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Adult Neurogenesis in the Hippocampus, Potential Roles in Memory and Learning

Bachelor thesis

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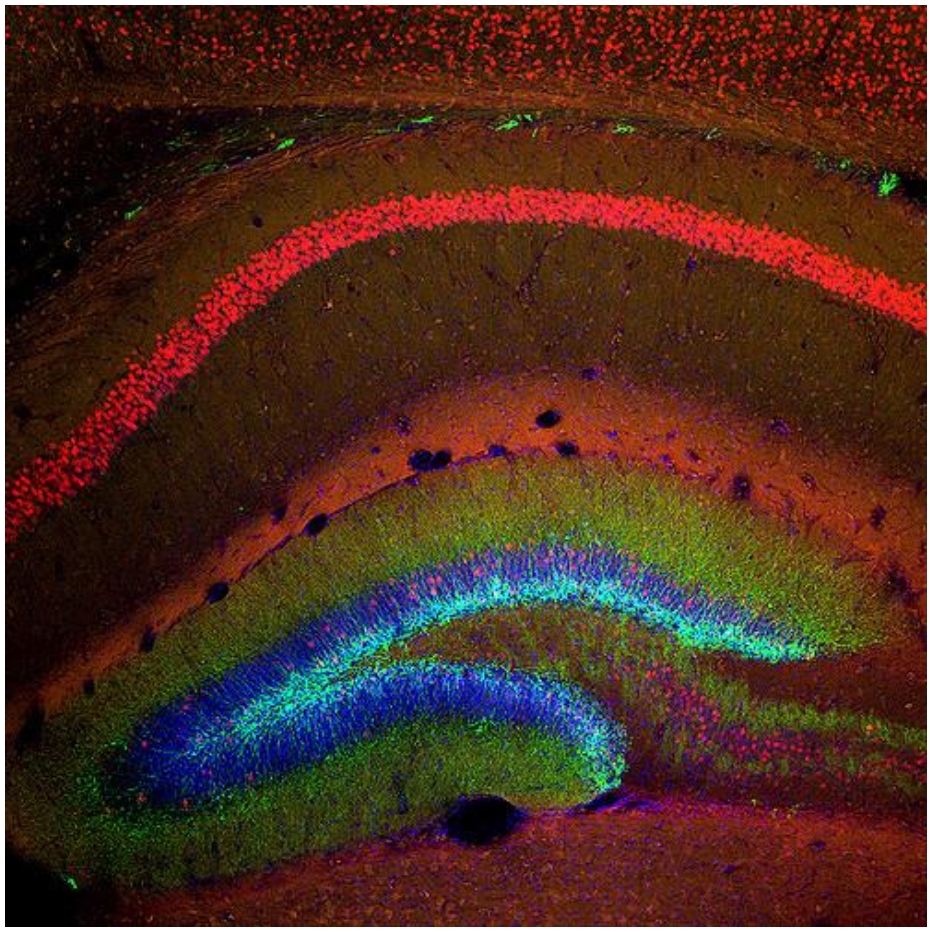

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Abstract

For a long time, we have thought that neurons in the adult brain do not proliferate, since a few decades ago this was proven to be false. The presence of newly generating neurons has been shown a lot of different species of mammals, humans included. It is exclusively present in the olfactory bulb and the dentate gyrus of the hippocampus. The function of adult neurogenesis has yet to be elucidated, and in this thesis, we will discuss several theories and evidence about the function of adult neurogenesis in the hippocampus.

To infer any potential function for neurogenesis it is important to consider Dentate gyrus anatomy and role in memory. The dentate gyrus receives input from the entorhinal cortex through the perforant pathway and projects to the CA3 region of the hippocampus through mossy fibers. It consists of three distinct cell layers. Hippocampus function is to play a role in memory encoding and recalling. The dentate gyrus is thought to be involved in the sparse and orthogonal presentation of processed sensory information encoding, and the separation of similar patterns. Indeed, it was found in behavioral studies that lesions of this region, or a knock-out of an essential receptor affected pattern separation abilities.

Neurogenesis is highly specific for this region, so it would make sense to hypothesize that neurogenesis is necessary to the function of the specific region. Impairment of neurogenesis in multiple ways has been shown to decrease efficacy in spatial separation tasks. On the other hand, augmenting neurogenesis by genetic modification enhances the ability of mice to perform contextual fear discrimination tasks.

Development and maturation of new neurons and factors that influence it could be important clues to unraveling the function and characteristics of neurogenesis. Proliferation of neural stem cells are controlled by GABA signaling, and later maturation, and migration of young neurons are enhanced by surrounding GABA, providing a potential negative feedback loop. Young granule cells die in large numbers around 2-3 weeks of age, and survival is activity dependent through NMDA receptor activation. After functional integration young neurons exhibit a sensitive period.

Evidence leads to potential functioning of neurogenesis in pattern separation. Also, temporal pattern separation and forgetting are hypothesized functions of neurogenesis. And finally, it is thought that neurogenesis plays a role in emotional memories and HPA-axis modulation.

Most of hypotheses mentioned are supported by behavior evidence, neuron characteristics and computational modeling. However, the subject matter makes it inherently difficult to prove direct causal relationships. Future studies and methods may however provide with stronger evidence.

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Introduction

The field of neurogenesis in the adult brain has seen a surge of new research in the past two decades. Before, it was believed that adult circuits were hard wired and new neurons could not be added to them. Renown neurobiologist Santiago Ramon y Cajal in 1913 proposed what has been the central dogma in neurobiology for years: "*In adult centers the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated.*". Until the 1990's it was thought that neurons could only be lost and not regenerated, and that this was the underlying problem of many neurodegenerative diseases. Adding new neurons to an existing network was thought to destabilize encoded information.

However, recently it has been demonstrated that this dogma does not apply to the whole brain. Though most of the brain is static and does only decrease in number of neurons with time, it has been demonstrated in a number of mammals that two areas display neurogenesis into adulthood. (Amrein 2015) This phenomenon seems to be highly specific in these regions of the brain and evolutionary conserved. This postulates the question; what is so special about these brain regions and their function that requires this physiological trait that is found in no other brain regions so far known in any species?

Given this it would come as no surprise that neurogenesis would be found in the human brain. And indeed, (Eriksson et al. 1998) was the first to report adult neurogenesis in humans in the sub granular zone of the dentate gyrus. It has first been shown post mortem, in cancer patients who had been injected with bromodeoxyuridine (BrdU) for diagnostic purposes. BrdU is a nucleoside that is built into DNA during the S-fase. All cells that light up after immunohistostaining for BrdU have been generated after injection. Since this discovery extensive research has been done to discover the functionality of neurogenesis in adult individuals

Neurogenesis has been shown to happen in just two sites in the brain. However, in this thesis the focus will be on what adult human hippocampal neurogenesis looks like and what its effects are on learning and memory. We will look at the current hypotheses regarding the function of adult neurogenesis in the hippocampus and what evidence there is that supports these ideas. Finally, we will discuss the direction that further research may go to further explain this phenomenon.

When and where are new neurons born?

Dentate gyrus anatomy

The hippocampus, named after a seahorse because of its shape, consists of two plate-like structures folded over each other. The Dentate Gyrus (DG) and Cornu Ammonis (CA). The DG is the region where neurogenesis occurs.

