negative, leading to either more or fewer new neurons in the system. Many of the factors that have been shown to induce changes in neurogenesis rate or survival of new neurons have also been shown to have effects on cognition. Such strong correlations between neurogenesis and cognition often suggest a causal role, but direct evidence linking the regulation of new neurons and learning has been lacking.

Two of the most studied regulators of neurogenesis function are aging and stress. Both of these processes decrease the number of granule cell precursor cells that are born in the DG, thereby lowering the rate of the incorporation of new neurons into the network. The relationship between an animal's age and its neurogenesis levels has been investigated since the work by Altman and Bayer [1], who showed that the levels of postnatal neurogenesis in the DG continued to decrease, but did disappear entirely, in the weeks after an animal's birth. Kuhn and colleagues systematically studied the effect of aging on neurogenesis specifically in older rats[2], showing that the proliferation rates were considerably lower in aged animals. Comparable observations have been made in mice as well [3]. These studies on aging have reliably shown that neurogenesis decreases considerably by the time mice and rats are "middle aged" (roughly 6 months old), and after a year old (an age where rodents are typically considered aged) neurogenesis almost disappears in the absence of other factors to offset the decrease. This decrease in neurogenesis is correlated with deficits in learning tasks - behavioral studies have

demonstrated that age-related decreases in cognitive abilities are partially correlated with the magnitude of the age-related decrease of neurogenesis in those animals [4].

Many factors likely affect this age-dependent reduction of new neurons[5]. Decreased proliferation is partially alleviated by removal of the adrenal glands, suggesting that increased stress with aging plays a role [6]. Changes in the local neurogenic niche and stem cell potency also have been implicated [7]. The effects of aging can be mitigated by other factors, including enrichment and running [3]. While aging is an irreversible process that all animals experience, stress is an experience dependent process that can come and go.

Along with aging, stress was one of the earliest factors shown to dramatically affect neurogenesis levels. During stressful experiences, regions such as the pre-frontal cortex and amygdala will activate the hypothalamic-pituitary-adrenal (HPA) system (for review of the stress response, see [8]). This activation of the HPA axis will lead to the release of glucocorticoids from the adrenal glands into the bloodstream. Glucocorticoids have effects on many systems throughout the body, including significant effects on many neural systems. The hippocampus is both tightly coupled to the HPA axis and greatly affected by the stress response, and this relationship is affected during chronic stress states, including depression [8, 9].

The effects of stress on neurogenesis are profound, with strong decreases observed after many different stress paradigms in many different species (reviewed in [10]). Both acute and chronic stress decrease the birth and initial survival of adult-born neurons by marked levels [10-12]. Importantly, once the stressors are removed, the animal's neurogenesis rates can be recovered, either naturally [11] or with the use of antidepressants [13]. This regulation of new neurons by stress is believed to be a key component of the relationship between depression, neurogenesis, and antidepressant treatment.

In addition to their effects on neurogenesis, aging and stress have both been associated with various cognitive impairments, including memory deficits. While both of these are complex

conditions likely affecting many neural systems, the model provides an opportunity to determine how their effect on neurogenesis would affect the function of the DG. In this study, simple decreases in neurogenesis rates were used to investigate what the general effects of changing neurogenesis rates could be on memory formation.

Experimental Procedures

Aging and stress were both simulated by decreasing the rate of new neurons "born" into the network. In the aging experiment, after growing with a constant neurogenesis rate to day 120, the neurogenesis rate was decreased by 5% every 10 days:

$$NG(d) = NG(d-10) \times 0.95 \quad \text{(VII.8)}$$

where d is the current day of the simulation.

The control network maintained a constant neurogenesis rate throughout the experiment. As with previous studies, environments were changed every 40 days until the networks were 520 days old, for a total of 13 trained environments. During growth, pattern separation was tested every 40 days, temporal separation was measured at the onset of aging (day 120) and at the end of growth (day 520), and network specialization was measured at day 520.

Stress was simulated by an acute 75% drop in the rate of neurogenesis on day 120 (2.5 neurons/day). This low rate was maintained in the network for 60 days (until day 180), where the rate was returned to baseline (10 neurons/day). The rate of neurogenesis (NG) was measured in new neurons/day according to the following equations:

$$if \left\{ \begin{array}{ll} 0 < d < 120 & NG = 10 \\ 120 < d < 180 & NG = 2.5 \\ 180 < d < 280 & NG = 10 \end{array} \right\} \quad \text{(VII.9)}$$

where d is the day of the simulation.

As with the aging study, environments changed every 40 days, and the network continued growing until day 280. Pattern separation was measured every 5 days, and temporal separation was measured on day 120 (stress onset), day 180 (stress end), and day 280 (experiment end). Network specialization was measured at day 280. All results were compared to a network growing up to day 280 at a constant rate of neurogenesis.

Pattern separation was measured in these networks in the same manner as is described in Chapter V. Briefly, all plasticity in the network was turned off (no synaptic learning, neurogenesis, maturation, or cell death). The response of the networks was measured at multiple locations tiling a series of test environments. These environments were designed to span a range of lEC and mEC similarities. The normalized dot product (NDP) similarity was calculated between EC and GC responses from different locations/environments. This permitted a comparison of input (EC) and output (GC) similarities. For each network, the mean of all responses with comparable EC similarities represented the separation by the DG for that level of input similarity. In addition, unlike the experiments described above, the environment used for testing pattern separation was not entirely random, but chosen from a set of random environments to ensure that the environment used for each network was minimally similar to the previously experienced familiar environments in order to reduce bias due to the effects of specific network learning. Ten potential test environments were selected, and the one with the least overlap (measured by NDP – eq. VII.7) with the training environments was chosen to use as the first test environment. The vector representing this environment was then shuffled to create the second environment for the pattern separation experiment.

Temporal separation was determined by a procedure similar to pattern separation, but the networks were grown in between measuring the responses to the test environments (a test-grow-re-test procedure, see Chapter V). The similarity of the networks' responses to different events

occurring at different times was then measured. The results were then computed by comparing the separation ability of the networks for different intervals of time between events.

Neuron specialization was observed according to the method described in Chapter VI. After growth in multiple environments, the responses of the network were measured to multiple locations tiling the previously experienced familiar environments (FEs). Total activity of each neuron in these environments after a 500ms exposure was then calculated. Finally, the cumulative responses for each neuron were plotted at each location for each environment.

Effect of aging on neurogenesis function

The effect of aging on neurogenesis function was measured by testing the three network behaviors described in Chapters V and VI. At the time of testing, the aged networks were considerably larger than the networks tested in previous studies, even in the networks with decreasing neurogenesis rates (Figure VII-1).

The pattern separation function of the model DG was the first behavior examined in the aged networks. Since neurogenesis rates were considerably lower by the end of aged growth, the expectation was that the aged networks would lack the pattern integration component seen in younger networks with neurogenesis. When measured at different times during the long-term growth of these networks, the networks with constant neurogenesis integrate highly dissimilar inputs at greater levels than networks with a gradually decreasing neurogenesis rate (Figure VII-3). Importantly, however, even the "aged" networks are pattern integrating rather than pattern separating, in spite of their almost nonexistent neurogenesis rates at this point.

This maintenance of pattern integration despite losing the population of immature neurons after aging is evident from looking at the full pattern separation curves of older networks. If neurogenesis rates are not decreased, pattern separation decreases as the network grows (Figure VII-4). Although not specifically investigated, this reduction of pattern separation is likely due to

the size of the GC layer in the network, whose larger size likely has implications in activating the feedback layers. The gradual decrease of neurogenesis used in the aging simulation actually maintains the younger pattern separation ability of the network (Figure VII-5), suggesting that one possible effect of the aging-induced decrease in neurogenesis may be the maintenance of pattern separation ability.

Even though pattern integration was not eliminated in the aged networks, investigation of the temporal separation ability of the network revealed that without neurogenesis, the amount of time elapsed between memories did affect the pattern integration (Figure VII-6). Rather, it appears that the pattern integration occurring in aged networks is not temporally dependent, but simply a function of the global network state. Networks without a decrease in neurogenesis rate maintained their ability to temporally separate information (Figure VII-7), consistent with their retention of a dynamic neurogenesis process.

Finally, the response of the network to each of the environments experienced during its maturation was measured. Without "aging," the growth in the multiple successive environments did not show a marked change in the network's response to familiar environments (FEs) (Figure VII-8). In contrast, the continuous decline in neurogenesis rates in the aged networks results in a dramatic reduction in the size of the specialized neuron population for FEs experienced later during growth (Figure VII-9).

Effect of stress on neurogenesis function

In contrast to the slow decline in neurogenesis rates used in the aging study, stress was modeled by an acute decrease in neurogenesis rate (75% reduction), a chronic state of low neurogenesis for 60 days, followed by an acute recovery. The behavior of this "stressed" network was compared to networks with constant neurogenesis over that time period.

Unlike the aging study, where the aging network initially had similar neurogenesis levels to the control network, the acute drop in neurogenesis rates in the stressed network led to a substantial decrease in pattern integration (Figure VII-12). This drop did not occur until about two weeks after the neurogenesis rate was affected, consistent with the role of 2-3 week old neurons in the pattern integration function. Pattern integration did not return until about two weeks after recovery of neurogenesis levels, again suggesting that a time delay is necessary for the effects of strong changes in neurogenesis rate to be seen behaviorally. Measuring the full pattern separation curves (Figure VII-13) revealed that during the stress period the network had stronger pattern separation overall. In addition, the stress may have helped stave off some of the global lowering of pattern separation seen in the control network with continuous neurogenesis (Figure VII-14).

As with the aging study, the temporal separation ability of the network was mostly eliminated during the stressed period (Figure VII-15; VII-16). Events occurring proximal in time at day 180, which was at the end of the stress period, were not separated. Recovery of the pattern integration ability in a once-stressed network returns the temporal separation function as well.

As suggested by Chapter VI, growth in successive environments with constant neurogenesis continues to form populations of GCs that are specialized to the FEs. However, just as the aging-induced decrease in neurogenesis led to FEs not being encoded towards the end of the aging process, stress led to a sharp decline in the development of specialized GCs to FEs that were experienced during times of stress (Figure VII-17). In particular, FE-5, which would have been experienced between days 160 and 200, failed to develop many specialized neurons. This finding is consistent with the greatly reduced levels of proliferation between days 120 and 180. FE-6, which the system was exposed to beginning on day 200, adequately developed a specialized set, demonstrating that the recovery of neurogenesis after stress leads to the future

recovery of DG specialization. As with the aging control network, the control network for the stress condition showed full environmental specialization (Figure VII-18).

Discussion

The effects of aging and stress on the previously described neurogenesis functions in the model indicate that long-term perturbations of neurogenesis can have significant ramifications on memory formation in the model. The reduction of neurogenesis in both cases affects the pattern integration and temporal separation functions described in the previous chapters. Interestingly, the model's results suggest that without an aging-related drop in neurogenesis, DG pattern separation becomes less effective, suggesting a possible purpose for decreasing neurogenesis rates. However, while the lack of neurogenesis keeps pattern separation effective in the aged DG, the temporal relationships between events encoded by the DG are lost, suggesting that memories dependent on temporal information would be most at risk under these conditions.

Stress's effects on neurogenesis function are more pronounced. The acute drop in neurogenesis rate essentially eliminates the pattern integration ability of the model, albeit with a brief time-delay after the onset of stress. Recovery of neurogenesis is sufficient to restore the pattern integration ability of the network. As with aging, temporal separation is greatly affected by this drop in neurogenesis, with temporal relationships being eliminated for events occurring during the stress period. Like the pattern integration effect, recovery of neurogenesis is sufficient to restore the temporal coding effect.

The observation that the long-term specialization of the DG is significantly different when neurogenesis is either absent or weakened due to stress or aging emphasizes an important point about the neurogenesis process. One of the concerns sometimes raised regarding assigning a significant role for neurogenesis in hippocampal function is that the number of young neurons at any given time is only a fraction of the much larger DG and especially hippocampal neuron

populations. Over the course of months, the immature neurons that are lost due to chronic stress or aging begin to represent a substantial number, and the result is that extended periods of the model's past fail to be encoded into the DG network. Further work is required to ascertain what the role of such network specialization may be, but if the purpose of these dedicated populations of neurons is to optimize encoding of those environments at later times, the network will be at a significant disadvantage if neurogenesis is lacking.

Both aging and stress have been associated with memory impairments [4, 9, 14]. It is difficult to directly attribute these psychological observations to neurogenesis, especially since both conditions are known to affect many brain regions. Nevertheless, loss of neurogenesis does provide a possible mechanism for these cognitive conditions, and importantly may be a potential target for therapies to treat these symptoms. While the baseline drop in neurogenesis due to aging is irreversible, positive neurogenesis behaviors, such as running and enrichment, may be strategies for mitigating aging's effects on neurogenesis-dependent memory. Likewise, in addition to the elimination of stressors, antidepressants may play a key role in recovering much of the loss functionality due to stress's effects on neurogenesis.

Chapter VII, in part, includes material that was published in the article "Computational Influence of Adult Neurogenesis on Memory Encoding," Aimone, James B; Wiles, Janet; and Gage, Fred H.; *Neuron*, January 2009. The dissertation author was the primary investigator and author of this paper.

