



Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: [www.elsevier.com/locate/bbr](http://www.elsevier.com/locate/bbr)



Research report

## Could adult hippocampal neurogenesis be relevant for human behavior?

Jason S. Snyder\*, Heather A. Cameron

Unit on Neuroplasticity, National Institute of Mental Health, National Institutes of Health, Building 35/3C911, MSC3718, Bethesda, MD 20892, USA

### ARTICLE INFO

#### Article history:

Received 9 March 2011  
Received in revised form 2 June 2011  
Accepted 20 June 2011  
Available online xxx

#### Keywords:

Adult neurogenesis  
Dentate gyrus  
CA3  
Circuits  
Human  
Rodent

### ABSTRACT

Although the function of adult neurogenesis is still unclear, tools for directly studying the behavioral role of new hippocampal neurons now exist in rodents. Since similar studies are impossible to do in humans, it is important to assess whether the role of new neurons in rodents is likely to be similar to that in humans. One feature of adult neurogenesis that varies tremendously across species is the number of neurons that are generated, so a key question is whether there are enough neurons generated in humans to impact function. In this review we examine neuroanatomy and circuit function in the hippocampus to ask how many granule neurons are needed to impact hippocampal function and then discuss what is known about numbers of new neurons produced in adult rats and humans. We conclude that relatively small numbers of neurons could affect hippocampal circuits and that the magnitude of adult neurogenesis in adult rats and humans is probably larger than generally believed.

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### 1. Introduction

Despite the growing number of studies demonstrating behavioral changes following inhibition of adult neurogenesis, the function of new granule neurons in the dentate gyrus is still far from clear. Logic suggests that any behavioral function of new neurons will reflect some subset of the behavioral roles for the specific regions in which they reside, in this case the hippocampus. Although there is still much to learn about the behavioral function of the hippocampus, it is clear that it plays an important role in spatial and episodic memory [1] and that the ventral hippocampus is important for mediating stress responses [2,3]. Very little is known about the specific role of the dentate gyrus, but recent studies in animals and humans point to a role in disambiguating related stimuli [4–8], often during emotional learning [9–11]. Potential roles for new granule neurons in emotional or spatial behavior are beginning to emerge, but the data remain very conflicted (see Snyder [12] for a comprehensive list, online). Eventually, the specific function or functions of adult-born neurons will likely become apparent from rodent studies. However, the ultimate goal is to apply the findings on adult neurogenesis in animal models to our understanding of human brain function. Adult neurogenesis does appear to exist in humans, but definitive experiments can be done in humans only in rare circumstances [13]. While the key experiments directly testing whether new neurons are behaviorally significant in humans

may never be performed, it is important to consider this possibility since it is the impetus for a great amount of animal research. In this review, rather than interpreting behavioral data from animal neurogenesis-ablation models, we instead focus on what is known about neurogenesis numbers, neuroanatomy, and hippocampal circuitry to assess the likelihood that functions of neurogenesis in rodents will be relevant for humans as well.

Before assessing the functional relevance of neurogenesis in rodents it is worth considering whether new neurons would be expected to perform a similar function in humans. Given the comparable anatomy between rodent and human hippocampus [14], it seems likely that the computational function is conserved. Whether this translates into similar behavioral functions is unclear; however consistent roles for the hippocampus in memory across species [15,16] suggests this to be the case.

Perhaps the biggest source of skepticism toward a functional role for adult neurogenesis is the perception that too few new neurons are added in adulthood to have a significant impact. Interestingly, this concern, while valid, is usually raised informally and rarely in the scientific literature; very few studies have addressed this issue [17]. It is difficult to generate even a rough estimate the number of neurons produced in the adult dentate gyrus without cumulative labeling, which has only been feasible in rats and mice and even then is cumbersome and rarely done. More importantly, there is no way to determine how many neurons might be needed to comprise a functional population without a sophisticated understanding of how the neurons function in the circuit, which is still lacking. While numbers offer a hint about function, for example loss of cells may suggest loss of function, even a small popula-

\* Corresponding author. Tel.: +1 301 451 8281; fax: +1 301 480 4564.  
E-mail address: [snyderjason@mail.nih.gov](mailto:snyderjason@mail.nih.gov) (J.S. Snyder).

tion of neurons could exert a significant impact. This possibility is supported by findings in other neuronal populations, e.g., evoked stimulation of individual CA3 interneurons and cortical pyramidal neurons have been shown to alter large-scale network dynamics and even behavior [18–20].