The Dentate Gyrus consists of three layers; A molecular layer that is occupied mostly by the dendrites of the granule cells. Fibers from the perforant pathway originating from the entorhinal cortex also end up here. A small number of interneurons also reside in this layer, receiving inputs from several other brain regions.

The granule cell layer contains the densely packed granule cells. Granule cells are the cells that are generated throughout life. Bordering the granule cell layer lies the polymorphic cell layer which constitutes of mossy cells amongst others. These mossy cells project to the CA3 region of the hippocampus through the mossy fibers [figure 1] (Amaral, Scharfman, and Lavenex 2007).

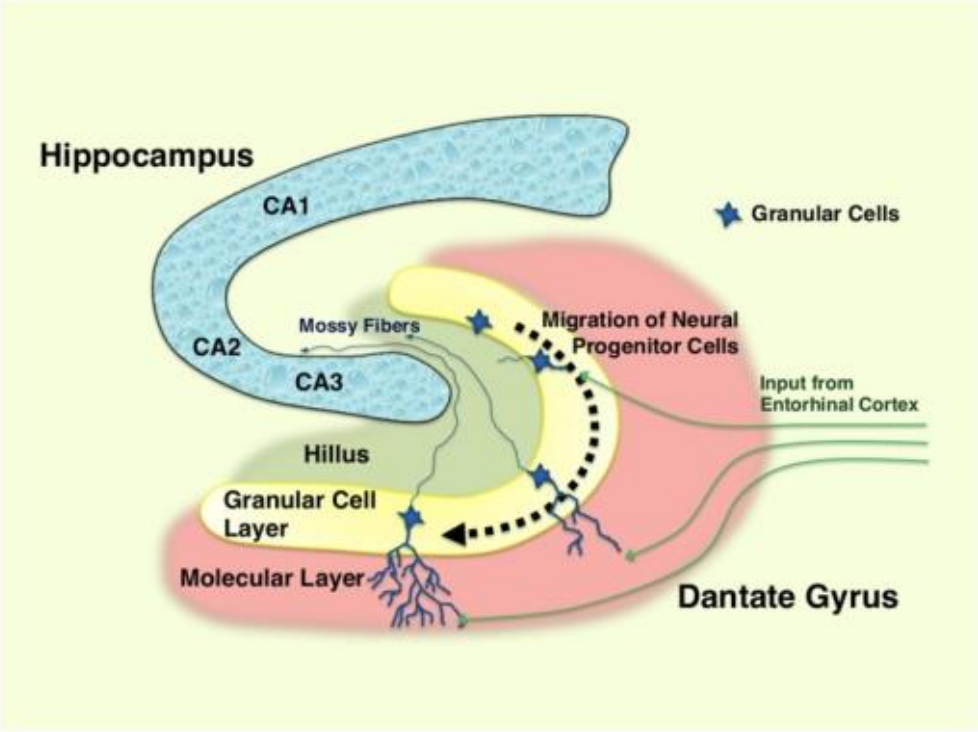

Figure 1. Hippocampus anatomy with migration of young neurons illustrated, image from (Kino 2015)

Dentate gyrus function

The brain region wherein the Dentate gyrus functions is the hippocampus, which has been shown to have a role in encoding and recalling of memory sequences. Memory sequences consist of a wide range of processed sensory inputs which are time and context dependent. For efficient recalling and association processes, these inputs need to be arranged in sparse patterns (Petrantonakis and Poirazi 2014).

(Rolls 1996) inferred from computational modelling that an important function of the DG is to separate patterns (i.e. spatial contexts). This would be achieved by creating different sparse and orthogonal presentations from a great amount of sensory information which could facilitate the association task performed by the CA3 region. These unique patterns are vital to the functioning of the hippocampus, thus if patterns were to overlap in an individual it would affect their ability to perform spatial discrimination tasks.

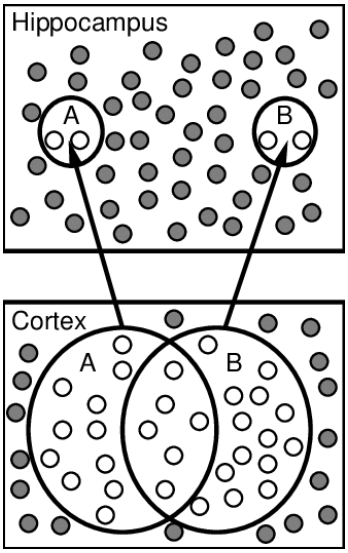

Figure 2. Theoretical rendering of contexts represented in enthorhinal cortex and the hippocampus, pattern separation took place making the pattern of activity smaller and not overlapping. From (Randall, O'Reilly, and Rudy 2001)

Another approach to determine function performed by a certain area are lesion studies. These have shown that the hippocampus may indeed be involved in context discrimination processes. It has been shown that after colchicine lesioning of the dorsal DG, it was more difficult for rats to discriminate similar object-place contexts for rewards (Lee and Solivan 2010). In another study it was shown that knocking out an essential subunit of the NMDA receptor exclusively in the DG resulted in disability to distinguish two similar contexts in a fear-context association task (McHugh et al. 2007). (Morris, Weeden, et al. 2013) showed that without a functional dorsal DG, rats were unable to associate olfactory cues with a certain context. Deficiencies in pattern separation resulted from lesions seem to be observed more readily when the contexts used in the experiments are more alike. Different kinds of lesions in the DG interfere with the ability to distinguish contexts from each other based on several sensory inputs.

Furthermore, we know the DG receives sensory inputs from the entorhinal cortex perforant pathway. It has been shown that cells within the hippocampus are stimulated by most sensory inputs, from auditory to

olfactory inputs. This further supports that an important function of the dentate gyrus is to process all kinds of sensory information, creating unique patterns of activity correlating to separate events. (Kesner 2013)

Could this specific function of the DG require the generation of neurons throughout life? Perhaps the function cannot be accomplished by a network that is more hardwired and perhaps the properties of the newborn cells are essential to fulfill this function.

Behavioral studies linked to neurogenesis

As previously mentioned, the hypothesized function of the DG is to produce separated representations of events that have close similarity to each other. Therefore, it could be that the neurogenesis that is displayed so specifically in this area contributes to this function. To demonstrate a causal effect of neurogenesis on pattern separation, experiments have been done observing the behavioral effects of enhanced or suppressed neurogenesis.

Effects of impaired neurogenesis on learning

Approaches that have been used often to test pattern separation abilities are Morris water maze tests and fear-context discrimination tests. Behavioral studies show that impairment of neurogenesis decreases the ability to discriminate fear contexts in rodents. In a study done by Tronel et al. (2012) a transgenic mouse model was used where neurogenesis ablation could be induced. The neurogenesis impaired mice were unable to discriminate between two similar contexts after training where one context was paired with foot shocks.

In other experiments, Clelland and collegues (2010) showed that after impairment of neurogenesis via irradiation, test groups compared to controls were unable to correctly perform spatial discrimination tasks where stimuli were presented with little spatial separation, but not when stimuli were more widely separated in space. One of the behavior tests that was used was a radial arm maze where effects of ablated neurogenesis were only observed when tested arms were near one another and mice, and therefore mice had to distinguish one from another. This served as a model for pattern separation as arms that are closer to each other share overlapping features.