Figures

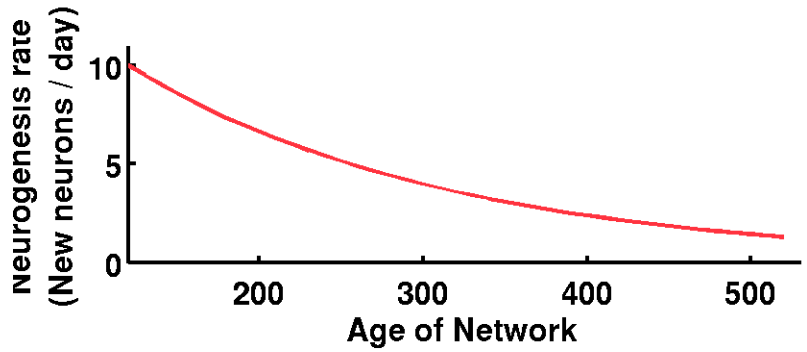

Figure VII-1: Schematic for Aging Study
In aging networks, neurogenesis was decreased gradually (10% drop in rate per week). As with other studies, environments were changed every 40 days. In control networks (not shown), the neurogenesis rate remained constant.

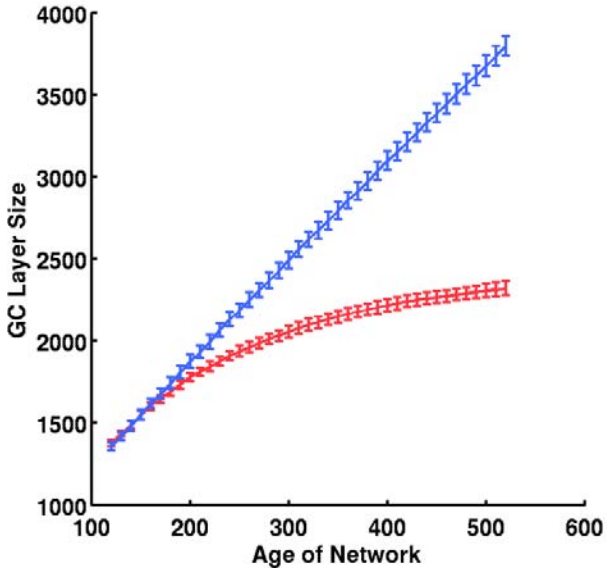

Figure VII-2: Plot of GC Size over Time with Aging
The drop in neurogenesis rate in aging networks limited the size that networks grow. Control networks, with constant neurogenesis, were substantially larger at later time points.

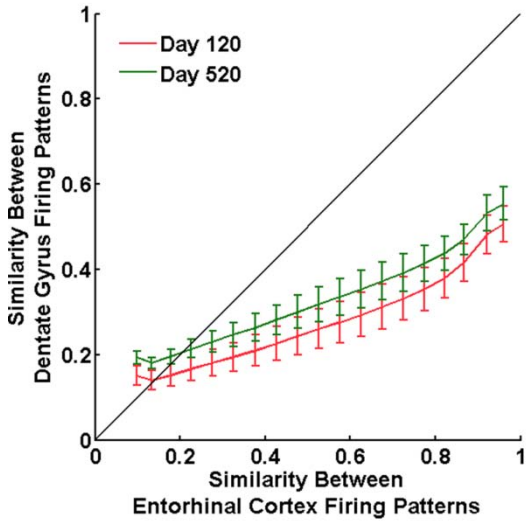

Figure VII-3: Pattern Separation in Aged Networks with Constant Neurogenesis
Pattern separation gradually lessens in networks with constant neurogenesis rates. The longer the network has grown with neurogenesis, the weaker the pattern separation ability of the DG.

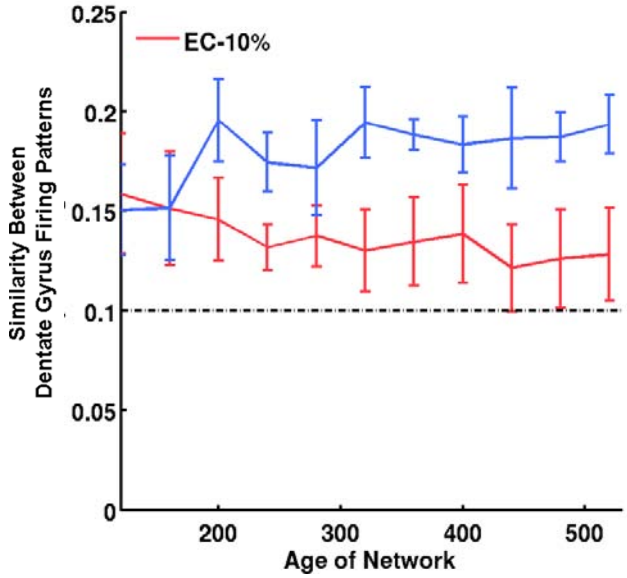

Figure VII-4: Pattern Integration Changes over Time with Aging
Temporal drift of pattern integration (pattern separation at 10% EC similarity) ability of networks with constant neurogenesis (blue) and gradually decreasing neurogenesis (red). Networks with constant neurogenesis rates showed significantly higher pattern integration than aged networks with decreasing neurogenesis rates.

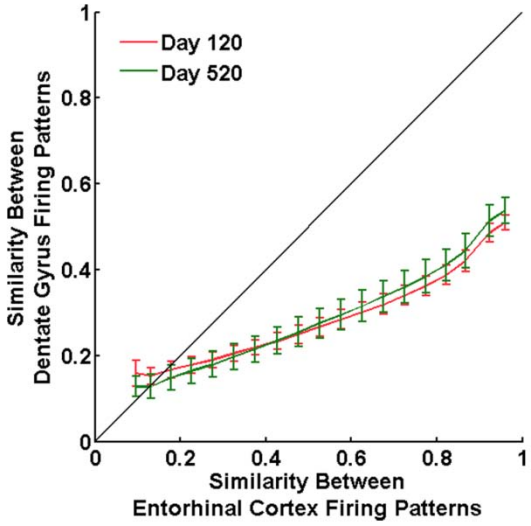

Figure VII-5: Pattern Separation in Aged Networks with Decreasing Neurogenesis
The gradual reduction of neurogenesis in aging networks mitigated the long-term drift in pattern separation in older networks (see Figure VII-3). Even though neurogenesis was very low at the end point (green, Day 520), the network still pattern integrated, unlike neurogenesis knockouts in younger networks (Figure V-2).

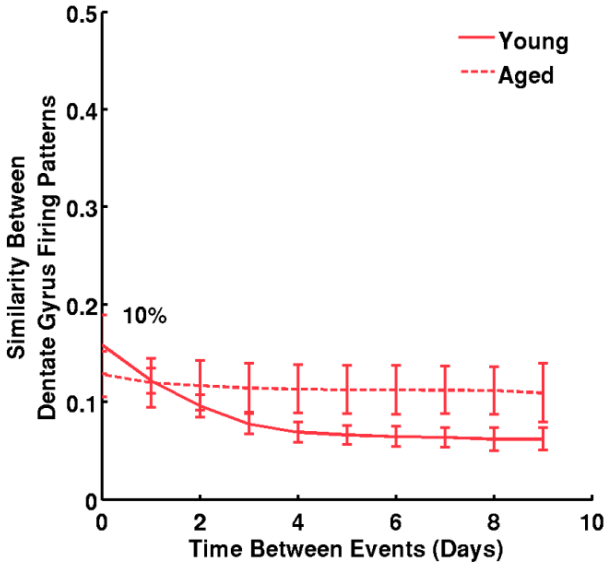

Figure VII-6: Temporal Separation in Aged Networks with Decreasing Neurogenesis
The temporal relationship between DG outputs was markedly reduced in aged networks with decreasing neurogenesis (dotted line) compared to younger networks (solid line).

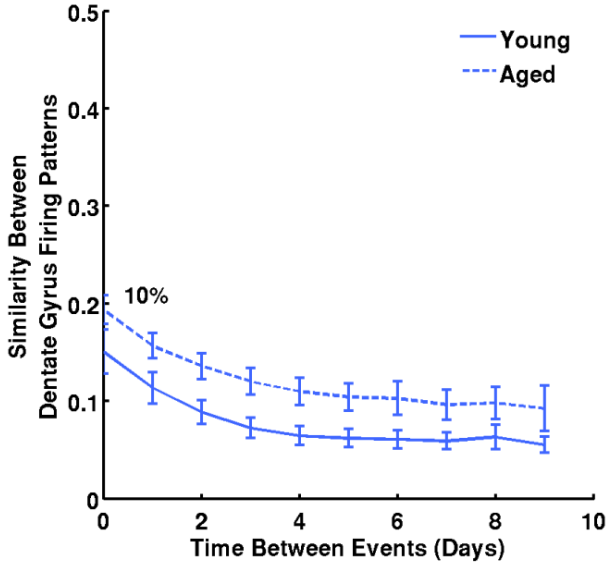

Figure VII-7: Temporal Separation in Aged Networks with Constant Neurogenesis
The temporal relationship between DG outputs was maintained in older networks with constant neurogenesis (dotted line) compared to younger networks (solid line). The worse pattern separation by older networks was apparent for all durations between events.

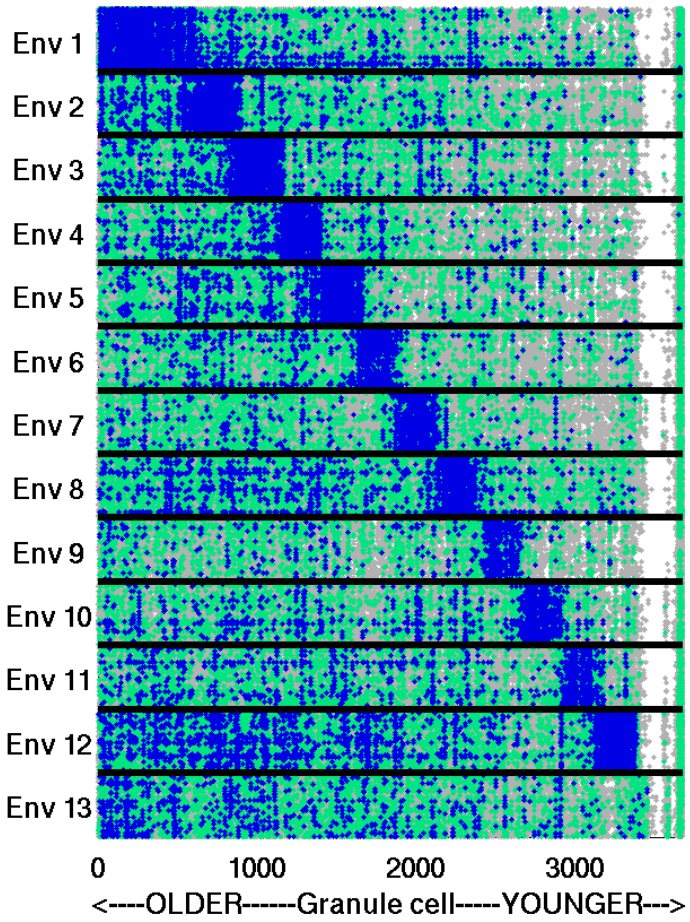

Figure VII-8: Environment Specialization in Aged Networks with Constant Neurogenesis
Older networks with continuous neurogenesis continued to form specialized populations of GC to environments experienced during maturation.

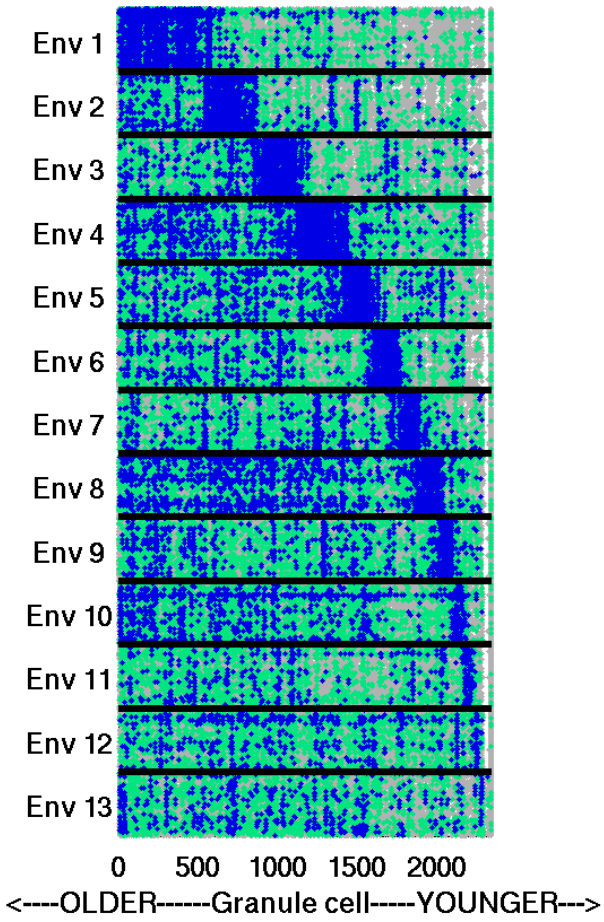

Figure VII-9: Environment Specialization in Aged Networks with Decreasing Neurogenesis Aged networks with decreasing neurogenesis failed to form strong specialized populations of GC to later environments experienced during maturation, but they did form smaller specialized groups to events earlier in network aging.