## 2. How do granule neurons contribute to circuit-level function?

Whether or not there are enough new neurons to affect hippocampal function depends on how granule cells function at the circuit level and how new neurons function within this population. Several studies have linked long-term potentiation of synaptic input to the dentate gyrus with learning and memory [11,21–24]. Synaptic plasticity has been studied primarily at the medial perforant path input, the primary source of spatial and contextual information to the hippocampus, but other inputs, namely the lateral perforant path and mossy cell inputs are also likely to contribute to hippocampal encoding processes. The lateral perforant pathway, for example, relays nonspatial sensory information and its convergence with the medial perforant path in the hippocampus is thought to underlie the association of the components of episodic memory [25,26].

One of the most distinctive components of hippocampal circuitry is the sparse mossy fiber-CA3 pathway. The granule cell population shows very sparse activity, with only 2–5% of granule cells active during exploration of any environment [27–29]. In addition, each granule cell contacts only ~10–15 CA3 pyramidal neurons [30]. This unique dentate gyrus-CA3 circuitry has led to speculation that this region of the brain performs a pattern separation function, whereby similar inputs arriving via the entorhinal cortex are given more distinct representations, in order to better distinguish related events during memory encoding and retrieval [4,31]. Importantly, the specialized contacts between granule cells and CA3 pyramidal cells, the giant mossy terminals, are so powerful that a single granule cell is able to trigger firing in downstream CA3 targets [32]. Because of this “detonator” action, and the extensive excitatory collateral connections between CA3 pyramidal neurons [33,34], a single granule neuron can potentially have a large impact despite representing only a tiny fraction of the population. Consistent with the disproportionate impact of small numbers of granule cells, learning deficits have been observed following overexpression of calbindin in only ~1% of dentate granule neurons [35]. If adult-born granule cells are more likely to be activated than mature granule cells, they could make up a significant proportion of the active granule cells at any given time, even if their numbers are small. Several lines of evidence suggest that adult-born granule cells are in fact reliably activated [36] and more likely to undergo long-term potentiation [37–41] in response to electrophysiological stimulation *in vitro*. Furthermore, they are up to five times more likely to be activated than older granule cells by hippocampus-dependent behaviors [42–45].

## 3. Do immature granule neurons have a unique function?

In the past several years, it has become clear that adult-generated granule neurons form synaptic connections and integrate into the hippocampal circuit. In rodents, adult-born neurons receive synaptic inputs from the perforant path that can be detected morphologically [46,47] and electrophysiologically [41,48–50]. The new neurons show immediate-early gene (IEG) expression, an indirect indication of synaptic responses, in response to specific aspects of behavioral stimulation [42,51–54] (see also Snyder et al., this issue) and contribute to long-term potentiation at the population-level [37,38,55,56]. Less is known about the output

of new neurons, but retrograde tracer studies [57–59] and morphological investigations at the level of the light [47] and electron microscope [60,61] suggest that new neurons form functional efferent synapses. More directly, driving channel rhodopsin-expressing new neurons with light generates postsynaptic currents in downstream CA3 neurons [60]. Taken together, these studies clearly demonstrate that individual adult-born granule cells can function as neurons. But they do not address the potential impact of adult-born granule neurons on hippocampal circuits. One key issue for determining whether adult neurogenesis could impact hippocampal circuits is how these late-born granule cells function relative to the rest of the granule cell population. Do they behave just like the granule neurons born during development, and serve to increase the size of the population, or do they have unique properties that confer a function distinct from that of pre-existing granule cells?

Findings from several studies point to similar electrophysiological properties in granule cells born in adulthood once the new granule cells have reached maturity [62–64]. This electrophysiological similarity is consistent with the nearly identical marker expression in mature granule cells, regardless of birth date, as well as their intermixing within the granule cell layer. Moreover, some studies of IEG activation suggest equivalent rates of behavioral activation in granule neurons born in development and adulthood [65] (but see below), further supporting an equivalent function for these two populations.