Another test that was used was also spatial dependent, but mice did not need to navigate a physical space. In a cage a touch screen was placed displaying 2 out of 5 boxes that were lit. The mice were rewarded when touching the right box. Irradiated mice were unable to learn this task correctly when the boxes were closer to each other. So, it has been consistently shown in multiple test methods that ablation of neurogenesis in the DG impairs discrimination tasks where contexts are more similar or closer to each other. Also, this effect is subtle and depends heavily on the testing parameters, which is why some studies have failed to find an effect.

In contrast to spatial pattern separation, it was hypothesized by Aimone, Wiles, and Gage(2006), that neurogenesis might contribute to temporal association processes, i.e. association events that are close in time to each other. To study this a new behavior study paradigm was created to test for the temporal separation abilities (Morris, Curtis, et al. 2013). This behavior study shows that after colchicine lesion of the dentate gyrus, mice are less able to associate events or contexts that were close in time to each other. This hypothesis will further be discussed in a following chapter where we discuss potential functions of the dentate gyrus.

Effects of increased neurogenesis on learning

Not only does ablating neurogenesis impair spatial discrimination tasks, augmenting it has been found to improve performance in these tasks (Sahay et al. 2011). In this study a genetically modified mouse was used

with enhanced survivability of adult born DG granule neurons as to increase the number of newborn neurons in the circuit. This increased efficiency of mice to perform contextual fear discrimination tests where contexts were alike.

Effects of different cell populations on learning

The effects of neurogenesis ablation or augmenting on pattern separation abilities has been shown not to be dependent on the functioning of old granule cells. Transgenic mice, which had input from older mature granule cells blocked, leaving mostly input from young sensitive granule cells, kept normal performances of pattern separation tasks (Nakashiba et al. 2012). Pattern separation abilities were tested through contextual fear conditioning where a fear response was associated with a context, and fear response was measured in a very similar yet on some points distinct context. However, these mice had their performance impaired in pattern completion tasks. These tasks include recall of a contextual fear conditioning test and a standard Morris water maze test. The idea is that for mice to recall and associate the context with fear or reward, an appropriate representation of this context must be available. Pattern completion in this regard means that separate cues of a certain context are readily available and recall of contexts is done rapidly. This study further suggests that the pattern separation behavior observed is dependent on young granule cell, and not the functioning of all of the dentate gyrus.

To conclude, behavioral studies show results that align with the theorized function for the dentate gyrus from computational models. Not only when neurogenesis is ablated, but also augmented, influences on pattern separation abilities have been observed.

Developmental time course of new neurons

To assess any potential function or purpose for neurogenesis in the DG, it is important to consider the processes influencing proliferation, migration and survival of these newborn cells. As discussed in the previous chapter, the rate of neurogenesis has influence on pattern separation abilities, however the mechanisms leading to this gain of function are not clear. To further investigate how these processes might affect neurogenesis and how this may contribute to the overall function, the next paragraphs are dedicated to the developmental time course of young neurons in the DG and what we know may influence each step in the process of functional integration of the neurons.

Neural stem cell proliferation

The site of neurogenesis, where the neural stem cells are located, is in the sub granular zone of the DG. Here neural stem cells reside in a largely quiescent population. They can be recruited to non-radial cells that can proliferate, neuroblasts. Whether they are recruited is dependent on their niche environment. The quiescent neural stem cells in this layer have been shown to express glial fibrillary acidic protein (GFAP), which is normally expressed by glial cells. They also exhibit cellular characteristics of astrocytes (Seri et al. 2001).

An important question to answer is how neural stem cells are recruited from the quiescent population and what factors control this proliferation. Although multiple niche-derived and intrinsic factors influence proliferation and differentiation of neural stem cells (Braun and Jessberger 2014; Zhao, Deng, and Gage 2008), NMDA activation of neural stem cells form an important part of proliferation inducing factors (Joo et al. 2007). Especially in the context of controlling neurogenesis on the level of the circuitry, because it tells us that neurogenesis is activity dependent.

Proliferation is not only activity dependent, but also sensitive to inhibition by GABA, as GABA has been identified to play an important role in regulating the tempo of neurogenesis. GABA is typically characterized as a neurotransmitter that has an inhibitory role in the central nervous system because it hyperpolarizes mature neurons. However, because of high Cl- concentrations in immature neurons and neural progenitor cells it has a depolarizing effect. GABA has been shown to have inhibitory effects on the proliferation of the quiescent neural progenitor cells of the sub granular zone (Ge et al. 2007; Giachino et al. 2014; Xiuxin Liu, Qin Wang, Tarik F. Haydar 2005).

Differentiation and migration

After division of the non-quiescent neuroblasts, most of the cells differentiate into new neurons, and a small part becomes glial cells. The radial glial cells have large processes that extend to the granular cell layer and into the molecular layer. They have lateral extensions that run through the granular cell layer which function as scaffolding for the migrating neuroblasts (Ihunwo, Tembo, and Dzamalala 2016)(G. Yang et al. 2015).

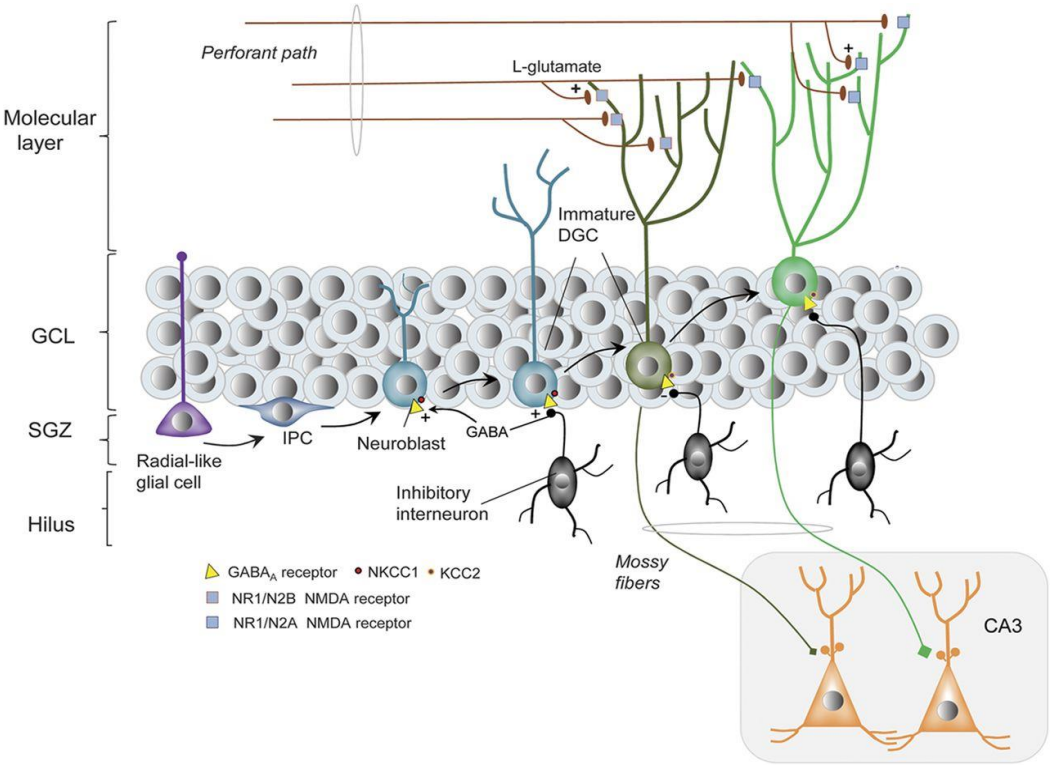

Figure 2. migration of neurons and differentiation from neural progenitor cells into young granule cells. Image from (Benarroch 2013)

After migration, the newborn neurons can integrate into functional networks. However, there is another step controlling the number of newborn neurons. It has been shown that a large proportion of newborn neurons die in their immature state. High frequency stimulation of the perforant pathway fibers causes more neurons to survive, by causing long-term potentiation (LTP) which is the strengthening of synapses between neurons based on activity. Kitamura et al. 2010 found that the window of this critical period of LTP dependent survival is between 7-10 days after birth of the neurons. It is thought that this is a form of use it or lose it plasticity where the newly integrated neurons must be contributing to the existing network to survive.