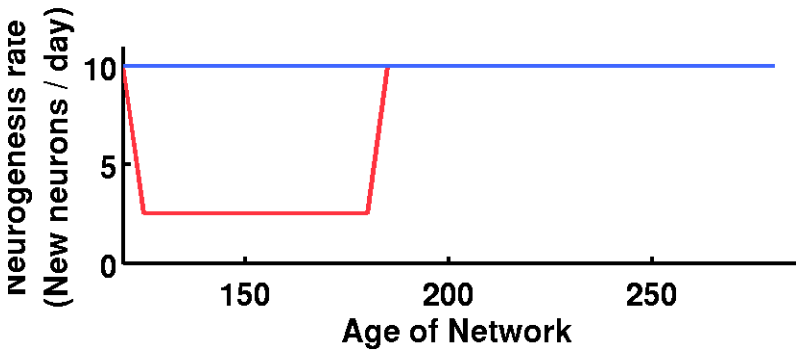

Figure VII-10: Plot of Neurogenesis Rates with Stress
Stress was modeled by an acute drop in neurogenesis rate (red), followed by an acute recovery 60 days later. The control networks (blue) maintained constant neurogenesis.

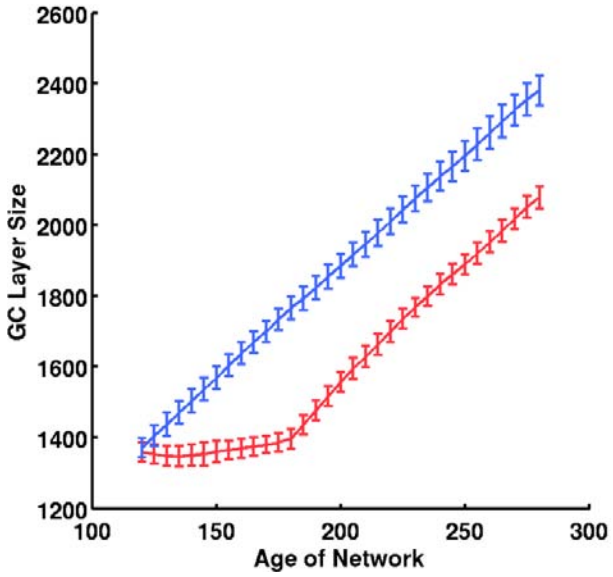

Figure VII-11: Size of GC Layer in Stress Study
Stressed networks showed only minimal growth during stress period, however after recovery the network size resumed growth.

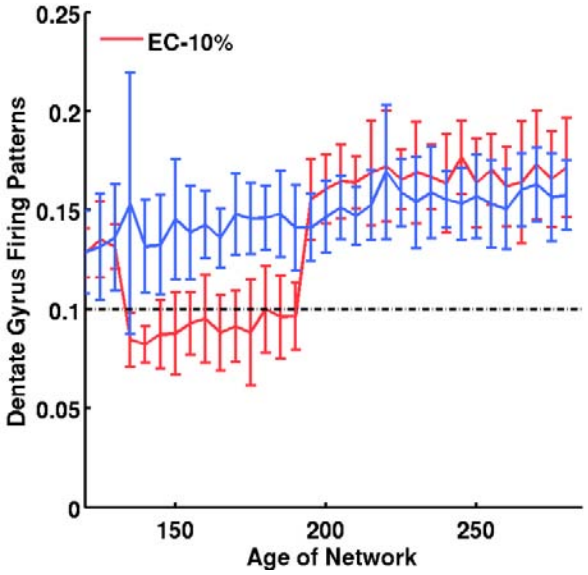

Figure VII-12: Effect of Stress on Pattern Integration over Time
Pattern integration (DG similarity at EC input similarity of 10%) was significantly lower for networks during the stress period with reduced neurogenesis. The drop in pattern integration was delayed by approximately 20 days from change in neurogenesis rate.

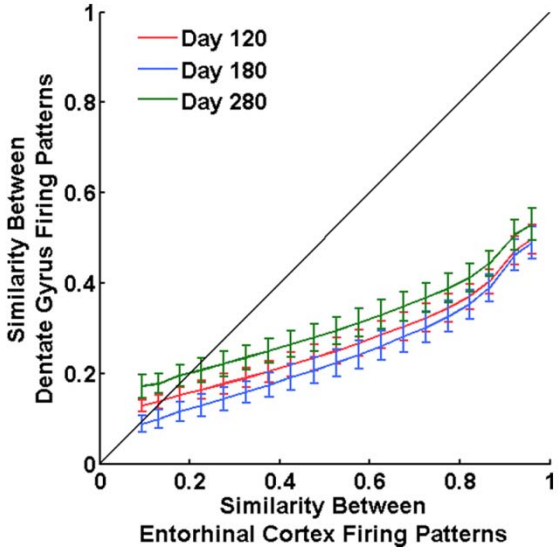

Figure VII-13: Pattern Separation in Stressed Network
The drop in neurogenesis rate caused by stress increased the pattern separation in the Stressed network. After recovery, the pattern separation decreased and showed the size-related effects observed in the aging study.

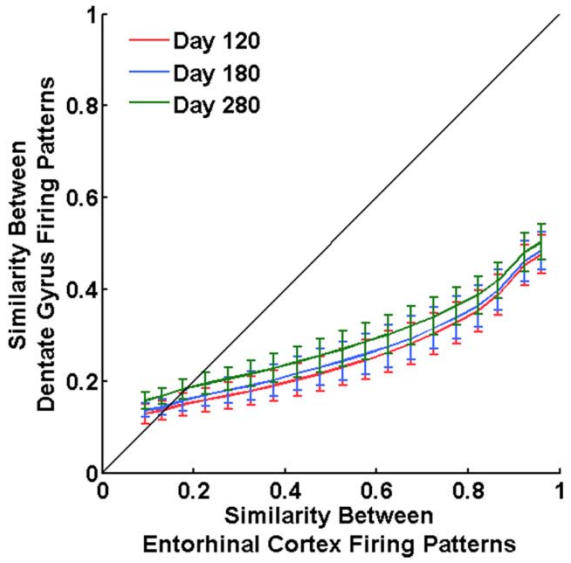

Figure VII-14: Pattern Separation in Non-stressed Network
Control networks growing through the same time as the stressed network showed only a gradual decrease in pattern separation due to network size increasing.

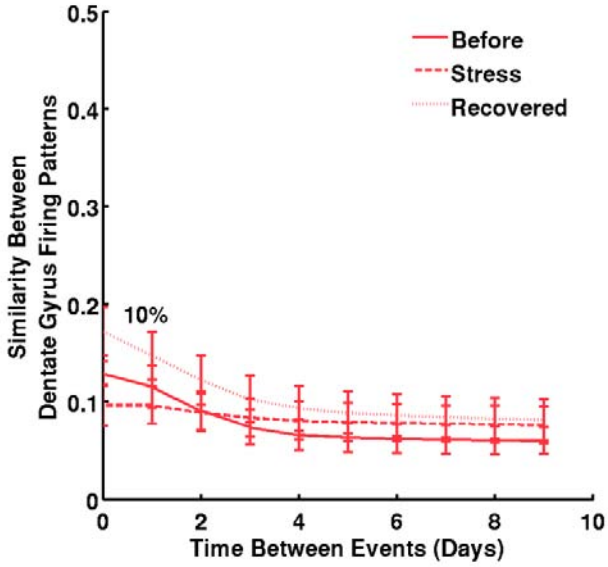

Figure VII-15: Temporal Separation in Stressed Network
Temporal separation was impaired during the stress period, with events separated in time showing strong pattern separation that was temporally independent.

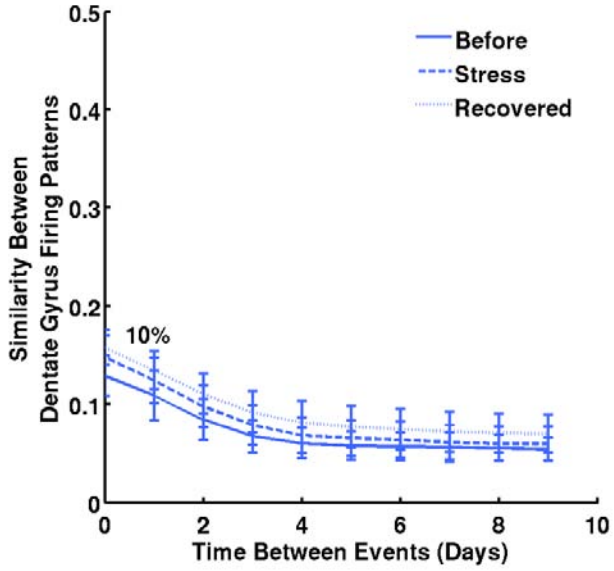

Figure VII-16: Temporal Separation in Non-stressed Network
Temporal separation was unchanged in control networks.

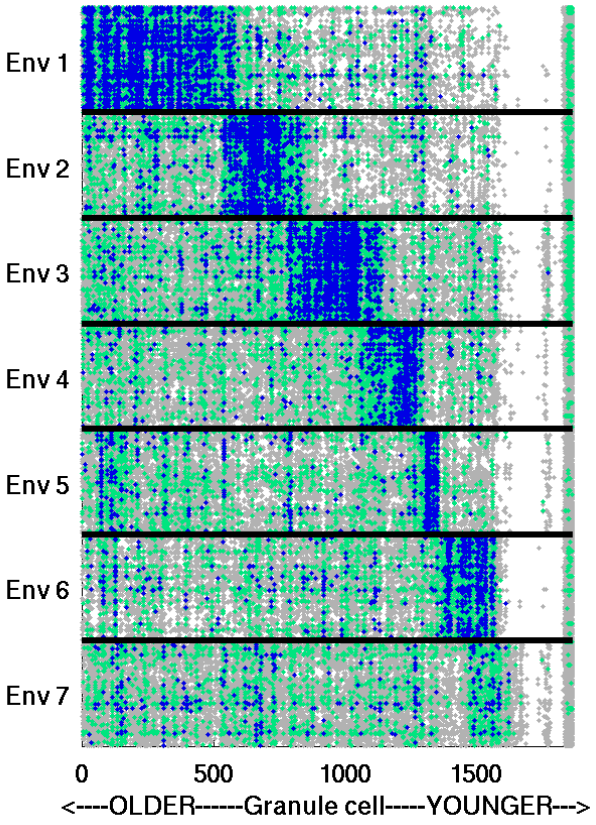

Figure VII-17: Environment Specialization in Stressed Network
The stress period for stressed networks, which occured during environment 4 and 5, results in few neurons specializing to the stressed environments, particularly environment 5.

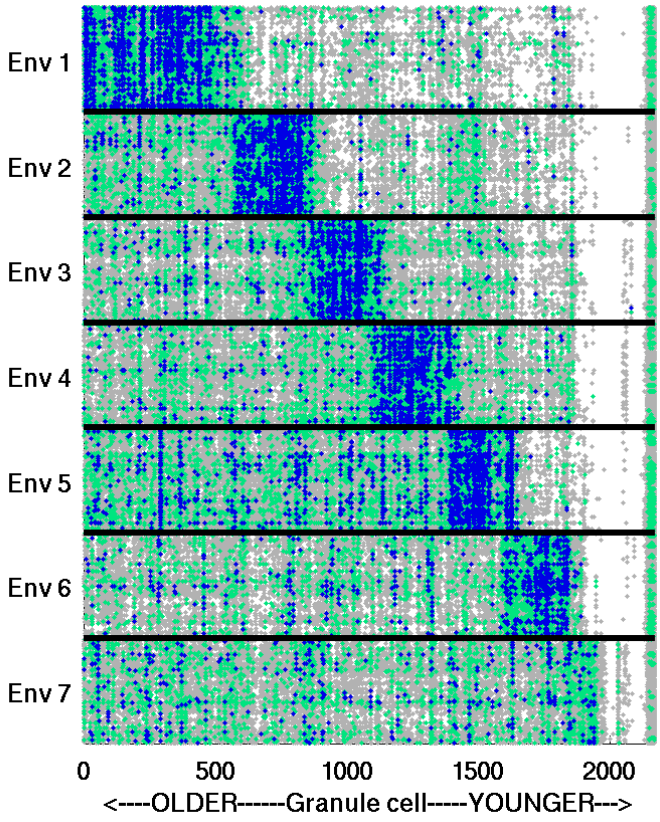

Figure VII-18: Environment Specialization in Non-stressed Network
The control, unstressed networks showed no differences in specialized environments.

References

1. Altman, J. and Bayer, S.A., *Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods.* J Comp Neurol, 1990. **301**(3): p. 365-81.

2. Kuhn, H.G., Dickinson-Anson, H., and Gage, F.H., *Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation.* J Neurosci, 1996. **16**(6): p. 2027-33.

3. Kempermann, G., Kuhn, H.G., and Gage, F.H., *Experience-induced neurogenesis in the senescent dentate gyrus.* J Neurosci, 1998. **18**(9): p. 3206-12.

4. Light, L.L., *Memory and aging: four hypotheses in search of data.* Annu Rev Psychol, 1991. **42**: p. 333-76.

5. Kempermann, G., *Activity Dependency and Aging in the Regulation of Adult Neurogenesis*, in *Adult Neurogenesis*, Gage, F.H., Kempermann, G., and Song, H., Editors. 2008, Cold Spring Harbor Laboratory Press: New York. p. 341-362.

6. Cameron, H.A. and McKay, R.D., *Restoring production of hippocampal neurons in old age.* Nat Neurosci, 1999. **2**(10): p. 894-7.

7. Hattiangady, B. and Shetty, A.K., *Aging does not alter the number or phenotype of putative stem/progenitor cells in the neurogenic region of the hippocampus.* Neurobiol Aging, 2008. **29**(1): p. 129-47.