However, an accumulation of recent evidence indicates that between the time that they acquire synapses and the time they reach full maturity, young granule neurons in the adult dentate gyrus have very different electrophysiological properties from the granule cell population as a whole, suggesting the possibility that they may serve a unique role in hippocampal signal processing. New granule neurons have a lower threshold for undergoing medial perforant path long-term potentiation [37–41,55]. This enhanced plasticity at the population level has been consistently observed – in both mice and rats, and using multiple methods for reducing neurogenesis – providing strong evidence that new neurons contribute uniquely to hippocampal circuit function. In addition, immature granule cells in the adult, like most developing neurons, are depolarized by GABA [66] and are therefore less likely to be inhibited by the strong GABAergic inhibition in the dentate gyrus [67]. Young neurons also appear to be more excitable than mature neurons [36,39]. This enhanced excitability is coupled with increased and more variable spike latency [36], suggesting that young neurons may differ from mature neurons in their firing patterns, a feature believed to be critical for encoding of experience by the dentate gyrus [29]. In addition, since low frequency firing of mossy fibers typically recruits inhibitory interneurons, while higher firing rates activate more CA3 pyramidal neurons [68], differential firing of young neurons could introduce a new mechanism by which the balance of CA3 excitation and inhibition is shifted during behavior. A circuit-level role is also supported by recent *in vivo* data showing that ablation of new neurons with either genetic or radiological approaches reduces the dentate gyrus synaptic response to perforant path stimulation and increases the magnitude and synchrony of spontaneous gamma bursts [69]. Given the role of oscillatory activity in shaping plasticity and behavior-specific neuronal firing [70], these findings suggest adult-born neurons may play a significant role in coordinating hippocampal network function. Finally, some evidence suggests that young granule neurons may be capable of synthesizing and releasing GABA in addition to glutamate [71,72], suggesting a unique neurotransmitter profile that could provide yet another mechanism through which young granule cells could function very differently from the rest of the granule cell population.

Studies examining IEG expression at a population level, which can bridge the gap between properties of adult-born neurons at a single-cell level and function in a behaving animal, also suggest distinct activation of young granule cells. IEGs such as Fos, zif268, and Arc are expressed by neurons in an activity-dependent fashion and show population-level patterns of activity similar to measures of in vivo spiking [73,74], suggesting that IEG analysis and electrophysiology are comparable and complementary in their ability to identify neurons involved in representing or encoding information. Adult-born granule neurons in rats undergo a peak in their zif268 expression in response to spatial water maze training at 3 weeks of age [43], suggesting an enhanced contribution of young neurons to behavior. This peak has not been observed in all studies [65,75], which could reflect differences between IEGs or functional differences between species. Indeed, in mice, peak responsiveness of immature neurons is revealed only after accounting for the fact that not all new neurons are functional at immature stages [43]. In addition, emerging data suggests that young granule neurons and mature granule neurons are maximally activated by different experiences or different aspects of experience. For example, immature neurons, unlike the general granule cell population, are primarily activated in the ventral dentate gyrus during water maze learning, consistent with a specific role in regulating the response to stress [42]. Adult-born neurons are also particularly activated by retraining at remote time points [52] and by training in multiple contexts in the spatial water maze (Snyder et al., this issue).