So as discussed above GABA has a controlling role in proliferation, however that is not the only regulating role it plays in the neurogenesis process. Young granule cells follow a developmental path where initially they

are depolarized by surrounding GABA as they do not have synapses yet for direct connections to GABAergic neurons, this depolarizing causes differentiation of new-born neurons. They then receive excitatory inputs from GABAergic neurons through synaptic activity, and finally they are activated by glutamatergic inputs, just like adult granule cells (figure 2)(Ge et al. 2007). When neurons become mature GABA will become a inhibitory neurotransmitter due to the increase in Cl- channels on the cell membrane and the decrease of intracellular Cl-. The depolarization of immature neurons by GABA turns into hyperpolarization after about 2 weeks of age.

The early tonic GABA activation of immature neurons has been shown to play a role in synaptic integration of the neurons in functional networks (Ge et al. 2006). If GABA's influence is restricted by a knock-out of a downstream chloride channel, it leads to significant defects in maturation of dendritic spines of new neurons. Which is another example of an activity dependent regulation of adult-born neurons that will eventually integrate into a functional network.

So, to summarize the effects of GABA on the different processes, it has an inhibiting effect on proliferation, yet a depolarizing effect on immature neurons. This depolarization influences the differentiation and functional integration of young neurons. It is of course noteworthy that GABA signaling in the SGZ mostly comes from interneurons. Also, the number of immature neurons influence the interneuron activity, because more excitatory synaptic contacts with these cells have been established (Ikrar et al. 2013). This evidence together points toward a potential negative feedback loop controlling proliferation depending on the number of immature neurons.

Survival and sensitive period

Besides GABA, another neurotransmitter that influences the different processes in neurogenesis is glutamate. Tashiro et al. 2006 showed NMDA receptors regulate survival of 2-3-week-old neurons that have formed synapses. By creating an NMDA receptor knock-out exclusively for immature neurons they showed that the survival of immature neurons is activity-dependent and cell-specific.

Not only do glutamatergic affect the survival of new-born neuron they also have an important functional significance when neurons are still young. Between 4 and 6 weeks of age, newborn neurons are in a sensitive period where they are more easily excited. (Ge et al., 2007) The window of time identified as the sensitive period is between 4 weeks and 6 weeks of age. This heightened sensitivity seems to be regulated by expression of NR2B containing NMDA receptors. This increases LTP amplitude and reduces LTP induction threshold. This is of importance because it shows a possible clue to the functionality of immature neurons, because they seem to be important during this sensitive period.

To summarize, proliferation, differentiation, maturation and survival of new-born neurons in the DG happen in an activity dependent manner. GABA-signaling plays an important role and has potential of being involved in a negative feedback loop on proliferation, although this is yet to be proven. And finally, neurons show a sensitive period where their influence on the neural circuitry is heightened, providing an important clue to possible functionality.

Controlling neurogenesis

To gain more insight on potential functions neurogenesis might have, it is important to consider the extrinsic and environmental factors that influence neurogenesis, as these may be adaptations that have functionality as well. Behavioral studies showed that several environmental or extrinsic factors affect neurogenesis, the most important being stress, exercise and enriched environment or learning (Garthe, Roeder, and Kempermann 2016; Kempermann, Kuhn, and Gage 1997; Van Praag 2008). To further explain results found in behavior studies it is important to unravel the molecular mechanisms involved. In the next paragraphs the progress in understanding these mechanisms will be reviewed.

The multiple factors that can increase neurogenesis that can act on different steps in the process. It is important in research to distinguish effects on neurogenesis into effects on proliferation rate, survival and integration, because this can all influence number of adult born functional integrated neurons in the granule cell layer.

Stress and stress hormones

Stress hormones called glucocorticoids have been shown to have decreasing effects on numbers of new born neurons. (Gould et al. 1992), this has been shown in acute stressors (Lagace et al. 2010) as well as in chronic stress (Czéh et al. 2002). However how stress hormones can reduce neurogenesis has yet to be fully understood. The question is how glucocorticoids influence neurogenesis, and on what steps in the process does this influence take place.

It has been shown that very few mitotic cells and young neurons in the dentate gyrus express glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) (Garcia et al. 2004). After 4 weeks of age neurons have been positively labeled with these receptors (Cameron, Woolley, and Gould 1993). This suggests that the effects of stress and stress hormones on proliferation and survival is an indirect one. And as it is known that the rate of neurogenesis is activity dependent it would not be inconceivable that afferent pathways activated by glucocorticoids influence the neural progenitor cells or their neurogenic niche. However, more research is needed to determine this.

Not only do glucocorticoids seem to influence neurogenesis, Snyder, Soumier, Brewer, Pickel, & Cameron, 2011, showed that neurogenesis might also influence the HPA axis itself. After inhibition of neurogenesis through radiation and genetic manipulation, mice show slower recover of glucocorticoid levels, suggesting a role of neurogenesis in HPA axis modulation. Neurogenesis inhibited mice showed more behavioral signs of depression, anhedonia and increased behavioral despair during forced swim test.

It is important to note that although stress and HPA activity overall seem to decrease cell proliferation in the dentate gyrus, it has also been shown that the low concentrations of corticosteroids can increase cell proliferation. MR's have a higher affinity for glucocorticoids, so low concentrations act as a MR agonist while higher concentrations also activate GR's (Anacker et al. 2013). This concentration dependent activity could be the reason stressors might be the reason that paradoxical effects on neurogenesis have been observed, and why some stressors might have no effect (Hanson et al. 2012), or an increasing effect. This theory is further supported by a study done one mice that were genetically modified to overexpress MR's in the hippocampus.

This overexpression protected against the decrease of neurogenesis after chronic stress (Kanatsou et al. 2015). It should however be researched whether glucocorticoids can have different effects on neurogenesis directly, or whether this is due to confounding factors or differences in experimental setups.

Activity, enriched environment and exercise

The consensus on neurogenesis is that it is activity dependent. Lesions in the fornix and other pathways have a diminishing effect on neural cell proliferation, whereas as previously discussed physical exercise has positive effects. Running has been shown to increase neural progenitor cell proliferation and thereby increasing neurogenesis. (Van Praag 2008)

It has been shown that living in an enriched environment increases the number of new neurons in the DG in rodents. This seems to be contributable to enhanced survivability. In (Kempermann et al. 1997) mice were either living in an enriched environment or a control cage. They were injected with BrdU for 40 days after which a subset of mice was sacrificed. The remaining mice lived for another 4 weeks to allow for maturation of the newborn cells, and several neurons would not survive this extra period. Subsequently the number of young neurons in the brains of the mice of the first group were compared to the amount in the group of mice that were sacrificed 4 weeks later. The group of mice that lived in the enriched environment showed more young neurons that survived compared to the control group, meaning that it increased the survivability of the neurons.