8. de Kloet, E.R., Joels, M., and Holsboer, F., *Stress and the brain: from adaptation to disease.* Nat Rev Neurosci, 2005. **6**(6): p. 463-75.

9. McEwen, B.S., *Stress and hippocampal plasticity.* Annu Rev Neurosci, 1999. **22**: p. 105-22.

10. Mirescu, C. and Gould, E., *Stress and adult neurogenesis.* Hippocampus, 2006. **16**(3): p. 233-8.

11. Heine, V.M., Maslam, S., Zareno, J., Joels, M., and Lucassen, P.J., *Suppressed proliferation and apoptotic changes in the rat dentate gyrus after acute and chronic stress are reversible.* Eur J Neurosci, 2004. **19**(1): p. 131-44.

12. Gould, E., McEwen, B.S., Tanapat, P., Galea, L.A., and Fuchs, E., *Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation.* J Neurosci, 1997. **17**(7): p. 2492-8.

13. Malberg, J.E., Eisch, A.J., Nestler, E.J., and Duman, R.S., *Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus.* J Neurosci, 2000. **20**(24): p. 9104-10.

14. Kim, J.J. and Diamond, D.M., *The stressed hippocampus, synaptic plasticity and lost memories.* Nat Rev Neurosci, 2002. **3**(6): p. 453-62.

CHAPTER VIII: STUDY 4: INTERACTION OF DOPAMINE AND NEUROGENESIS

Background

Overview of neuromodulation and neurogenesis

Although much of the research into neurogenesis, as with the rest of the hippocampus, has focused on the standard excitatory glutamatergic and GABAergic connections between neurons, there is strong reason to consider the effects of other neurotransmitters on neurogenesis function. The hippocampus, the DG in particular, is the recipient of many projections from subcortical regions that release the neurotransmitters acetylcholine, serotonin, dopamine, and norepinephrine [1, 2]. The information relayed from these subcortical areas is most likely related to the affective state of the animal, and acts on a longer time scale than the contextual and spatial information provided by the cortical inputs to the hippocampus. Furthermore, these neurotransmitters often act through metabotropic receptors which interact with intracellular signaling cascades, such as Ca^{2+} and cAMP, that are known to be important for progenitor cell differentiation and survival [3, 4].

Of these modulatory neurotransmitters, serotonin has been the most studied in relation to neurogenesis. Neurogenesis has been implicated in the antidepressant actions of selective serotonin reuptake inhibitors (SSRIs), both as a target of the drugs - SSRIs increase neurogenesis [5] – and as a mechanistic requirement – neurogenesis is required for the efficacy of SSRIs [6]. The serotonin system has been directly implicated, as lesions of the raphe nucleus lower neurogenesis levels [7] and $5HT_{1A}$ receptor activation has been shown to induce proliferation of neural precursors [6, 8].

While serotonin has been of particular interest due to the interaction of neurogenesis and SSRIs, other neurotransmitters affect neurogenesis proliferation and survival as well. Dopamine

denervation lowers the rate of neurogenesis [9]. Knockouts of the nicotinic acetylcholine receptors [10, 11] and damage to the cholinergic forebrain areas [12, 13] show decreased neurogenesis, and infusion of the acetylcholinesterase inhibitor donepezil increases the number of new neurons one month after BrdU labeling, whereas chronic infusion of the muscarinic receptor inhibitor scopolamine decreases the number of new neurons [14]. Norepinephrine may also have an effect on neurogenesis, as dexefaroxan, an antagonist to α2-adrenoceptors, increases the survival of adult-born neurons [15].

Dopamine and adult neurogenesis

Despite evidence that different neuromodulators have an effect on adult neurogenesis, there is little physiological evidence about their direct effect on immature neurons. Recently, Yangling Mu in the Gage Laboratory has acquired data concerning the effects of dopamine (DA) on adult-born granule cells (Mu et al., *in preparation*). Since this data is unpublished, it will be briefly summarized here.

Despite the relatively sparse dispersion of axons in the DG, Mu's data suggests that DA has a strong effect on both adult-born GCs of several ages as well as mature GCs. Specifically, transient application of DA leads to a long lasting decrease in the firing rate of GCs in response to perforant path stimulation. This depression effect is not a function of intrinsic neuron properties, as direct current application can continue to induce reliable firing after DA application, suggesting that the mechanism for depression is synaptic.

Mu subsequently isolated the individual receptors responsible for this effect. Application of the D1 agonist SKF38393 specifically decreases EPSCs from the perforant path in only immature neurons at roughly 4 weeks post injection (wpi). After a 5 minute exposure, the agonist is washed out, with synaptic current gradually decreasing to about 25% less than baseline. This depression is maintained for the duration of the recording (~1 hour). Application of the D2 agonist quiniprole does not have an effect on young neurons. The paired pulse ratio of EPSCs

was not affected by SKF38393 in immature neurons, suggesting that the effect is not a function of pre-synaptic release. This suggests that the EPSC depression observed in immature neurons may have a post-synaptic source.

In contrast to 4 wpi neurons, 8 wpi and mature neurons were selectively depressed by quiniprole. The effect of D2 agonism was similar to that of the D1 agonist in immature neurons: EPSCs were decreased by roughly 25% for a prolonged amount of time after transient application of the agonist. However, unlike D1 agonism in immature neurons, D2 agonism in older GCs does increase PP ratio considerably. This suggests that the depression observed in older neurons is manifested by a pre-synaptic mechanism that decreases synaptic release.

Modeling dopamine's effect on the DG

The electrophysiology data presented here suggests that the effects of DA on synaptic transmission onto immature and mature neurons are comparable, albeit through different receptors. This observation would appear to suggest that there is no interaction between DA and neurogenesis function under these conditions. However, the results in previous chapters suggest that the effects of neurogenesis on dentate gyrus function is complex. Therefore, while both young and old granule cells are suppressed by DA, it is possible that the cumulative effect on the network is biased toward either young or old neurons.

To investigate the population effects of DA in the DG, the DA-induced decrease in EPSC amplitude was included in the computational model. The effects of DA on pattern separation was of particular interest, since the decrease of EPSC amplitudes from the lEC and mEC inputs should result in a sparser activation of GC neurons in the model. Since the pattern separation function of the DG arises, in part, from the sparse activity of the GC layer, it is likely that the depression brought on by DA results in increased pattern separation. Since DA affects immature neurons similarly to mature neurons (at least in the experimental conditions outlined here), at the onset of

the experiment it was unclear whether the neurogenesis effect on pattern separation would be affected.

Modeling Dopamine in the Computational Model

In order to test how DA affects pattern separation, DA modulation was incorporated into the existing DG model. Although DA affected young neurons and mature neurons via D1 and D2 receptors, respectively, the effect of DA was the same for all neurons: an indefinite decrease in the synaptic weights of the input PP pathways. As in the previous studies, the model developed within a series of environments with full neurogenesis and without DA, and then continued to develop within a fourth environment either with (NG) or without (No NG) neurogenesis. Once fully developed, the networks were then examined in an environment designed to test the effect of DA on network activity.

After the NG and No NG networks were developed (day 160), pattern separation was examined as described previously. Briefly, the activity of GC neurons was measured in a range of environments designed to cover a large range of EC similarities. The similarity of GC layer activity (as measured by normalized dot products) was then measured between responses to different locations and environments.

In addition to measuring the similarity between the NG and No NG networks, the responses of the outputs and the similarity of the responses was measured for cases where lEC and mEC synaptic inputs were decreased. This decrease represented the effects of DA in the system. Pattern separation was measured by testing the networks for 500 ms in 400 locations within a series of seven environments.

Temporal separation was measured by growing the network for one day (with plasticity) between testing sessions (without plasticity). Pattern separation was then measured between events occurring at different times. On day 5 of the temporal separation study, the networks were

tested both with and without DA to examine how the presence of DA in the encoding of an event affects how it is temporally associated with other events. DA was simulated during the test phase by decreasing perforant path inputs (lEC to GC and mEC to GC) by 10%. In the pattern separation study, DA responses were only compared to other DA responses, essentially measuring separation only within that event. In the temporal separation study, responses to DA events were compared to responses to non-DA events occurring at different days.

Results

The effects of DA on DG activity were examined by incorporating the observed effects on synaptic transmission into the computational model. As in the previous studies, artificial DG networks were generated using a biologically-derived neurogenesis process. After the equivalent of 120 "days" in three successive environments (for 40 days each), neurogenesis was suppressed in some networks (No NG networks) and retained in other networks (NG networks). After growing for another 40 days in a fourth environment, the behavior of the model was then examined.

The significant effects of DA on GC activity by attenuating perforant path EPSCs by 10% indicated that the overall network activity would be reduced considerably when synaptic transmission was weakened to simulate DA's effects. Indeed, neuronal activity in the model is considerably lower in all GC neurons (0.3Hz Vs 0.144 Hz; $p<0.01$) as well as in young GC neurons (0.16 Hz vs. 0.11 Hz; $p<0.05$) (Figure VIII-2).

The pattern separation ability of the networks was then tested with and without DA. The proposed pattern separation function of the DG is believed to emerge from the low activity levels of granule cells in the network. Therefore, the expected implication of the reduced activity of GCs due to DA would be significantly stronger pattern separation in the network. Consistent with this hypothesis, pattern separation was much stronger in the networks after DA than before

(Figure VIII-3). The effects of DA on pattern separation appear to remove the pattern integration contribution by immature neurons, as NG networks with DA have greater pattern separation than No NG networks without DA (although the No NG networks with DA have even stronger pattern separation).

Dopamine and temporal separation

The effects of DA on temporal separation were then measured in the model. The results described in Chapter V suggested that the presence of new neurons caused events close in time to have increased similarity during encoding. This effect is symmetrical, with events before and after a reference event (without DA) being associated by the immature neuron population (Figure VIII-4). The reduction of DG activity by DA affects how DA events are encoded relative to other temporally proximal events. Events that occur prior to DA release show increased similarity to temporally proximal events without DA, causing a retroactive bias in the temporal relationships if there is more DA for later events compared to earlier ones (Figures VIII-5-6). This temporal bias would potentially cause events that induce DA release to be associated with prior events as opposed to following events.

Likewise, when DA is present for an event preceding the reference event, the reference event will have stronger temporal associations to events that follow it as opposed to the DA events before (Figures VIII-7-8). It appears that DA provides a disruption of the temporal contiguity of information passing through the DG, and that its effects are order dependent.

While the presence of DA before or after a non-DA reference event affects the temporal symmetry of the reference event, the temporal symmetry of the similarities around the DA event is unchanged (Figure VIII-9). Notably, the temporal associations around a DA event are weaker than those around a reference event without DA. As with networks without DA, the temporal associations present in DA networks are dependent on neurogenesis (Figure VIII-10).

Discussion

There are two key findings in these results. First, DA acts as a potent sparsifier, dramatically increasing the separation of GC outputs. This large drop in global DG activity (50%) is due to only a minor 10% decrease in EPSC amplitude. The sensitivity of GCs is consistent with the premise that their tuning is very tight. Even though different GCs in a local region may receive inputs from a similar cohort of EC neurons, the level of tonic inhibition in the network is so high that only those neurons that are heavily depolarized by a given input will be activated. When DA has depressed perforant path inputs to the network, an even smaller set of neurons whose inputs were sufficiently strong to overcome the decrease in EPSCs will be activated. This tight tuning of GC neurons suggests that even minor global alterations to synaptic inputs can have substantial implications on DG function.

The second important aspect of these results is that the drop in similarity caused by DA disrupts the temporal structure of memories encoded by the DG. Because DA decreases pattern separation in the network, marginalizing the pattern integration contribution by new neurons, the DA events are not temporally associated as strongly as non-DA events. According to these results, events encoded under DA conditions are encoded without the temporal reference that other events typically are encoded with.

Overall, the model suggests that decreased activity of GC neurons will result in greater pattern separation and no temporal relationships for memories formed with DA-induced synaptic depression. Importantly, because of the time course observed in the DA physiology, these effects would be expected *after* an actual DA-releasing event, such as a rewarding stimulus. This suggests that there may be a significant difference for how the DG would encode memories before and after DA release. Events prior to a major reward (or punishment) would be encoded normally, whereas information for a time period after the event would be encoded more sparsely and separately, and without temporal context.

Dopamine modeling considerations

There are several caveats to this modeling result. First, it is unclear whether the concentration of DA used in the physiology study is comparable to the biological concentrations present in typical dopaminergic activity in vivo. There is considerable evidence that DA has dose-dependent effects arising from differential binding affinities for the D1 and D2 receptors, which have opposing functionality in certain systems [16]. Although the dose-dependence of DA on the DG system is unclear (and a focus of current experimental work), the possibility does exist that lower concentrations of DA will result in a fundamentally different type of behavior.

A second caveat is the unknown effect of DA on other neurons in the network. Although DA's effect was limited to perforant path EPSCs in this study, it is possible, or even likely, that DA affects different DG neuron populations differently, as is the case with serotonin and acetylcholine [17]. If DA is affecting other populations as well as the GC layer, it is possible that the decreased input to GC neurons can be offset by an accompanying decreased level of inhibition. Such a possibility would lower the sparsity of the GC layer and attenuate the observed pattern separation effect.