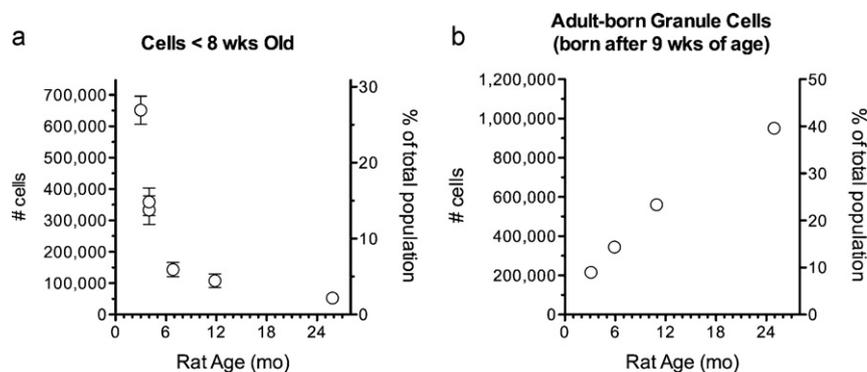
The studies above provide compelling evidence that young, presumably immature, granule neurons have different properties than older granule cells, consistent with the possibility that young granule cells make a unique contribution to hippocampal circuits. Alternatively, it is possible that the unique physiological properties of young granule cells simply reflect synaptic activation that is required for neuronal maturation but does not actually contribute to circuit function. That is, the new granule cells may only truly integrate into hippocampal circuits and contribute to network activity once they are fully mature and identical to existing granule cells. However, a third possibility is that granule cells born in adulthood never become truly identical to those born in development. One intriguing study has reported that even 5-month-old adult-born neurons, i.e. assumed to be well beyond their immature plastic stage, are more likely than the general population to express Arc following exploratory behavior [45]. Similarly, granule neurons located in the deepest aspect of the granule cell layer, which are primarily adult-born though not necessarily immature [76,77], are preferentially activated during water maze learning [42]. Dendritic architecture varies with position in the granule cell layer [78–81], suggesting that granule cells born in adulthood, located deep in the granule cell layer, have a different morphology from those born during development even after they are mature. Since dendritic architecture plays a role in the integration and processing of information [82,83], these morphological differences could lead to permanent differences in the way adult-born granule cells contribute to behavior.

Future studies will be needed to determine whether adult-born granule neurons do in fact have a function distinct from that of existing granule neurons and whether this role is played by young neurons with immature features or by mature adult-born neurons that have different properties from granule cells generated during development. The answers to these questions will influence the numbers of neurons necessary to impact function. If new granule cells function like existing cells, and their role is to increase the population, they need to be added in significant numbers relative to the existing granule cells. If, on the other hand, young granule neurons function as a distinct population, they could potentially impact function even if they are few in number, in the manner of small but powerful subtypes of interneurons.

#### 4. How many new neurons are produced in rodents and humans?

The perception that only small insignificant numbers of new neurons are added in adulthood likely comes from the idea that a stable population of neurons is necessary to preserve memories [84,85]. This idea is reinforced by immunohistochemical images, which typically show new neurons labeled using a small number of BrdU injections or transiently expressed endogenous markers of immaturity. These images therefore provide only a snapshot of neurogenesis, labeling only those neurons born within a few hours (with BrdU) or a few weeks (with markers of immature neurons such as doublecortin). Even retroviral and genetic methods for labeling new neurons, which might be expected to cumulatively label newly generated neurons, frequently label only a fraction of the dividing cells and stop labeling additional neurons after a period of time, perhaps due to lingering uncertainty about the identity and targeting of true stem cells [86,87]. To directly assess the magnitude of adult neurogenesis, we calculated cumulative numbers of neurons born in the adult rat dentate gyrus. We started with cell proliferation rates at different ages based on 2 h post-BrdU survival data from previous studies in our lab [17,88,89], then calculated the number of new cells generated per day based on the number generated in young adults [17] and evidence that the cell cycle is unchanged with age [88]. We then integrated across time to obtain total numbers of accumulated new neurons, accounting for the fact that approximately half of all neurons die during the month after their birth [43,76,90]. Since adult-generated neurons may play important functional roles when they are young, and have different properties from existing granule cells (see above), we generated a curve that illustrates the number of immature neurons (8 weeks and younger) present at various times throughout the life of the rat (Fig. 1a). These calculations reveal that the number of young granule cells in 3-month-old rats is quite large, i.e., greater than 650,000 cells. The curve also shows the well-established decrease in adult neurogenesis with age [91,92], suggesting that if young neurons play a role distinct from that of existing granule neurons, this function is likely to be inhibited with age. However, it is possible that the plastic period may be extended in older animals, offsetting this loss to some extent [44]. Moreover, even in rats greater than 2-years-old, well past the life expectancy, there are more than 50,000 young granule cells in the dentate gyrus. Since there are approximately 500,000 CA3 pyramidal cells [93], and each granule cell contacts 11–15 pyramidal cells [30], this suggests that even in the oldest animals, each CA3 pyramidal cell could potentially receive a direct contact from a young granule cell.