In another experiment, Garthe et al. (2016) showed that enriched environment improves spatial learning and flexibility in Morris water maze tasks and that this is dependent on neurogenesis. After ablation of neurogenesis with temozolomide, the performance enhancing effects of an enriched environment were absent.

Exercise is also linked to increased numbers of proliferating neurons in the dentate gyrus (van Praag, Kempermann, and Gage 1999). Exercise has different potential ways to influence neurogenesis, as exercise can influence multiple neurotransmitters, monoamines, and growth factors. However, here we will highlight one important possible mechanism by which higher proliferation of neurons might be achieved. According to Molteni, Ying, and Gómez-Pinilla (2002), genes associated to the glutamatergic system are upregulated after periods of exercise, more specifically NMDA receptor 2A and 2B subunits. NMDA receptors play an important role in mediating rates of proliferation in the dentate gyrus (Joo et al. 2007). Even more interestingly a connection has been shown between exercise increased levels of NMDA activation and neurogenesis. When mice were lacking the NMDA receptor ε1 subunit, exercise no longer had any positive effects on proliferation and survival (Kitamura, Mishina, and Sugiyama 2003).

There is a difficulty in determining a causal relationship between several extrinsic effects and neurogenesis due to the complex effects exercise, stress and enriched environment can bring with them. There can be an overlap and interaction between these effects. Enriched environments in these studies, for example, not only entail a larger cage, more toys and playmates for rats, but usually also a running wheel. Also, increasing the amount of exercise has effects on HPA axis activity and receptor numbers (Fediuc 2006). In a system this complex these interactions may act as confounding factors, and moreover causal relations may be difficult to point out as exercise and enriched environment cause an array of effects.

Potential functionality of adult hippocampal neurogenesis

In previous chapters, we have reviewed behavior studies and adult born neuron characteristics and development. This chapter will try to explain the hypotheses about neurogenesis function and relate this to previous chapters.

Pattern separation and sparse representations

From behavior studies and theorized role of the dentate gyrus, an increase in neurogenesis is thought to enhance pattern separation, and pattern separation functioning is dependent on the presence of new born neurons. The sensitive period that neurons undergo after maturation however, could theoretically create an overlap between patterns, because all contexts have a higher probability to activate these sensitive neurons.

To further investigate pattern separation abilities beyond the limitations of behavior study, Ikrar et al. (2013) looked at how neurogenesis affects patterns of activation in the DG. This was achieved by using fast voltage-sensitive dye imaging, laser photostimulation and electrical stimulation. They show that with increased functional young neurons, activation is decreased with a reduced spread. And with impaired neurogenesis the opposite was observed; an increase in activation with widened spread.

This seems paradoxical, as an increase in neurons which have lower thresholds for activation and long-term potentiation would expectedly result in more activation overall and a wider spread pattern. The reason why this is not happening and increased active neurons result in sparse representations is hypothesized by Mcavoy, Besnard, and Sahay (2015) to be due to interaction with inhibiting mossy cells and interneurons. And indeed, in the same study it was shown that when they then investigated the effects of neurogenesis on GABAergic inhibition and connectivity of adult-born neurons to inhibiting interneurons, neurogenesis had no effects on post-synaptic currents or inhibitory tone, but there was an increase in connectivity to interneurons with increased adult-born neurons (Ikrar et al. 2013).

As discussed earlier according to computational theory, for the CA3 to perform its associative task, it needs to receive sparse orthogonal representations from sensory inputs of the entorhinal cortex (Kesner and Rolls 2015). The way the dentate gyrus is thought to achieve this according to this paper, is by acting as a competitive net that ensures that only the most active neurons are left after competitive feedback inhibition. The GABAergic signaling and survival dependent integration might provide such a competitive network.

Potential role in forgetting

In a study discussed earlier it was suggested that old granule cells are responsible for pattern completion and young granule cells are responsible for pattern separation (Nakashiba et al. 2012). Pattern completion here practically means being able to remember specific details from a context. Theoretically old granule cells encode for consolidated older memory engrams in a stable network. Changes to this deterministic network would be able to compromise already consolidated memories (Knobloch and Jessberger 2011).

As discussed when young neurons enter the DG network, they show an increased sensitivity for activation and LTP, which is thought to enable pattern separation. The addition of more young neurons to the DG could compromise older memory engrams to better encode new information. Therefore, a trade-off is thought to exist between the pattern completion and pattern separation, dependent on the balance of new-born granule cells and old mature granule cells (Guskjolen, Epp, and Frankland 2017).

Temporal pattern separation

Another hypothesized function of the dentate gyrus and neurogenesis is temporal pattern separation, where events that happened around the same time are associated with each other. Young granule neurons only show a temporary sensitive phase and new neurons are born throughout adult life. This might cause certain periods of time to be linked together through this linked neural activity (Aimone, Wiles, & Gage, 2006). How memory engrams of different contexts are formed and temporally associated is schematically represented in figure 2.

Empirically this makes sense as well, as soon as you are reminded of a memory like a certain song or food you ate in a certain period of time, other memories which seem otherwise unrelated resurface as well. In the previous chapter we discussed a learning paradigm created to test abilities to associate events that took place close in time, and evidence was shown that the DG is linked to this function (Morris, Curtis, et al. 2013).

A problem with this hypothesis as suggested in (Friedman 2007), might be that this assumes an automatical association of events that happened during the same time and that this association did not occur because of some logical relation between the two events. For example, the smell of a certain tea might trigger someone to think about studying for a specific course. Here the two events, drinking the tea and studying served the same goal; preparing for an exam. The person starts making the tea to sit down and study, there a reason why these events would be associated without needing the explanation of an automatic association based on time only. This idea should be excluded by creating a paradigm for testing association between events close in time that are unrelated.

So, to summarize, the hypothesis that temporal association is an important function of neurogenesis makes sense based on the properties of young neurons. However, the behavioral evidence is still sparse and a causal relationship between this seemingly emergent property of neurons and temporal associations.

