

# Advances on Learning-modulated Adult Neurogenesis

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## Abstract

During the last decade neuroscientists have shown an increasing interest in adult neurogenesis and a number of studies have been focused on the role that it could play in learning and memory processes. One of the brain structures that generates new neurons along adulthood is the Dentate Gyrus of the hippocampus. This structure is involved in learning and memory processes, and a plausible role for these newly born cells at the information processing level has been suggested. Nevertheless, actual evidences do not provide a clear picture on the type of cognitive processes supported by adult-born neurons. The present work reviews the last evidences related to the possible role of neurogenesis in learning and memory mechanisms.

**Keywords:** Neurogenesis, Hippocampus, Learning, Memory.

## 1. Introduction

For a long time, it was assumed that the adult mammalian brain was composed of a fixed number of neurons without capacity to divide after the end of development. This dogma, restricting neurogenesis to a developmental phenomenon has, however, been challenged by the discovery that new neurons are produced throughout the lifespan in specific brain areas [1,2]. This discovery led to a fascinating but still controversial hypothesis about the integration of adult-born neurons in existing brain circuits.

The dentate gyrus (DG) of the hippocampal formation is one of these few structures where adult neurogenesis occurs in mammals, including humans [1,3-10], and it has been estimated that several thousand new cells are generated daily [11-13]. The DG is part of an integrated network that participates in memory processes, especially in the establishment and use of spatial representations [14].

Neurogenesis in the DG is a complex process that starts with the proliferation of neural precursors residing in the dentate subgranular layer. At least 50% of the new cells die during the first days [15]. The majority of those cells that survive this initial

period differentiate into granule neurons and survive for some months within the DG [16,17]. These adult-born granule neurons are then integrated into the hippocampal circuitry and exhibit physiological properties similar to those of mature granule neurons [18].

A number of years ago, Altman and colleagues suggested a role for postnatally generated neurons in learning processes [19,20]. However, such idea of adult-generated neurons involvement in learning was discussed and studied first by Nottebohm and collaborators in relation to song learning in birds [21,22]. Later studies considering seed caching behavior and the involvement of the avian homolog of the hippocampus led to the idea that adult neurogenesis is important for learning and memory of spatial information [23,24]. However, the potential relevance of these findings for learning in mammals was not generally accepted until it was clear that new neurons in the DG become synaptically integrated [25-27], attain morphological and biochemical characteristics of neurons [4,28] and generate action potentials [29].

Although interest in adult neurogenesis has grown exponentially in recent years, evidence for a role of adult-born granule cells in learning and memory remains controversial. Based on a number of correlative pieces of evidence it has been hypothesized that adult-born hippocampal neurons contribute to memory processes. First, the rate of neurogenesis is positively correlated to hippocampal-mediated learning abilities [30]. Second, conditions that improve learning ability, such as enriched environment or middle-age adrenalectomy enhance neurogenesis [9,31], whereas conditions that impair learning, such as prenatal stress or lesions of cholinergic septo-hippocampal pathway, decrease neurogenesis as well [32,33]. Third, spatial learning has been shown to increase both cell proliferation [34] and the survival of newborn neurons [35].

Despite these compelling pieces of evidence, other attempts to demonstrate a causal relationship between neurogenesis and spatial learning have produced opposite results. Remarkably, spatial learning in the water maze has been linked to a decrease in the number of newborn neurons in the DG [36,37]. Indeed, a decline in neurogenesis has

been correlated with spatial abilities, i.e., rats with the lowest number of newly born cells showed better memory performances, indicating that learning, and not training, decreased the number of adult-born cells [37]. These complex results provide a puzzling picture in which increases and decreases in the number of newborn neurons are both correlated with learning. In this review, possible evidences in favor and against a role for adult neurogenesis in learning are presented.

## **2. Correlations between the number of new neurons and learning abilities**

Positive correlations between the number of new neurons and learning performance would imply a relationship between neurogenesis and learning, although not necessarily a causal one. Several lines of evidence have suggested a positive correlation between neurogenesis and learning in mammals. Thus, strain differences in the rate of adult neurogenesis in mice have been shown to parallel strain differences in learning. That is, the mice with the fewest number of new neurons performed most poorly in a spatial navigation task in the water maze [38]. In addition, some mutant mice with decreased hippocampal neurogenesis showed impaired performance on hippocampus-dependent learning tasks [39,40]. Similar results have been found in aged rats by Drapeau et al. [41]. Nevertheless, there are some other reports in which such relationship has been dissociated or appears to be reversed. For instance, unlike in mice, strain-dependent differences in hippocampal neurogenesis do not correlate with spatial navigation learning in rats [42].