If adult neurogenesis exists to increase the size of the granule cell population, or if adult-born neurons function differently regardless of their level of maturity, then the key question is how many additional neurons they add. To address this question, we calculated the total number of adult-born neurons in rats of different ages (Fig. 1b). Since adult-born granule neurons more than 4 weeks old rarely die [76,94], the total number of adult-born cells grows with age. By 6 months, the cumulative number of adult-born neurons is equal in size to the 359,000 afferent neurons in layer 2/3 of the entorhinal cortex (using numbers from [95]) and nearly as large as the efferent population of CA3 pyramidal neurons. The population of adult-born neurons, despite the diminishing rate of adult neurogenesis, eventually grows more than twice as large, reaching nearly 1,000,000 cells, or 40% of the total population by the end of the animal's life (Fig. 1b). It is worth noting that, while no studies have cumulatively labeled new neurons for such extended periods, one study has found ~50,000 labeled cells in 4-month-old rats that were injected with a subsaturating dose of BrdU for 12 days, consistent with our predictions [96]. These numbers of adult-born neurons clearly seem large enough to have a major impact on the circuitry of the den-



**Fig. 1.** Estimates of adult neurogenesis numbers in the rat hippocampus. (a) The estimated number of young ( $\leq 8$ -week-old) granule neurons, at various times throughout adulthood. This number decreases significantly due to the reduction in adult neurogenesis with age. Numbers were calculated using bilateral BrdU+ cell counts 2 h after injection (proliferating population) at 5-weeks-old [17], 9-weeks-old [17,88], 5-months-old [89], 10-months-old [88], and 24-months-old [89]. Counts were converted to numbers of cells generated per day by assuming that 4912 BrdU+ cells give rise to 9089 cells/day [17] and that this ratio is constant for all ages because the cell cycle time is constant across age [88]. The number of cells generated each day was multiplied by 7 to calculate the number generated each week. To account for cell death during maturation the number of cells born in one week multiplied by the % surviving for that week [43]. For week 1 it was assumed that all cells survived, since continued proliferation and dilution of BrdU beyond the limit of detection make it difficult to estimate cell death during this period [76]. After 4 weeks, it was assumed that death is complete and that 62% of cells born more than 4 weeks earlier survived [43]. Cells surviving during each of the 8 weeks were summed to estimate the total number of cells  $\leq 8$ -week-old. (b) The estimated cumulative number of granule neurons born in adulthood throughout the lifespan. Numbers were calculated by adding the numbers of  $< 4$ -week-old (still dying) cells at each age to the number of cells generated/surviving since the previous time point, assuming a linear decline in cell proliferation between adjacent tested ages.

tate gyrus. It is worth noting that several studies employing genetic labeling techniques have estimated that the cumulative contribution of adult neurogenesis to the granule cell population plateaus earlier (by 4–6 months of age) and is significantly smaller than our estimates (1–10%) [86,97,98]. This discrepancy could reflect differences between mice and rats or the labeling of transit amplifying populations that may not generate new neurons indefinitely. Modeling net neurogenesis based on Ki67+ labeling across the lifespan in mice has produced intermediate estimates [99].

To extend the rodent data into humans we can assess whether adult neurogenesis occurs in humans, compare its magnitude across species, and determine whether the process is qualitatively similar to that of rodents. For many obvious reasons, the human data is limited. However, a small number of studies have emerged over the last 10 years to enable us to begin to assess whether neurogenesis may contribute to human brain function as it does in rodents. Endogenous markers of immature neurons such as doublecortin and TUC-4 have been used to identify new neurons born in the adult human dentate gyrus and to look for changes in neurogenesis in several pathological conditions [100–102]. Although these endogenous markers appear to label neuronal populations that are not newly born in some brain regions [103], in the dentate gyrus they are exclusively expressed by newborn neurons in the rodent [96,104,105] and show every indication of labeling new neurons in humans based on the similarity of the location and morphology of labeled cells to that in rodents [106] (keeping in mind that morphology can change depending on the fixation delay in post-mortem tissue [107]). The number of doublecortin-expressing cells in the human dentate gyrus shows a dramatic reduction in new neurons with age [106]; this parallel with the age-related decreases in rodents and non-human primates [92,108,109] provides evidence that the regulation of adult neurogenesis in humans is similar to that in other species. However, since doublecortin and TUC-4 are lost as cells mature or die, they provide little information on the numbers of neurons that survive to maturity. The number of mature neurons produced in the adult human dentate gyrus could be much larger than expected based on the relatively small doublecortin-expressing population if maturation and loss of doublecortin is relatively rapid and/or the survival rate of young neurons is relatively high.