In addition to the effects of concentration and other neuron populations, there is a fundamental drawback to modeling DA in isolation. DA release typically coincides with norepinephrine release [18]. Indeed, it is believed that DA release in cortical areas may originate as much from terminals releasing norepinephrine as well as DA-specific terminals [19]. Norepinephrine has been shown to have distinct effects on perforant path synapses, suggesting that there may be an interaction between DA, NE, and the mPP and lPP inputs to GCs [2]. Similarly, serotonin and acetylcholine (as well as less studied peptides, such as opiates) have both been associated at times with reward and salience signaling. Such a caveat applies to most physiology and modeling studies in the brain, but it is nonetheless important to acknowledge when considering the effects of DA on DG function.

Dopamine and signaling to CA3

The effects of DA on DG output must be considered with reference to the DG's function in CA3. The dramatic increase in pattern separation due to very sparse activity would likely have a significant effect on how memories are formed in the associative CA3 network. According to the Treves and Rolls schema that suggests that the DG dictates which CA3 neurons learn a given cortical input [20, 21], the expectation would be that a more separate, sparser DG signal would result in sparser, more distinct CA3 attractor states.

However, there may be drawbacks to having a sparser attractor network in CA3. Ultimately, the amount of information stored in a given memory is a function of the number of neurons responsible for its encoding, essentially the size of the attractor. If a memory is comprised of fewer neurons, it would be more difficult to associate with other events and would possibly be more sensitive to disruption.

In addition to the direct effects of a sparser DG output, there is also the direct effect of DA on DG to CA3 transmission. A recent study [22] showed that application of DA and the D1 agonist SKF81297 affects the release probability of some mossy terminals onto CA3 neurons. This function is important in the context of the general perception that DG transmission to CA3 is gated by the bursting behavior of the mossy fiber. Recordings from CA3 neurons show that single mossy fiber spikes have a very low probability of release, whereas bursting of a single mossy fiber axon input is sometimes sufficient for firing of the CA3 pyramidal cell [23]. In contrast, mossy fiber synapses onto CA3 interneurons are initially effective and have depressing inputs. This dichotomy suggests that a weak GC signal (e.g., a single spike) will preferentially activate the feed-forward interneuron populations of the CA3, inhibiting much of the network, whereas a bursting GC neuron will preferentially activate its target CA3 pyramidal cells at a level that can overcome the feed-forward inhibition [24, 25].

If DA is decreasing GC activity dramatically, one possibility is that signals that otherwise would activate pyramidal neurons would instead activate the inhibitory network in the presence of DA. The Kobayashi results suggest a mechanism that may counteract this possible side effect, allowing the sparser GC output to be more effective in activating CA3 principal neurons. An alternative interpretation of the Kobayashi results is that the bias observed is actually occurring on immature neuron axons. This possibility is supported by the observation that the increase in release probability is dependent on D1 receptors, which is responsible for DA's effects on immature GC physiology. In addition, not all axons show the DA induced potentiation.

Chapter VIII, in part, includes material that is being prepared for publication. Mu, Yangling; Aimone, James B, and Gage, Fred H. The dissertation author was the primary investigator and author of those aspects of this manuscript that are included in this chapter.

Figures

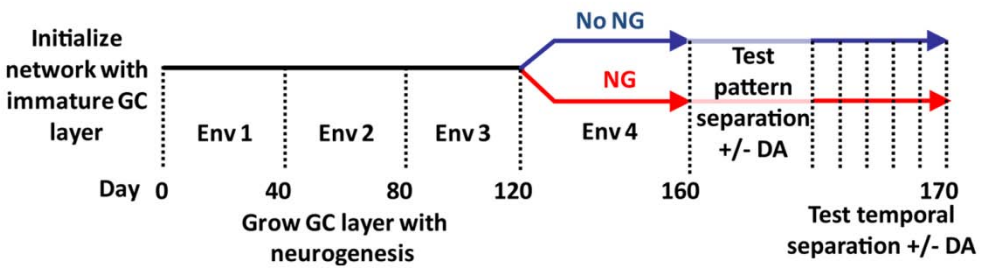

Figure VIII-1: Schematic of Dopamine Study
Networks were initialized and grown similar to previous studies. After growth in 3environments, neurogenesis (NG) and no neurogenesis (No NG) networks were generated. After day 160, pattern separation was tested with and without dopamine (DA). Temporal separation was tested by growing the network to Day 170 and measuring network responses daily. DA was simulated by decreasing EC inputs to the model by 10%.

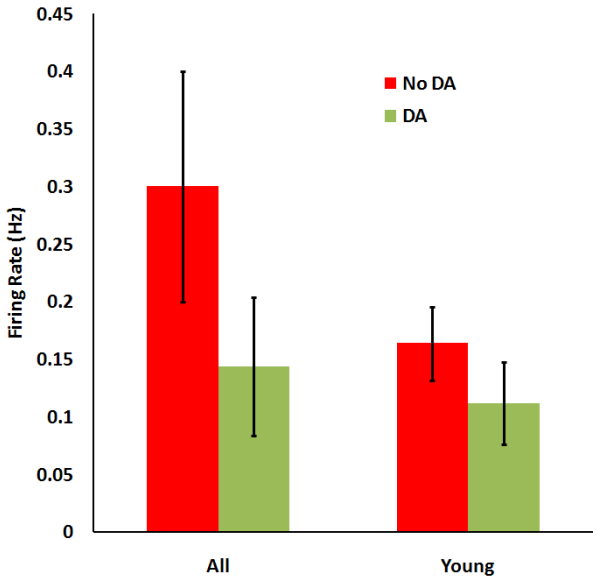

Figure VIII-2: Effect of Dopamine on DG Activity Levels
DA decreased the firing rate of all GCs in the model (left) as well as young neurons (<6 weeks old). Both differences are significant ($p<0.05$).

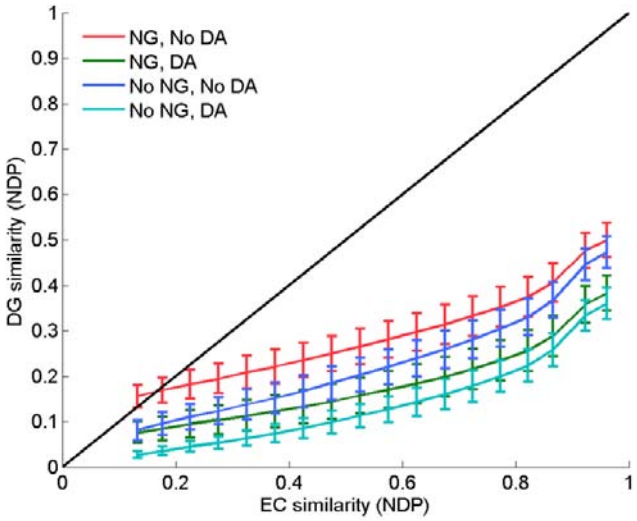

Figure VIII-3: Pattern Separation in DG with and without Dopamine
Compared to control networks, pattern separation was increased in networks lacking neurogenesis. Dopamine made pattern separation stronger in both NG and No NG networks, with the networks with DA and neurogenesis having stronger pattern separation than simple neurogenesis knockouts.

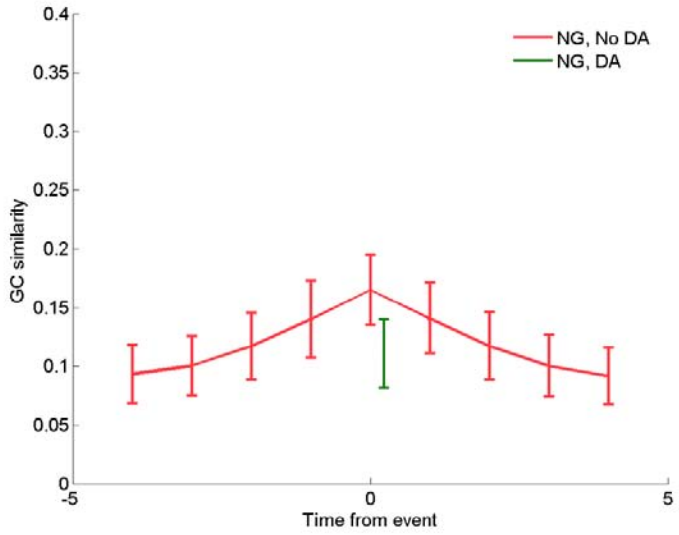

Figure VIII-4: Symmetry of Temporal Responses around Reference Event on Day 5, Dopamine on Day 5
Temporal relationships are shown between GC patterns of a reference event occurring at Day 5 without dopamine and other events occurring on other days before and after. The relationship of the reference event to a DA event occurring on Day 5 is shown.

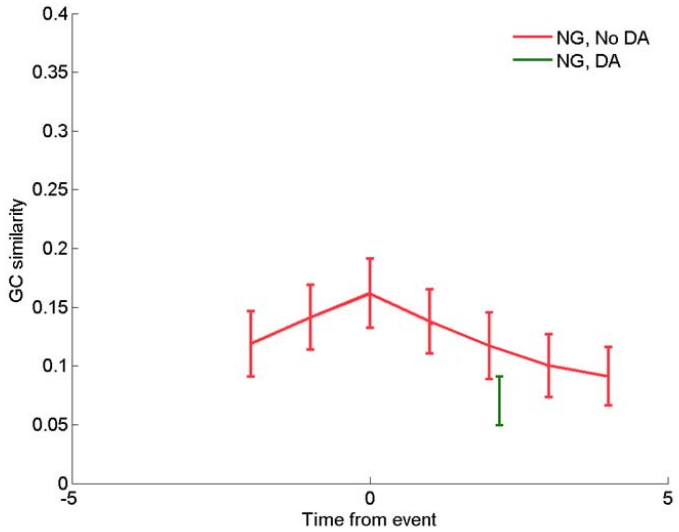

Figure VIII-5: Temporal Similarities around Reference Event on Day 3, Dopamine on Day 5
Temporal relationships are shown between GC patterns of a reference event occurring at Day 3 without dopamine and other events occurring on other days before and after. The relationship of the reference event to a DA event occurring on Day 5 is shown.

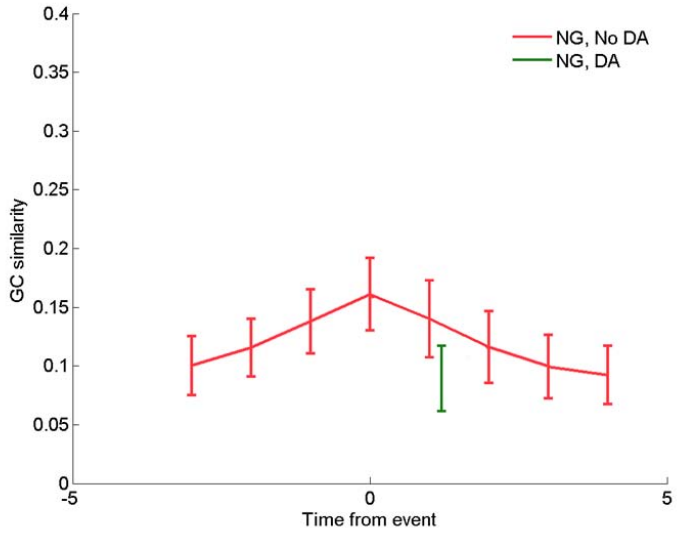

Figure VIII-6: Temporal Similarities around Reference Event on Day 4, Dopamine on Day 5

Temporal relationships are shown between GC patterns of a reference event occurring at Day 4 without dopamine and other events occurring on other days before and after. The relationship of the reference event to a DA event occurring on Day 5 is shown.

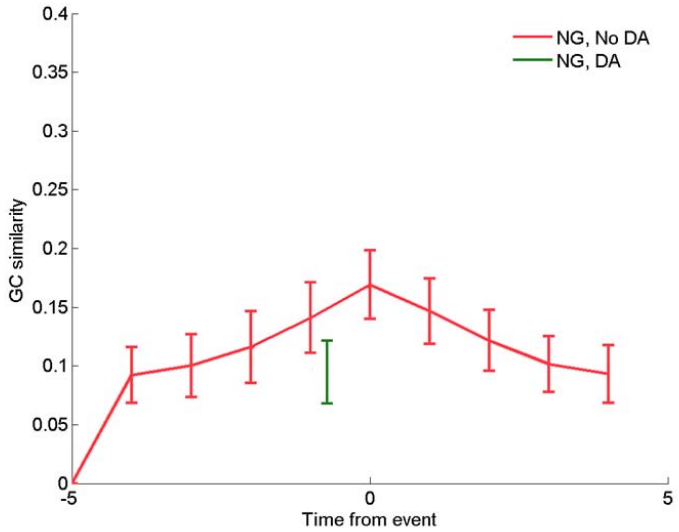

Figure VIII-7: Temporal Similarities around Reference Event on Day 6, Dopamine on Day 5

Temporal relationships are shown between GC patterns of a reference event occurring at Day 6 without dopamine and other events occurring on other days before and after. The relationship of the reference event to a DA event occurring on Day 5 is shown.