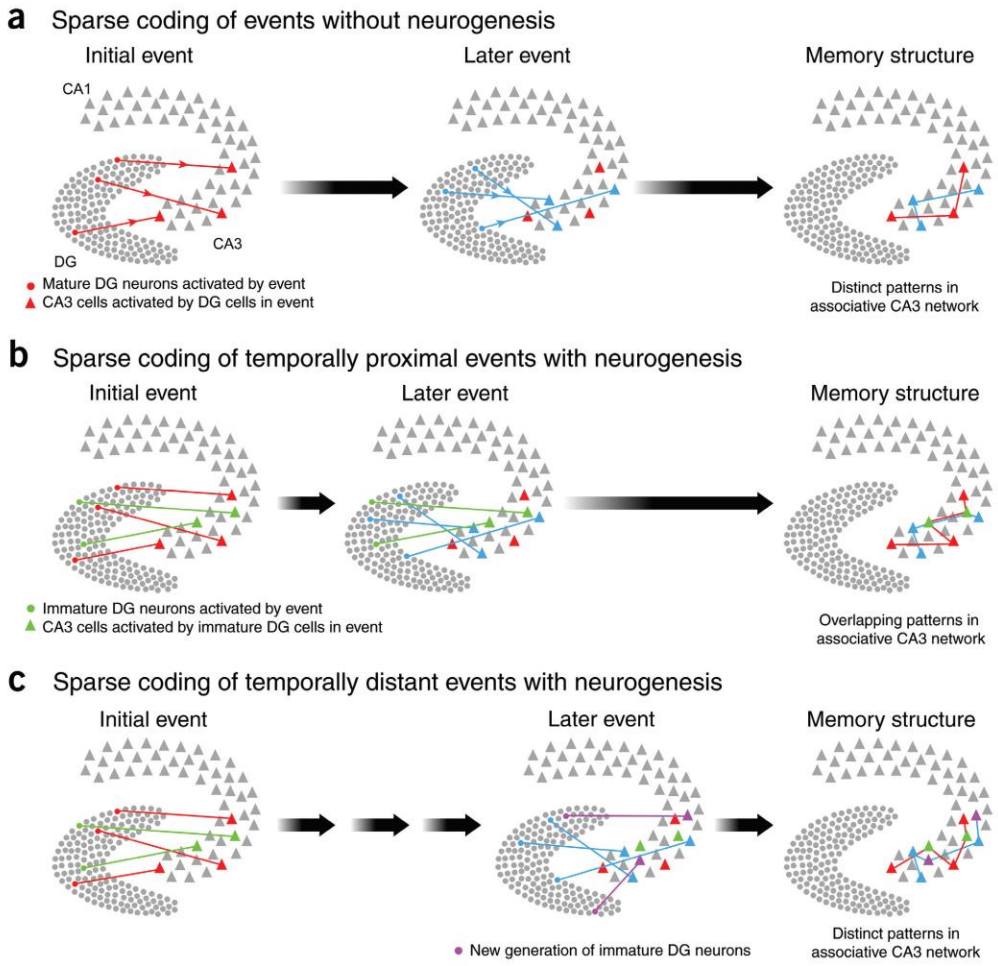

Figure 2. neurogenesis as possible mechanism for separating memory based on time. From (Aimone et al. 2006)

Emotional memory consolidation and HPA-axis modulation

Theories about functionality of neurogenesis in the dentate gyrus so far have been giving possible explanations of the characteristics of new born neurons and how they can be adaptive. However, the interactions of neurogenesis and stress responses that have been mentioned in the chapters about behavior and control of neurogenesis have not been explained in a similar matter. The following paragraphs therefore propose a function neurogenesis has in emotional memory forgetting by HPA-axis modulation.

There is an interaction where glucocorticoid concentrations affect neurogenesis rate and neurogenesis modulates HPA-axis activity as discussed in previous chapters (Snyder et al. 2011). Also emotional responses are used as input for memory formation (Leal et al. 2014). This leads to an interesting theory, because in times of high stress neurogenesis is downregulated. This results in contexts being more generalized. Individuals in times of high stress will now view more contexts as stressful and act more vigilantly. This can in some cases be an adaptive strategy. Of course, the other side of this story is that as a maladaptation this can result in chronic stress (Baptista and Andrade 2018). A consequence of decreased neurogenesis might also be that as discussed before older memory engrams are less easily forgotten. This can result in stressful experiences to be harder to forget, which enables chronic stress (Baptista and Andrade 2018).

This theorized double effect on chronic stress also predicts that decreased neurogenesis and stress increases chance of developing depression. The effects and connection between chronic stress, stress coping

mechanisms and depression have been well established (L. Yang et al. 2015). However, a causal relationship between decreased neurogenesis and development of depression should be shown to further support this theory. While indeed in some studies neurogenesis ablation results in depressive-like behaviors (Petrik, Lagace, and Eisch 2012; Snyder et al. 2011), results have been conflicting (Petrik et al. 2012).

A clue that also led to this hypothesis is the interaction of adult neurogenesis with antidepressants. Fluoxetine was able to reverse the decrease in neurogenesis after acute stress (Bessa et al. 2009; Malberg and Duman 2003). This suggested that neurogenesis plays a role in the development of stress and anxiety disorders and depression. However, studies have shown that the effects of antidepressants on depressive-like behavior are not neurogenesis dependent (Bessa et al. 2009). This suggests that an increase of neurogenesis after treatment with antidepressants does not have a role to play in modulation of emotion and stress induced depression.

So even though evidence for causal effects of neurogenesis on depression are scarce and somewhat conflicting, interactions between neurogenesis and stress seem to play important roles in memory and HPA-axis modulation. This warrants more research into causal effects and mechanisms of neurogenesis to stress and emotional memories. Furthermore, research is necessary to prove the idea that stress causally modulates forgetting of other stressful memories as this is just based on modelling and stress correlating to pattern separation abilities and neurogenesis.

Discussion

To assess how manipulations in neurogenesis influence pattern separation, it is necessary to find a behavioral paradigm that tests this ability specifically. It is difficult to set up a test that is sensitive for one functional aspect that may be attributed to adult neurogenesis so that an effect may be observed. For this reason, slight differences in experimental set-up might lead to missing the effects of manipulated neurogenesis on behavior.

If an effect is found however, it is important to make a distinction between the found behavioral aspect and the function the researcher would like to ascribe to it. For example, if a rat is very good at discriminating similar contexts by associating the right ones with fearful experiences, it would be jumping to conclusions to say that pattern separation is enhanced. This is because this term refers to the computational theoretical process of creating distinct and sparse representations of similar events, and this cannot be demonstrated in a behavior test.

However, even though speaking of pattern separation based on behavioral observations may not be very accurate, it does stimulate thinking about how neural representations affect the outcome of behavior. It also a way of connecting the computational work that has been done on hippocampal and DG function to the biological context. As these behavioral studies can provide some evidence to back up the hypothesized function. It is good to keep this in mind though, and eventually it would be nice to demonstrate that neuronal representations in the DG really are sparse and distinct even though events are similar.

To solve many limitations posed by behavior studies, better ways to quantify the amount of pattern separation are necessary. Liu et al. (2014) Have been working on a method to identify and even manipulate specific memory engrams in DG granule cells. It seems they have successfully created a false memory by artificially learning mice to act fearful in a context where no foot shocks ever happened. Meaning they have successfully identified the neurons of the DG that are activated and encode a specific context.

They did so by infecting the cells with a virus expressing channelrhodopsin on the c-fos promotor, an immediate early gene expressed by active neurons (Ramirez et al. 2013). Channelrhodopsing is a light sensitive ion channel that causes depolarization once light hits it. Neurons that have expressed the c-fos after a fearful

event could then later be triggered to depolarize, even though the context they were in was not associated before with fear.

This method could be applied to identifying memory engrams of specific contexts and investigate if patterns overlap or not. Instead of expressing an ion channel to later activate a specific memory engram, perhaps a fluorescent protein could be expressed. This provides a unique opportunity to investigate representations and their spread, and to test hypotheses based on neural circuitry level.