BrdU and its analogues are the only markers of new neurons that can be followed for weeks, allowing conclusive identification of newborn neurons that survive and mature. These markers are very difficult to use in human studies, however, since they must be injected into living subjects. There is to date only one study of BrdU-labeled cells in the adult hippocampus, by Eriksson et al. [13], who examined brains containing BrdU used for diagnostic purposes in non-brain cancer patients. This study provides solid evidence that adult neurogenesis occurs in humans by demonstrating that BrdU+ cells are located in the subgranular zone of the dentate gyrus, the same region in which new neurons reside in rodents and non-human primates, 6 months to 2 years after BrdU administration in adults. In addition, the quantitative data presented in the study suggest that adult neurogenesis may occur at rates at or above those in rodents. The human subjects received a very small dose of BrdU compared to typical rodent studies; each subject was given 250 mg, or roughly 3.3 mg/kg (assuming average human body weight of 75 kg). This dose is 15–60 times lower than the 50–200 mg/kg generally used in rodent studies, but simple conversions adjusting only for weight differences do not usually provide equivalent doses in different species. One rule for translating pharmacological doses across species, based on normalizing body surface area, divides mouse doses by 12 and rat doses by 6 to give an equivalent human dose [110]. According to this general rule, the human dose in the Eriksson study may be equivalent to 40 mg/kg in mice or 20 mg/kg in rats – doses that fail to detectably label 40% and 90% of S-phase cells, respectively, using the immunohistochemical methods employed in these studies [17,111]. However, the pharmacokinetics of a thymidine analogue that has been extensively studied, azidothymidine, show that similar plasma levels are achieved at doses only 50% higher in mice than in humans [112–114], suggesting that the BrdU dose used in the Eriksson study may be equivalent to vanishingly low doses in rodents. Given this low dose of BrdU it is remarkable that  $\sim 25$ – $325$  BrdU+ cells/mm<sup>3</sup> were found in the in the human subgranular zone and granule cell layer. It should also be noted that the subjects in this human study were terminally ill, the stress of which may inhibit adult neurogenesis, and were advanced in age (mean = 64 years). Densities of labeled cells in middle-aged rats fall within this same range after adjusting for multiple BrdU injections [44,92], suggesting that the actual number of new neurons in humans may be significantly higher than

in rats. Eriksson et al. also co-immunostained BrdU+ cells for phenotypic markers, and found that approximately 25% of BrdU+ cells expressed neuronal markers. This is lower than the proportion of BrdU+ cells that label with NeuN in many young adult rodent studies but agrees with findings showing reduced NeuN expression in older animals [115–119], perhaps due to prolonged maturation with age [47,120,121]. Alternatively, it could reflect conservative labeling cutoffs [43], low signal to noise in NeuN staining due to species differences in expression [43] or use of formamide in staining [122], and/or species-dependent differences in maturation [43]. It is worth noting that other studies have quantified aspects of neurogenesis in non-human primates and humans and, collectively, have reported tremendous variation in the magnitude of neurogenesis (comprehensive lists compiled online, primates: [123], humans: [124]). While comparisons between the rodent and human literature provide some hints about the amount of neurogenesis in humans, numerous methodological differences between the studies preclude solid conclusions. Technological advances will hopefully provide better estimates of the rate of neurogenesis in humans in the near future.

### 5. Are these numbers potentially sufficient to exert a functional impact in humans?

We feel that the answer to this question is an overwhelming “yes”. The number of new neurons added to the adult dentate gyrus is likely to be large enough to make an impact on hippocampal function both in rodents and in humans. While it is not clear what behavioral role new neurons play, the unique properties of adult-born neurons may enable them to process information in novel ways or serve as naïve substrates for information storage across all mammalian species.

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