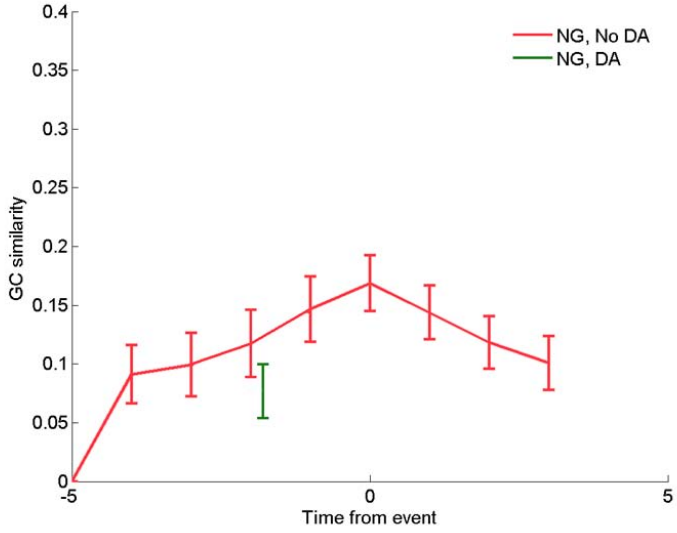

Figure VIII-8: Temporal Similarities around Reference Event on Day 7, Dopamine on Day 5

Temporal relationships are shown between GC patterns of a reference event occurring at Day 7 without dopamine and other events occurring on other days before and after. The relationship of the reference event to a DA event occurring on Day 5 is shown.

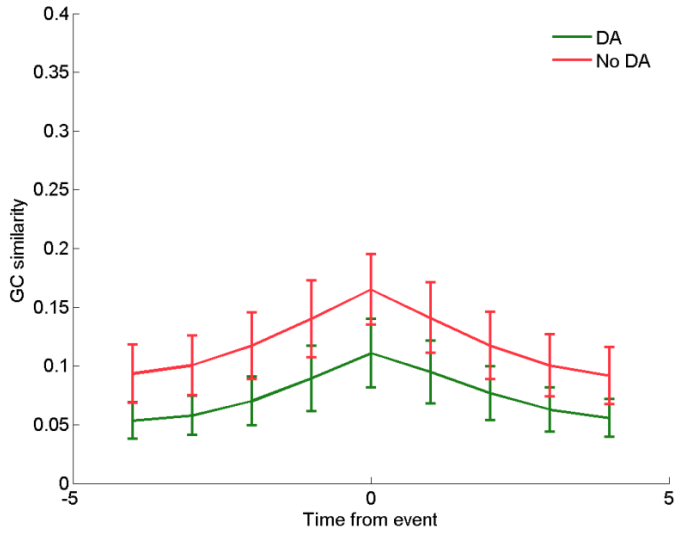

Figure VIII-9: Temporal Similarities around DA and No DA Reference Events on Day 5
Temporal relationships are shown between GC patterns of a reference event occurring at Day 5 with (green) and without dopamine (red) and other events occurring on other days before and after.

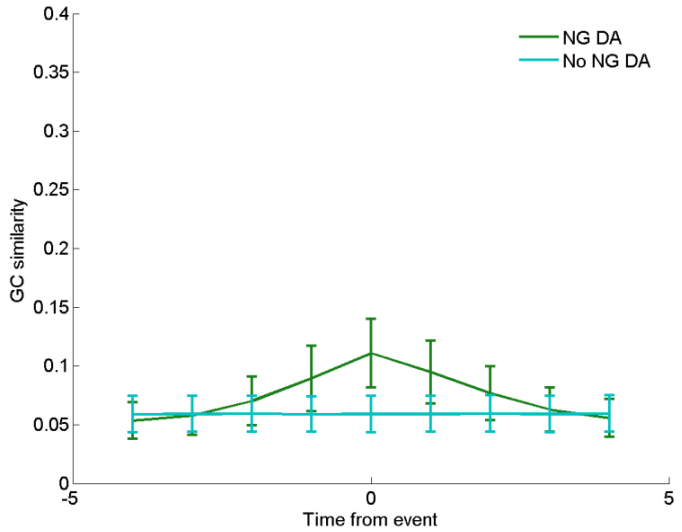

Figure VIII-10: Temporal Similarities around DA Reference Events on Day 5, NG and No NG
Temporal relationships are shown between GC patterns of a reference event occurring at Day 5 with (green) dopamine for networks with (green) and without neurogenesis (light blue). Relationships are shown with other events encoded without neurogenesis.

References

1. Leranth, C. and Hajszan, T., *Extrinsic afferent systems to the dentate gyrus.* Prog Brain Res, 2007. **163**: p. 63-84.

2. Harley, C.W., *Norepinephrine and the dentate gyrus.* Prog Brain Res, 2007. **163**: p. 299-318.

3. Deisseroth, K., Singla, S., Toda, H., Monje, M., Palmer, T.D., and Malenka, R.C., *Excitation-neurogenesis coupling in adult neural stem/progenitor cells.* Neuron, 2004. **42**(4): p. 535-52.

4. Nakagawa, S., Kim, J.E., Lee, R., Malberg, J.E., Chen, J., Steffen, C., Zhang, Y.J., Nestler, E.J., and Duman, R.S., *Regulation of neurogenesis in adult mouse hippocampus by cAMP and the cAMP response element-binding protein.* J Neurosci, 2002. **22**(9): p. 3673-82.

5. Malberg, J.E., Eisch, A.J., Nestler, E.J., and Duman, R.S., *Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus.* J Neurosci, 2000. **20**(24): p. 9104-10.

6. Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., and Hen, R., *Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants.* Science, 2003. **301**(5634): p. 805-809.

7. Brezun, J.M. and Daszuta, A., *Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats.* Neuroscience, 1999. **89**(4): p. 999-1002.

8. Banasr, M., Hery, M., Printemps, R., and Daszuta, A., *Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone.* Neuropsychopharmacology, 2004. **29**(3): p. 450-60.

9. Hoglinger, G.U., Rizk, P., Muriel, M.P., Duyckaerts, C., Oertel, W.H., Caille, I., and Hirsch, E.C., *Dopamine depletion impairs precursor cell proliferation in Parkinson disease.* Nat Neurosci, 2004. **7**(7): p. 726-35.

10. Koike, K., Hashimoto, K., Okamura, N., Ohgake, S., Shimizu, E., Koizumi, H., Komatsu, N., and Iyo, M., *Decreased cell proliferation in the dentate gyrus of alpha 7 nicotinic acetylcholine receptor heterozygous mice.* Prog Neuropsychopharmacol Biol Psychiatry, 2004. **28**(3): p. 517-20.

11. Caldarone, B.J., Harrist, A., Cleary, M.A., Beech, R.D., King, S.L., and Picciotto, M.R., *High-affinity nicotinic acetylcholine receptors are required for antidepressant effects of amitriptyline on behavior and hippocampal cell proliferation.* Biol Psychiatry, 2004. **56**(9): p. 657-64.

12. Mohapel, P., Leanza, G., Kokaia, M., and Lindvall, O., *Forebrain acetylcholine regulates adult hippocampal neurogenesis and learning.* Neurobiol Aging, 2005. **26**(6): p. 939-46.

13. Cooper-Kuhn, C.M., Winkler, J., and Kuhn, H.G., *Decreased neurogenesis after cholinergic forebrain lesion in the adult rat.* J Neurosci Res, 2004. **77**(2): p. 155-65.

14. Kotani, S., Yamauchi, T., Teramoto, T., and Ogura, H., *Pharmacological evidence of cholinergic involvement in adult hippocampal neurogenesis in rats.* Neuroscience, 2006. **142**(2): p. 505-14.

15. Rizk, P., Salazar, J., Raisman-Vozari, R., Marien, M., Ruberg, M., Colpaert, F., and Debeir, T., *The alpha2-adrenoceptor antagonist dexefaroxan enhances hippocampal neurogenesis by increasing the survival and differentiation of new granule cells.* Neuropsychopharmacology, 2006. **31**(6): p. 1146-57.

16. Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., and Caron, M.G., *Dopamine receptors: from structure to function.* Physiol Rev, 1998. **78**(1): p. 189-225.

17. Freund, T.F., *Interneuron Diversity series: Rhythm and mood in perisomatic inhibition.* Trends Neurosci, 2003. **26**(9): p. 489-95.

18. Sara, S.J., *The locus coeruleus and noradrenergic modulation of cognition.* Nat Rev Neurosci, 2009. **10**(3): p. 211-23.

19. Devoto, P. and Flore, G., *On the Origin of Cortical Dopamine: Is it a Co-Transmitter in Noradrenergic Neurons?* Curr Neuropharmacol, 2006. **4**(2): p. 115-25.

20. Rolls, E.T., *A theory of hippocampal function in memory.* Hippocampus, 1996. **6**(6): p. 601-20.

21. Treves, A. and Rolls, E.T., *Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network.* Hippocampus, 1992. **2**(2): p. 189-99.

22. Kobayashi, K. and Suzuki, H., *Dopamine selectively potentiates hippocampal mossy fiber to CA3 synaptic transmission.* Neuropharmacology, 2007. **52**(2): p. 552-61.

23. Henze, D.A., Wittner, L., and Buzsaki, G., *Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo.* Nat Neurosci, 2002. **5**(8): p. 790-5.

24. Lawrence, J.J. and McBain, C.J., *Interneuron diversity series: containing the detonation--feedforward inhibition in the CA3 hippocampus.* Trends Neurosci, 2003. **26**(11): p. 631-40.

25. Acsady, L., Kamondi, A., Sik, A., Freund, T., and Buzsaki, G., *GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus.* J Neurosci, 1998. **18**(9): p. 3386-403.

CHAPTER IX - DISCUSSION OF MODEL RESULTS

Summary of hypotheses for neurogenesis function

The studies described here suggest that adult-born GC have multiple functions during their development (Figure IX-1). The results in Chapter V show that the electrophysiology properties of new GC may alter the pattern separation function of the DG by providing a level of similarity to events that would otherwise be separated. This process is referred to as *pattern integration,* and implies that pattern separation is limited for events that utilize the same population of immature neurons. The subsequent maturation of this immature population over several days reduces this integration effect, suggesting that the contribution of immature neurons to memory encoding is dependent on when the event is encoded. With reference to the DG's pattern separation function, it appears that immature neurons are contributing to temporal separation of memories.

Chapter VI describes results that suggest that the immature neurons involved in these pattern integration and temporal separation functions eventually mature into unique dimensions that may be used to improve memory encoding in the future. Further analysis using dimensional reduction techniques, such as PCA, suggests that immature GCs grow into unique dimensions that are distinct from those used to encode previous environments. In contrast, without neurogenesis, novel environments are encoded using combinations of mature neurons.

Although these functions for new neurons occur at different times during their development, they are not independent. Each emerges from the young neurons' experience-dependent maturation process. During maturation, new GC transition from progenitor cells to fully functional neurons. For the first few weeks of this process, the electrical properties of immature neurons are quite different from those of mature GC [1]. The pattern integration

function emerges from the observation that the immature neurons with increased excitability may decrease the separation function performed by the DG. The transition of these immature neurons out of this increased excitability phase, provides the mechanism for encoding the temporal relationship between events.

While a significant fraction of immature neurons dies before they are fully mature, a non-trivial proportion of them remains alive indefinitely [2-4]. In the model, the activity-dependent maturation of these surviving neurons results in the generation of specialized groups of GC that may improve the encoding of that environment in the future, consistent with biological studies using immediate-early genes that showed that neurons responded preferentially to events that occurred during their maturation [2, 5]. These results suggest that these populations of neurons may represent new dimensions that the DG can use to encode new memories – dimensions that are "custom-built" for the information contained in those memories. Indeed, the same neurons that perform pattern integration between events when they are young in the model ultimately comprise the new dimensions to better encode those events when they are older. While pattern integration is adding similarity to the encoding of current events, the new neurons are gaining specificity that will lead them to improve the encoding of future events.

In addition to the dynamics of maturation possibly leading to new neuron function, the model suggests that outside dynamics acting on the neurogenesis process itself can lead to effects on hippocampal function. The results in Chapter VII suggest that changes in neurogenesis rate due to chronic conditions such as aging and stress may affect the DG's function over long periods of time. These results suggest that the memory problems associated with aging may be partially due to significant changes in DG structure and function due to the diminishing levels of neurogenesis. Furthermore, the recovery of neurogenesis after stress rescues the model's phenotype from the low-neurogenesis stress state, suggesting a possible mechanism for the cognitive effects of anti-depressants, which are known to increase neurogenesis [6, 7].

In addition to systemic effects on neurogenesis, behavior and experience may have direct modulatory effects on immature neurons. Chapter VIII describes how dopamine in the network can have a complex interaction with the neurogenesis system in the DG. The slice physiology observation that dopamine decreases perforant path input to GCs were applied to the model, revealing that this decrease in network inputs can have a significant effect on increasing the separation of the dopamine event from other events. Even though immature neurons were less affected by dopamine directly in the modeling results, their effect on DG pattern separation was somewhat marginalized due to population effects.

Relationship of results to theories of hippocampal function

The idea that DG sparse coding leads to pattern separation has been developed over recent decades. Modeling studies have provided clear arguments for why pattern separation is useful, or even necessary, for hippocampal function [8-11]. By producing sparse, orthogonal codes the DG is an ideal input to the downstream CA3, which is believed to be store memories in its associative network.

Many of these theoretical notions of DG function have been confirmed by observations that GCs are sparsely active [12], and are capable of strongly depolarizing downstream CA3 neurons [13]. Behavioral studies have also implicated pattern separation as a possible function for DG [14-16]. Not all evidence is as clear, in vivo recording during spatial exploration of different environments suggest that DG neurons are not as contextually discriminant as other hippocampal neurons, though still can be considered “pattern separating” at a spatial, population level [17].

The results presented here support the possibility that mature neurons in the DG perform pattern separation. However, the presence of neurogenesis in the model suggests that this function is not as straightforward as other models suggest. The separation effect in the model is

significantly affected by immature neurons in the GC layer, whose broad tuning serves to act counter to the strong separation in the network by older neurons for temporally proximal events, but not temporally distinct events. This contribution by young neurons makes the pattern separation function of the neurogenic DG in this model more sophisticated than that previously considered. These differences may have significant effects on hippocampal function.