Concluding remarks

Several promising theories exist that try to explain neurogenesis functionality. These theories were formed from computational work and previous knowledge on hippocampal functions and are further supported by behavior studies and details about development and characteristics of young granule cells. However, there is an inherent difficulty in trying to explain the function of a specific brain region in vivo, as it is difficult to determine causality or effects flowing from dependencies on other brain regions. With new methods becoming available for research, it may be possible to test hypothesis on the level of neuronal circuitry, as the hypotheses discussed here predict on this level.

References

Aimone, James B., Janet Wiles, and Fred H. Gage. 2006. "Potential Role for Adult Neurogenesis in the Encoding of Time in New Memories." *Nature Neuroscience* 9(6):723–27.

Amaral, David G., Helen E. Scharfman, and Pierre Lavenex. 2007. "The Dentate Gyrus: Fundamental Neuroanatomical Organization (Dentate Gyrus for Dummies)." *Progress in Brain Research* 163:3–22.

Amrein, Irmgard. 2015. "Adult Hippocampal Neurogenesis in Natural Populations of Mammals." 7(5):a021295. Retrieved (http://cshperspectives.cshlp.org/lookup/doi/10.1101/cshperspect.a021295).

Anacker, Christoph et al. 2013. "Glucocorticoid-Related Molecular Signaling Pathways Regulating Hippocampal Neurogenesis." *Neuropsychopharmacology* 38(5):872–83. Retrieved (http://dx.doi.org/10.1038/npp.2012.253).

Baptista, Pedro and José P. Andrade. 2018. "Adult Hippocampal Neurogenesis : Regulation and Possible Functional and Clinical Correlates." 12(June):1–23.

Benarroch, Eduardo E. 2013. "Adult Neurogenesis in the Dentate Gyrus: General Concepts and Potential Implications." *Neurology* 81(16):1443–52.

Bessa, J. M. et al. 2009. "The Mood-Improving Actions of Antidepressants Do Not Depend on Neurogenesis but Are Associated with Neuronal Remodeling." *Molecular Psychiatry* 14(8):764–73. Retrieved (http://dx.doi.org/10.1038/mp.2008.119).

Braun, S. M. G. and S. Jessberger. 2014. "Adult Neurogenesis: Mechanisms and Functional Significance." *Development* 141(10):1983–86. Retrieved (http://dev.biologists.org/cgi/doi/10.1242/dev.104596).

Cameron, H. A., C. S. Woolley, and E. Gould. 1993. "Adrenal Steroid Receptor Immunoreactivity in Cells Born in the Adult Rat Dentate Gyrus." *Brain Res* 611(2):342–46. Retrieved (http://www.sciencedirect.com/science/article/pii/000689939390524Q).

Clelland, C. D. et al. 2010. "A Functional Role for Adult Hippocampal Neurogenesis in Spatial Pattern Separation." *Science* 325(5937):210–13.

Czéh, Boldizsár et al. 2002. "Chronic Psychosocial Stress and Concomitant Repetitive Transcranial Magnetic Stimulation: Effects on Stress Hormone Levels and Adult Hippocampal Neurogenesis." *Biological Psychiatry* 52(11):1057–65.

Eriksson, Peter S. et al. 1998. "Neurogenesis in the Adult Human Hippocampus." 4(11):1313–17.

Fediuc, S. 2006. "Effect of Voluntary Wheel Running on Circadian Corticosterone Release and on HPA Axis Responsiveness to Restraint Stress in Sprague-Dawley Rats." *Journal of Applied Physiology* 100(6):1867–75. Retrieved (http://jap.physiology.org/cgi/doi/10.1152/japplphysiol.01416.2005).

Friedman, William J. 2007. "Comment on "' Potential Role for Adult Neurogenesis in the Encoding of Time in New Memories ."""" 504:503–4.

Garcia, Ana, Barbara Steiner, Golo Kronenberg, Anika Bick-Sander, and Gerd Kempermann. 2004. "Age-Dependent Expression of Glucocorticoid- and Mineralocorticoid Receptors on Neural Precursor Cell Populations in the Adult Murine Hippocampus." *Aging Cell* 3(6):363–71.

Garthe, Alexander, Ingo Roeder, and Gerd Kempermann. 2016. "Mice in an Enriched Environment Learn More Flexibly Because of Adult Hippocampal Neurogenesis." *Hippocampus* 26(2):261–71.

Ge, Shaoyu et al. 2006. "GABA Regulates Synaptic Integration of Newly Generated Neurons in the Adult Brain." *Nature* 439(7076):589–93.

Ge, Shaoyu, Dennis A. Pradhan, Guo li Ming, and Hongjun Song. 2007. "GABA Sets the Tempo for Activity-Dependent Adult Neurogenesis." *Trends in Neurosciences* 30(1):1–8.

Giachino, C. et al. 2014. "GABA Suppresses Neurogenesis in the Adult Hippocampus through GABAB Receptors." *Development* 141(1):83–90. Retrieved (http://dev.biologists.org/cgi/doi/10.1242/dev.102608).

Gould, E., H. a Cameron, D. C. Daniels, C. S. Woolley, and B. S. McEwen. 1992. "Adrenal Hormones Suppress Cell Division in the Adult Rat Dentate Gyrus." *The Journal of Neuroscience* 12(September):3642–50.

Guskjolen, Axel, Jonathan R. Epp, and Paul W. Frankland. 2017. "Hippocampal Neurogenesis and Forgetting." *The Hippocampus from Cells to Systems: Structure, Connectivity, and Functional Contributions to Memory and Flexible Cognition* 95–121.

Hanson, Nicola D., Michael J. Owens, Katherine A. Boss-williams, and Jay M. Weiss. 2012. "Several Stressors Fail to Reduce Adult Hippocampal Neurogenesis." 36(10):1520–29.

Ihunwo, Amadi O., Lackson H. Tembo, and Charles Dzamalala. 2016. *The Dynamics of Adult Neurogenesis in Human Hippocampus*.

Ikrar, Taruna et al. 2013. "Adult Neurogenesis Modifies Excitability of the Dentate Gyrus." *Frontiers in Neural Circuits* 7(December):1–15. Retrieved (http://journal.frontiersin.org/article/10.3389/fncir.2013.00204/abstract).

Joo, J. Y. et al. 2007. "Activation of NMDA Receptors Increases Proliferation and Differentiation of Hippocampal Neural Progenitor Cells." *Journal of Cell Science* 120(8):1358–70. Retrieved (http://jcs.biologists.org/cgi/doi/10.1242/jcs.002154).

Kanatsou, Sofia et al. 2015. "Overexpression of Mineralocorticoid Receptors Partially Prevents Chronic Stress-Induced Reductions in Hippocampal Memory and Structural Plasticity." *PLoS ONE* 10(11):1–17.

Kempermann, Gerd, H. Georg Kuhn, and Fred H. Gage. 1997. "More Hippocampal Neurons in Adult Mice Living in an Enriched Environment." *Nature* 386(6624):493–95.

Kesner, R. P. and E. T. Rolls. 2015. "A Computational Theory of Hippocampal Function, and Tests of the Theory: New Developments." *Neuroscience and Biobehavioral Reviews* 48:92–147.

Kesner, Raymond P. 2013. "An Analysis of the Dentate Gyrus Function." *Behavioural Brain Research* 254:1–7. Retrieved (http://dx.doi.org/10.1016/j.bbr.2013.01.012).

Kino, T. 2015. "Hippocampus DG."