First, with neurogenesis, the extent that the DG can pattern separate any two inputs at a given time is bounded. Regardless of how distinct two inputs may be, there would likely be some overlap in the population of immature neurons activated. Given that the CA3 is itself believed to be capable of pattern separating largely dissimilar inputs[17], the effect of immature neurons would likely not be disruptive. However, the presence of some residual similarity in the DG signal may be important to ensure that associations can be formed between events if necessary. Therefore, one possible function for pattern integration may be to ensure that pattern separation does not overwhelm the system.

Second, while the hippocampus has long been considered critical for the encoding of temporal information, these studies have focused mostly on recurrent network dynamics in the CA3 and spike-timing dependent plasticity [18, 19]. Both these effects operate at time scales considerably shorter (seconds and milliseconds) than the temporal associations proposed here (hours and days). If an associative memory network is designed to capture possible sources of causation in its associations, then it is necessary for the system to detect relationships occurring at a wide range of time scales. Causal relationships between events would be expected to be far more common for events occurring within very short time frames, which would explain why many regions of the brain have plasticity operating at these time scales. But there are important relationships occurring between more temporally distant events, and it is not surprising that a structure critically involved in memory formation would have a mechanism that has dynamics at the time scale of neurogenesis.

Finally, in addition to adding a lower-bound and temporal separation to the pattern separation function of DG, the long-term incorporation of new neurons into the network has the potential to have a more global effect on its function. The results suggesting that new neurons specialize to environments indicate that at an individual level new neurons may be learning about their environments. But at a population level, the effect is far more pronounced. As opposed to previous computational and theoretical models of DG, where the networks are initialized randomly or neuron activity simply follows a Poisson process [10], these results suggest that most DG neurons develop specifically to capture features of their environments. Experience-dependent development of brain structure is a process studied in many systems [20], but most of these systems are capturing basic statistics about the world. Embryonic-born and developing DG neurons probably develop to encode basic aspects of the world, but the large populations of post-natal and adult-born GCs are most likely capturing more individually-dependent features of the world. While every animal may be expected to learn the statistics associated with color range and auditory range, the events experienced by animals diverge substantially after birth. The DG, in contrast to almost all other brain regions, is well situated to acquire those unique characteristics.

Relationship of results to other models of neurogenesis

The "bottom-up" approach to modeling adult neurogenesis described here differs considerably from that of previous models of adult neurogenesis (for review, see [21]). These distinctions likely underlie the differences between these results and previous theoretical results. The model presented here has at least three major features that distinguish it from previous modeling studies: (1) the inclusion of details about the maturation process; (2) simulation over long time scales, allowing successive generations of new neurons to populate the DG; and (3) assaying DG function by measuring pattern separation while using biologically derived inputs. The extent of biological detail included in this model is in contrast to previous computational

studies of neurogenesis that investigated the effect of either neuron addition or turnover on specific network functions in less complicated models. While those models have revealed several possible functions for the addition of new neurons in simple network architectures, the inclusion of biological details was likely important for the observation of several network behaviors heretofore not described.

In some cases, the results were similar to those of other models. For example, FEs attaining dimensional independence due to the maturation process is similar to the hypotheses put forth by two previous computational studies that suggested that new neurons protect old memories by increasing the capacity available for encoding new memories [22, 23]. However, these studies suggest that the acute effects of stopping neurogenesis would be substantial, potentially leading to the collapse of previously encoded memories, whereas this model predicts that the cessation of neurogenesis would result in a more subtle deficit: new environments would continue to be encoded using a combination of previous environments, but their transition to being familiar would be impaired.

Relationship of neurogenesis hypotheses to animal behavioral studies

As described extensively in the overview (Chapter II), behavioral studies on neurogenesis knockdown studies in rodents have provided mixed results. The variability associated with these studies likely arises from several sources, including species differences, neurogenesis levels in different mouse strains [24], different knockout approaches, and methodological differences. While many of the behavioral tasks designed to dissociate functions for hippocampal subregions were motivated by computational theories regarding these functions [16, 25-30], neurogenesis studies have relied mostly on general hippocampal tasks, such as water maze and context fear conditioning.

Although previous behavioral studies of neurogenesis have lacked specific theoretical motivation, several studies have results that may be explained by the theoretical results presented here. Of the previous behavioral results using knockdown models of neurogenesis, perhaps the most relevant to the model is the observation that irradiated animals have improved performance on a working memory [31]. One prediction of the pattern integration hypothesis is that reducing neurogenesis might result in an increase of pattern separation during memory encoding. As a result, behaviors that benefit from greater separation may show an improvement after the elimination of new neurons. One interpretation of the working memory results is that normal mice have difficulty distinguishing between the current trial and recent trials, whereas irradiated mice have a better ability to segregate their current actions from those of the past. While pattern integration may make pattern separation more difficult, it may be necessary for other behaviors that require the animal to integrate information across several learning trials.

Design of new behavioral tasks to examine the model

Explicit testing of these hypotheses will require the design of new behavioral tasks. One possibility for testing the model is to simultaneously examine both pattern integration and temporal associations. The hypotheses suggest that events occurring close in time will be associated with one another, whereas events occurring several days apart will be encoded separately. An example behavioral paradigm using fear conditioning would be to present multiple contexts to an animal over time with one context coupled to an aversive stimulus (i.e., shock). The model would predict that animals would fear both the context where the shock occurred and those contexts that were proximal in time. One drawback to this specific example is that context fear conditioning is affected in neurogenesis knockdowns in certain conditions, so care must be taken to ensure the underlying fear memory is present.

The final hypothesis - that adult-born neurons mature to encode new dimensions - can also be examined behaviorally. One implication of developing specialized groups of GCs may be

an increased ability to acquire new memories that can utilize those new dimensions. Animals that live extensively within an enriched environment have an increased survival of new neurons that may specialize to features of that environment [2, 4]. Given the DG's presumed role in memory encoding, one would predict that these animals may have a greater ability to learn within that environment than animals for which the environment is novel. One possible behavioral task would be to pre-expose an animal to several contexts over several weeks, which should induce populations of specialized GC. Later, the animal would be trained to fear one of these contexts, but not the others. The prediction would be that neurogenesis would improve the discrimination of the feared context from the other pre-exposed environments.

Relationship of neurogenesis hypotheses to human memory

Ultimately, the relevance of a neurogenesis function will be judged by whether the role of new neurons can be demonstrated in human memory. Experiments on the structure and mechanism of human memory are fundamentally different from the studies performed in rodent models that have motivated much of the theoretical work on the hippocampus. With the exception of intracranial recordings performed alongside diagnostic medical procedures and gross lesion studies, most human experimentation is limited to extracranial imaging studies that provide limited temporal and spatial resolution. These limitations are offset by the significantly greater range of cognitive tasks. The development of functional imaging measures that are believed to correlate with adult neurogenesis [32] provide an opportunity to associate neurogenesis levels performance on psychological tasks that investigate the structure of human memories [33-35].

As with the examination of neurogenesis function in animal models, studies of neurogenesis in humans would benefit from having specific hypotheses relating to neurogenesis to examine. The hypotheses presented here suggest that while the dentate gyrus pattern separation function would be preserved, there would be some specific changes in how

information is encoded. As such, effects would be substantially more moderate than what is observed in hippocampal lesion patients, but may still be significant when studied properly. In addition to the functional hypotheses outlined here, the model studies regarding aging and stress demonstrate what functions of the neurogenesis process would be expected to be compromised during these conditions. Since both aging and stress are associated with memory deficits in individuals, these conditions may represent natural knock-down models for human neurogenesis.

How pattern integration may be manifested in human memory

Although most hippocampal network theories assume the DG's role is limited to pattern separation, some more general ideas for the structure of human memories suggest a use for the added similarity that pattern integration provides. One example is the "constructive memory hypothesis," which postulates that memories are composed of distinct elements that are stored separately and reconstructed at the time of retrieval, as opposed to a pure reproduction of a past event [36]. If memories are indeed stored in a distributed form, there is probably a requirement for some additional information that binds the distributed pieces together. While the pattern completion circuitry in the hippocampus would be effective at forming and recapitulating associations between items that occur at the same time or in sequence [27], complex memories might require a different mechanism to bind distributed components together. Although the classical view of the DG is that it would separate context from this information, immature neurons may limit the amount of separation performed at the time of encoding. Memories encoded by the network would still be adequately separated to the extent that effective attractors could be formed, but the attractor states of these memories would remain related to one another. Additionally, such associations would only be meaningful if the added similarity was temporally constrained, as there would be little benefit if all memories were linked to one another.

The results presented here suggest that the acute drop in neurogenesis due to stress greatly eliminates the pattern integration provided during memory encoding. In the

aforementioned constructive memory framework, one could anticipate that this lack of pattern integration may result in a decreased ability to combine distinct memory components into uniform memories and may be revealed by an improved performance on tasks designed to confuse information with contextual clues. The effect of aging on pattern integration is less dramatic in this model; however, the increased similarity occurring in older networks is not temporally dependent, suggesting that, while the ability to bind memories together remains with aging, this process loses its temporal precision.

How temporal separation may be incorporated into human memory

The structure of temporal information in human memory is unclear. Although the constructive memory framework suggests that memories consist of separate pieces that are associated in the generation of a coherent episode, the theory is somewhat agnostic to where those associations come from. Research into the structure of temporal information has focused more on the psychological domain as opposed to biological mechanisms. Friedman collated ten properties of temporal memory observed from psychological studies [37]. These properties relate to the relationships between different memories in time and under what conditions time is accurately recalled or encoded. In addition, the evidence suggests that temporal information is not a simple "time-stamping" process; rather, time is encoded more relatively. This perspective on how time would be encoded is consistent with time simply being one component of a larger, more sophisticated memory framework. How the temporal memory described in Chapters III and V relates to psychological studies of memory remains to be determined [38, 39]. One of the key features from these studies, however, is that temporal information is complex and appears to exist at multiple time scales. This is consistent with neurogenesis being only one type of neural dynamic that may provide temporal information to the system.

While these previous psychological studies have sought to explore how time is encoded, this theory makes specific predictions about how neurogenesis may contribute to the temporal

structure of memories. One prediction would be that subjects with putative low neurogenesis conditions, such as depressed individuals pre-treatment or patients following radiation therapy, would have difficulty in encoding temporal relationships between past events. The design of such studies would need to account for the information available during memory formation, as there are substantial contextual cues that may allow later reconstruction of temporal relationships.

How dimensional specialization of the DG affects human memory

In addition to a role for pattern integration, the possibility that novel environments are encoded using a combination of neurons previously used to encode familiar environments also fits nicely into the constructive memory framework. Consistent with the idea that memories are encoded in a distributed manner, the DG's representation of an FE included not only those neurons that matured within that environment, but also neurons that showed a preference for other, previously experienced, environments (Chapter VI). One possibility is that those neurons that are used in multiple environments encode features that are invariant between the two contexts. Furthermore, NEs were initially encoded entirely by using "familiar" dimensions. Without having developed a set of neurons customized to the current inputs, it appears that the network approximated the entire context by utilizing other neurons that matured in previous environments. Such a process is similar to recent proposals about the process of imagination: that thinking about the future consists of constructing a new combination of old memories into a new package [36]. These results suggest that recently experienced environments will not transition to being familiar after aging, as there are few new neurons to commit to those contexts. A failure of environments to transition to familiar may affect how memories are formed in aged or chronically stressed individuals; even environments that should be familiar may be considered novel if there is little neurogenesis available when previously experienced.

Limitations of the computational approach

While the complexity of this model was important for the generation of novel, behaviorally testable predictions, both the accuracy and completeness of the model are issues that remain to be addressed by both biological studies and future modeling work. Adult neurogenesis is a dynamic area of research and, as is the case with all computational models, future results may make it necessary to revisit certain assumptions made in the model. This caveat does not negate the validity of the results proposed here, but it underscores the importance of future biological investigation of these hypotheses, as described in the next section.

This modeling and theoretical work has focused principally on the DG, and it is possible that neurogenesis has unknown implications on other hippocampal regions. For example, the relationship between GC and CA3 neurons is complex, as it appears that CA3 pyramidal neurons and interneurons respond differentially to bursting of GC [13, 40]. If new neurons do not fire in the same manner as mature cells, it is possible that the CA3 will not respond as predicted. Until the mossy fiber projection is fully investigated *in vivo*, the precise effect new neurons have on CA3 is not entirely clear, though recent work has shown that they make functional connections [41].

In addition to mechanistic details, it is not yet clear how changing the pattern separation function in the DG will affect information processing in the rest of the hippocampus. While generally considered an associative network, the CA3 has been shown to also contribute to pattern separation, though this is believed to be fundamentally different from the separation function of the DG [17]. Neurogenesis would appear to be one source of this difference, as these results suggest that DG is separating inputs according to time as well as specific features of the events. In addition, further modeling work may reveal how neurogenesis affects the network dynamics of the DG. A more sophisticated understanding of the network dynamics associated with pattern separation in the DG network may clarify how this separation function affects the attractor dynamics in the CA3.

In addition to more complex analyses of the network dynamics, continued examination of the model's behavior considering other perspectives on hippocampal function will be revealing, particularly with regard to how neurogenesis affects the hippocampal representation of space and neurogenesis' relationship to depression. The DG is believed to be important in the formation of hippocampal place representations, and GC have distinct spatial behaviors, though how they affect hippocampal spatial processing is still unclear [17]. Similarly, the role of DG in affective conditions, such as depression, is unknown, though a strong relationship between neurogenesis and certain anti-depressant drugs suggests that adult-born neurons play a role in affective state [42]. The functional role of neurogenesis in encoding space and affect is unknown, and further work is required to relate the results of the model to these hippocampal functions.

Finally, as with other computational models, this study is limited by details of the system that have not yet been fully described. For instance, although the spatial properties of mEC neurons have been well characterized [43], the structure of the lEC input to the DG remains unclear [44]. For instance, GC in this model has a spatial structure that is obviously influenced by the grid structure of the mEC neurons. While *in vivo* studies have shown spatial structure to GC responses, it has not been reported as significantly grid-like [12, 17]. This difference in model behavior emerges from the grid cells being the only input population with a spatial structure. Furthermore, more examination is required to determine how immature neurons influence *in vivo* measurements of DG neurons during behaviors and exploration.

Conclusion

In summary, the studies described here provide the computational basis for several new hypotheses, both for the function of neurogenesis during cognition as well as the interactions of neurogenesis with other neurobiological processes. According to these hypotheses, new neurons born within the adult brain may make several contributions to memory formation, such as

temporal separation and long-term specialization, which have not previously been considered important features of memory. The results describing how aging, stress, and reward state may affect these functions demonstrate the complexity of the relationship between the neurogenesis process and the overall cognitive and physical state.

As with any new hypothesis, the potential functions proposed here require direct biological examination. These results will hopefully encourage the development of new behavioral and psychological approaches for examining these newly proposed aspects of memory. While this validation process and future experimental observations may require that certain aspects of the model be revisited, the process of testing and updating the theoretical framework will be a powerful process for advancing the understanding of neurogenesis.

Chapter IX, in part, includes material that was published in the article "Computational Influence of Adult Neurogenesis on Memory Encoding," Aimone, James B; Wiles, Janet; and Gage, Fred H.; *Neuron*, January 2009. The dissertation author was the primary investigator and author of this paper.

Figure

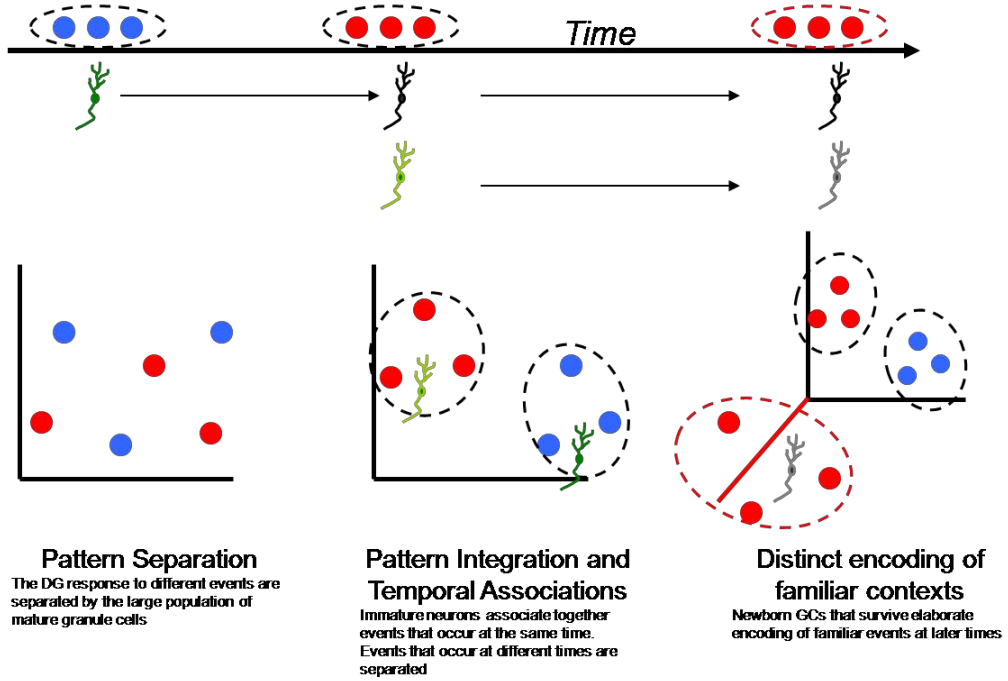

Figure IX-1: Schematic of different functions for immature neurons over time
Mature neurons in the DG are capable of separating events in memory. Immature neurons will pattern integrate over those events that occur during their youth (for the top neuron, the blue events, for the bottom neuron, the red events). Different populations will encode different events, leading to temporal separation. At later times, the previously experienced events can utilize the now mature neurons for more specialized encodings.

References

1. Esposito, M.S., Piatti, V.C., Laplagne, D.A., Morgenstern, N.A., Ferrari, C.C., Pitossi, F.J., and Schinder, A.F., *Neuronal Differentiation in the Adult Hippocampus Recapitulates Embryonic Development.* Journal of Neuroscience, 2005. **25**(44): p. 10074-10086.

2. Tashiro, A., Makino, H., and Gage, F.H., *Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage.* J Neurosci, 2007. **27**(12): p. 3252-9.

3. Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., and Gage, F.H., *Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice.* Development, 2003. **130**(2): p. 391-9.

4. Kempermann, G., Kuhn, H.G., and Gage, F.H., *More hippocampal neurons in adult mice living in an enriched environment.* Nature, 1997. **386**(6624): p. 493-5.

5. Kee, N., Teixeira, C.M., Wang, A.H., and Frankland, P.W., *Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus.* Nat Neurosci, 2007. **10**(3): p. 355-362.

6. Malberg, J.E., Eisch, A.J., Nestler, E.J., and Duman, R.S., *Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus.* J Neurosci, 2000. **20**(24): p. 9104-10.

7. Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., and Hen, R., *Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants.* Science, 2003. **301**(5634): p. 805-809.

8. Rolls, E.T., *A theory of hippocampal function in memory.* Hippocampus, 1996. **6**(6): p. 601-20.

9. O'Reilly, R.C. and McClelland, J.L., *Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off.* Hippocampus, 1994. **4**(6): p. 661-82.

10. Treves, A. and Rolls, E.T., *Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network.* Hippocampus, 1992. **2**(2): p. 189-99.

11. McNaughton, B.L. and Morris, R.G.M., *Hippocampal synaptic enhancement and information storage within a distributed memory system.* Trends in Neurosciences, 1987. **10**(10): p. 408-415.

12. Jung, M.W. and McNaughton, B.L., *Spatial selectivity of unit activity in the hippocampal granular layer.* Hippocampus, 1993. **3**(2): p. 165-82.

13. Henze, D.A., Wittner, L., and Buzsaki, G., *Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo.* Nat Neurosci, 2002. **5**(8): p. 790-5.

14. McHugh, T.J., Jones, M.W., Quinn, J.J., Balthasar, N., Coppari, R., Elmquist, J.K., Lowell, B.B., Fanselow, M.S., Wilson, M.A., and Tonegawa, S., *Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network.* Science, 2007. **317**(5834): p. 94-9.

15. Kesner, R.P., *A behavioral analysis of dentate gyrus function.* Prog Brain Res, 2007. **163**: p. 567-76.

16. Gilbert, P.E., Kesner, R.P., and Lee, I., *Dissociating hippocampal subregions: double dissociation between dentate gyrus and CA1.* Hippocampus, 2001. **11**(6): p. 626-36.

17. Leutgeb, J.K., Leutgeb, S., Moser, M.B., and Moser, E.I., *Pattern separation in the dentate gyrus and CA3 of the hippocampus.* Science, 2007. **315**(5814): p. 961-6.

18. Dan, Y. and Poo, M.M., *Spike timing-dependent plasticity of neural circuits.* Neuron, 2004. **44**(1): p. 23-30.

19. Dragoi, G. and Buzsaki, G., *Temporal encoding of place sequences by hippocampal cell assemblies.* Neuron, 2006. **50**(1): p. 145-57.

20. Katz, L.C. and Shatz, C.J., *Synaptic activity and the construction of cortical circuits.* Science, 1996. **274**(5290): p. 1133-8.

21. Aimone, J.B. and Wiskott, L., *Computational Modeling of Adult Neurogenesis*, in *Adult Neurogenesis*, Gage, F.H., Kempermann, G., and Song, H., Editors. 2008, Cold Spring Harbor Laboratory Press: Woodbury, NY. p. 463-481.

22. Becker, S., *A computational principle for hippocampal learning and neurogenesis.* Hippocampus, 2005. **15**(6): p. 722-38.

23. Wiskott, L., Rasch, M.J., and Kempermann, G., *A functional hypothesis for adult hippocampal neurogenesis: avoidance of catastrophic interference in the dentate gyrus.* Hippocampus, 2006. **16**(3): p. 329-43.

24. Kempermann, G., Kuhn, H.G., and Gage, F.H., *Genetic influence on neurogenesis in the dentate gyrus of adult mice.* Proc Natl Acad Sci U S A, 1997. **94**(19): p. 10409-14.

25. Tonegawa, S. and McHugh, T.J., *The ins and outs of hippocampal circuits.* Neuron, 2008. **57**(2): p. 175-7.

26. Nakashiba, T., Young, J.Z., McHugh, T.J., Buhl, D.L., and Tonegawa, S., *Transgenic inhibition of synaptic transmission reveals role of CA3 output in hippocampal learning.* Science, 2008. **319**(5867): p. 1260-4.

27. Rolls, E.T. and Kesner, R.P., *A computational theory of hippocampal function, and empirical tests of the theory.* Prog Neurobiol, 2006. **79**(1): p. 1-48.

28. Nakazawa, K., Sun, L.D., Quirk, M.C., Rondi-Reig, L., Wilson, M.A., and Tonegawa, S., *Hippocampal CA3 NMDA receptors are crucial for memory acquisition of one-time experience.* Neuron, 2003. **38**(2): p. 305-15.

29. Nakazawa, K., Quirk, M.C., Chitwood, R.A., Watanabe, M., Yeckel, M.F., Sun, L.D., Kato, A., Carr, C.A., Johnston, D., Wilson, M.A., and Tonegawa, S., *Requirement for hippocampal CA3 NMDA receptors in associative memory recall.* Science, 2002. **297**(5579): p. 211-8.

30. Lee, I. and Kesner, R.P., *Encoding versus retrieval of spatial memory: double dissociation between the dentate gyrus and the perforant path inputs into CA3 in the dorsal hippocampus.* Hippocampus, 2004. **14**(1): p. 66-76.

31. Saxe, M.D., Malleret, G., Vronskaya, S., Mendez, I., Garcia, A.D., Sofroniew, M.V., Kandel, E.R., and Hen, R., *Paradoxical influence of hippocampal neurogenesis on working memory.* Proc Natl Acad Sci U S A, 2007. **104**(11): p. 4642-6.

32. Pereira, A.C., Huddleston, D.E., Brickman, A.M., Sosunov, A.A., Hen, R., McKhann, G.M., Sloan, R., Gage, F.H., Brown, T.R., and Small, S.A., *An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus.* Proc Natl Acad Sci U S A, 2007. **104**(13): p. 5638-43.

33. Shohamy, D. and Wagner, A.D., *Integrating memories in the human brain: hippocampal-midbrain encoding of overlapping events.* Neuron, 2008. **60**(2): p. 378-89.

34. Bakker, A., Kirwan, C.B., Miller, M., and Stark, C.E., *Pattern separation in the human hippocampal CA3 and dentate gyrus.* Science, 2008. **319**(5870): p. 1640-2.

35. Schacter, D.L. and Slotnick, S.D., *The cognitive neuroscience of memory distortion.* Neuron, 2004. **44**(1): p. 149-60.

36. Schacter, D.L. and Addis, D.R., *The cognitive neuroscience of constructive memory: remembering the past and imagining the future.* Philos Trans R Soc Lond B Biol Sci, 2007. **362**(1481): p. 773-86.

37. Friedman, W.J., *Time in Autobiographical Memory.* Social Cognition, 2004. **22**(5): p. 591-605.

38. Friedman, W.J., *Comment on "Potential role for adult neurogenesis in the encoding of time in new memories".* Hippocampus, 2007. **17**(6): p. 503-4.

39. Aimone, J.B., Wiles, J., and Gage, F.H., *Potential role for adult neurogenesis in the encoding of time in new memories.* Nat Neurosci, 2006. **9**(6): p. 723-7.

40. Lawrence, J.J. and McBain, C.J., *Interneuron diversity series: containing the detonation--feedforward inhibition in the CA3 hippocampus.* Trends Neurosci, 2003. **26**(11): p. 631-40.

41. Toni, N., Laplagne, D.A., Zhao, C., Lombardi, G., Ribak, C.E., Gage, F.H., and Schinder, A.F., *Neurons born in the adult dentate gyrus form functional synapses with target cells.* Nat Neurosci, 2008.

42. Sahay, A. and Hen, R., *Adult hippocampal neurogenesis in depression.* Nat Neurosci, 2007. **10**(9): p. 1110-5.

43. Hafting, T., Fyhn, M., Molden, S., Moser, M.B., and Moser, E.I., *Microstructure of a spatial map in the entorhinal cortex.* Nature, 2005. **436**(7052): p. 801-6.

44. Hargreaves, E.L., Rao, G., Lee, I., and Knierim, J.J., *Major dissociation between medial and lateral entorhinal input to dorsal hippocampus.* Science, 2005. **308**(5729): p. 1792-4.