Kitamura, T., M. Mishina, and H. Sugiyama. 2003. "Enhancement of Neurogenesis by Running Wheel Exercises Is Suppressed in Mice Lacking NMDA Receptor E1 Subunit." *Neuroscience Research* 47(1):55–63.

Kitamura, Takashi, Yoshito Saitoh, Akiko Murayama, Hiroyuki Sugiyama, and Kaoru Inokuchi. 2010. "LTP Induction within a Narrow Critical Period of Immature Stages Enhances the Survival of Newly Generated Neurons in the Adult Rat Dentate Gyrus." *Molecular Brain* 3(1):1–8.

Knobloch, Marlen and Sebastian Jessberger. 2011. "Perspectives on Adult Neurogenesis." *European Journal of Neuroscience* 33(6):1013–17.

Lagace, D. C. et al. 2010. "Adult Hippocampal Neurogenesis Is Functionally Important for Stress-Induced Social Avoidance." *Proceedings of the National Academy of Sciences* 107(9):4436–41. Retrieved (http://www.pnas.org/cgi/doi/10.1073/pnas.0910072107).

Leal, Stephanie L., Sarah K. Tighe, Craig K. Jones, and Michael A. Yassa. 2014. "Pattern Separation of Emotional Information in Hippocampal Dentate and CA3." *Hippocampus* 24(9):1146–55.

Lee, I. and F. Solivan. 2010. "Dentate Gyrus Is Necessary for Disambiguating Similar Object-Place Representations." *Learning & Memory* 17(5):252–58. Retrieved (http://learnmem.cshlp.org/cgi/doi/10.1101/lm.1678210).

Liu, Xu, Steve Ramirez, Roger L. Redondo, and Susumu Tonegawa. 2014. "Identification and Manipulation

of Memory Engram Cells." *Cold Spring Harbor Symposia on Quantitative Biology* 79(Hebb 1949):59–65.

Malberg, Jessica E. and Ronald S. Duman. 2003. "Cell Proliferation in Adult Hippocampus Is Decreased by Inescapable Stress: Reversal by Fluoxetine Treatment." *Neuropsychopharmacology* 28(9):1562–71.

Mcavoy, Kathleen, Antoine Besnard, and Amar Sahay. 2015. "Adult Hippocampal Neurogenesis and Pattern Separation in DG : A Role for Feedback Inhibition in Modulating Sparseness to Govern Population-Based Coding." 9(August):1–7.

McHugh, Thomas J. et al. 2007. "Dentate Gyrus NMDA Receptors Mediate Rapid Pattern Separation in the Hippocampal Network." *Science* 317(5834):94–99.

Molteni, Raffaella, Zhe Ying, and Fernando Gómez-Pinilla. 2002. "Differential Effects of Acute and Chronic Exercise on Plasticity-Related Genes in the Rat Hippocampus Revealed by Microarray." *European Journal of Neuroscience* 16(6):1107–16.

Morris, Andrea M., Brian J. Curtis, John C. Churchwell, David W. Maasberg, and Raymond P. Kesner. 2013. "Temporal Associations for Spatial Events: The Role of the Dentate Gyrus." *Behavioural Brain Research* 256:250–56. Retrieved (http://dx.doi.org/10.1016/j.bbr.2013.08.021).

Morris, Andrea M., Christy S. Weeden, John C. Churchwell, and Raymond P. Kesner. 2013. "The Role of the Dentate Gyrus in the Formation of Contextual Representations." *Hippocampus* 23(2):162–68.

Nakashiba, Toshiaki et al. 2012. "Young Dentate Granule Cells Mediate Pattern Separation, Whereas Old Granule Cells Facilitate Pattern Completion." *Cell* 149(1):188–201. Retrieved (http://dx.doi.org/10.1016/j.cell.2012.01.046).

Petrantonakis, Panagiotis C. and Panayiota Poirazi. 2014. "A Compressed Sensing Perspective of Hippocampal Function." *Frontiers in Systems Neuroscience* 8(August):1–13. Retrieved (http://journal.frontiersin.org/article/10.3389/fnsys.2014.00141/abstract).

Petrik, David, Diane C. Lagace, and Amelia J. Eisch. 2012. *The Neurogenesis Hypothesis of Affective and Anxiety Disorders: Are We Mistaking the Scaffolding for the Building?*

van Praag, H., G. Kempermann, and F. H. Gage. 1999. "Running Increases Cell Proliferation and Neurogenesis in the Adult Mouse Dentate Gyrus." *Nat Neurosci* 2(3):266–70. Retrieved (http://www.ncbi.nlm.nih.gov/pubmed/10195220%255Cnhttp://www.nature.com/neuro/journal/v2/n3/pdf/nn0399_266.pdf).

Van Praag, Henriette. 2008. "Neurogenesis and Exercise: Past and Future Directions." *NeuroMolecular Medicine* 10(2):128–40.

Ramirez, S. et al. 2013. "Creating a False Memory in the Hippocampus." *Science* 341(6144):387–91.

Randall, C., O'Reilly, and Jerry W. Rudy. 2001. "Conjunctive Representations in Learning and Memory: Principles of Cortical and Hippocampal Function." *Psychological Review* 108(1):83–95.

Rolls, E. T. 1996. "A Theory of Hippocampal Function in Memory." *Hippocampus* 6(6):601–20.

Sahay, Amar et al. 2011. "Increasing Adult Hippocampal Neurogenesis Is Sufficient to Improve Pattern Separation." *Nature* 472(7344):466–70.

Seri, B., J. M. Garcia-Verdugo, B. S. McEwen, and A. Alvarez-Buylla. 2001. "Astrocytes Give Rise to New Neurons in the Adult Mammalian Hippocampus." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 21(18):7153–60.

Snyder, Jason S., Amélie Soumier, Michelle Brewer, James Pickel, and Heather A. Cameron. 2011. "Adult Hippocampal Neurogenesis Buffers Stress Responses and Depressive Behavior." *Nature* 476(7361):458–61. Retrieved (http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3162077&tool=pmcentrez&rendertype=abstract).

Tashiro, Ayumu, Vladislav M. Sandler, Nicolas Toni, Chunmei Zhao, and Fred H. Gage. 2006. "NMDA-Receptor-Mediated, Cell-Specific Integration of New Neurons in Adult Dentate Gyrus." *Nature* 442(7105):929–33.

Tronel, Sophie et al. 2012. "Adult-Born Neurons Are Necessary for Extended Contextual Discrimination." *Hippocampus* 22(2):292–98.

Xiuxin Liu, Qin Wang, Tarik F. Haydar, and Angélique Bordey. 2005. "Nonsynaptic GABA Signaling in Postnatal Subventricular Zone Controls GFAP-Expressing Progenitor Proliferation." 8(9):1179–87.

Yang, Guang, Cora Sau, Wan Lai, Joseph Cichon, and Wei Li. 2015. "Adult Mammalian Neural Stem Cells and Neurogenesis: Five Decades Later." 344(6188):1173–78.

Yang, Longfei et al. 2015. "The Effects of Psychological Stress on Depression." *Current Neuropharmacology* 13(4):494–504. Retrieved (http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1570-159X&volume=13&issue=4&spage=494).

Zhao, Chunmei, Wei Deng, and Fred H. Gage. 2008. "Mechanisms and Functional Implications of Adult Neurogenesis." *Cell* 132(4):645–60.