Apart from genetic determinants, environment has a major impact on hippocampal neurogenesis [43]. Some studies have shown that voluntary running increases cell proliferation, whereas exposure to an enriched environment promotes the survival of 1- to 3-week old immature neurons, which are the likely substrates for experience-specific modulation [44,45]. Both, voluntary exercise and environmental enrichment improve the performance of young and aged mice in the water maze [46,47]. Environmental enrichment also leads to better recognition memory [48]. Additional studies of mice deficient in presenilin-1, with reduced hippocampal neurogenesis induced by environmental enrichment, suggest a function of neurogenesis in the clearance of memory traces [49]. Running induced neurogenesis, which correlates with an induction of LTP in the DG, can be also modulated by the social status of animals and is likely to be mediated by molecules such as BDNF and VEGF [43]. In contrast, factors such as normal aging, stress and diabetes impair both neurogenesis and learning [50-55]. In other cases, however, although conditions of elevated glucocorticoids decreased cell proliferation in the

DG, they did not result in learning deficits on hippocampal-dependent tasks [56,57]. In fact, stressor exposure enhances learning of certain hippocampal-dependent memory tasks [16,58,59] which might suggest an inverse relationship between the number of new neurons and learning. Additionally, there are a number of drugs that are associated with decreases in neurogenesis, such as alcohol, nicotine and opiates [60-62], all of which can, in the appropriate doses, result in performance deficits during some learning tasks [63-65]. Nevertheless, although many studies suggest that decreases of neurogenesis are associated with impaired learning, most of these studies cannot provide statistical correlations. Thus, it is possible that other factors, such as structural plasticity and neurotrophin and hormone levels, also contribute to environmentally-induced changes in hippocampal-dependent learning and memory [43]. Correlative evidence can be quite refined when the experimental design allows for correlation of specific learning performance with the exact age of newborn neurons. This approach was initiated by Gould et al. [35] who discovered that neurons at 1-2 weeks of age survive as a result of training.

## **3. Influence of learning on the number of new neurons**

The regulation of neurogenesis by neural activity suggests that learning might induce the activation of newborn neurons and subsequently enhance their survival and incorporation into circuits. It has also been reported that hippocampal-dependent learning increases the proliferation of new cells, but surprisingly, decreases in their number have also been reported.

It seems plausible that the activation of new neurons enhance the production and/or survival of these cells. Thus, training on learning tasks that require the hippocampus has been shown to modify the number of neurons in the DG, although the direction of the effect is not always the same. For instance, DG neurogenesis is only enhanced by learning tasks that depend on hippocampus such as trace eyeblink conditioning, spatial learning in the water maze and conditioned food preference [16,32,35,37,66-68]. Hippocampus-independent tasks, such as delay eyeblink conditioning and active shock avoidance does not affect DG neurogenesis. Moreover, pretraining in delayed conditioning, which makes the subsequent trace conditioning independent of the hippocampus, renders trace conditioning ineffective in promoting the survival of newborn neurons [69]. Moreover, it is learning and not simple training that elicits the survival effect [70]. Similarly, learning appears to promote the survival of newborn neurons only in cognitively unimpaired aged rats [53]. Thus, according to Dupret et al. [71], learning elicits

different influences on neural precursors at different developmental stages.

In contrast to these studies demonstrating a stimulatory effect of learning on adult neurogenesis, other authors report that training on various learning tasks either does not alter the number of new neurons in the hippocampus [42,46,72] or actually decreases it [36,37,68,73]. One possibility for these discrepant findings is that the effect of learning on the number of new neurons depends on the age of the BrdU labeled cells at the time of learning. Some authors, postulate that trace eyeblink conditioning and water maze training enhance the survival of new neurons born one week prior to training, coinciding with the early stages of differentiation and major susceptibility to cell death [35,66]. In contrast, learning would decrease the survival of older and perhaps more mature neurons [36]. Recently, Dupret et al. [71] reported that spatial learning elicits different influences on neural precursors at different developmental stages. These authors studied the effect of training at different moments of learning. They found learning-induced apoptosis of newly born cells that were produced during the early phase of learning, survival of relatively mature neurons, and finally, proliferation of neural precursors. Learning-induced cell death was specific to the DG (subgranular layer), and no changes were observed in CA1 or CA3. According to the authors, learning-induced apoptosis becomes critical for spatial memory. Thus, the inhibition of apoptosis in animals that begin to master the task impaired the memory for the platform location and reduced cell proliferation induced by the late phase of learning. Therefore, according to Dupret et al. [71], spatial learning involves a mechanism very similar to the selective stabilization process observed during brain development, in which the production of new neurons is followed by an active selection of some and removal of others. As a consequence, learning-induced increases in survival and apoptosis of newborn cells would be interrelated processes.

During training in the water maze, two phases can be distinguished: an early phase during which performance improves rapidly, and a late phase during which performance stabilizes reaching an asymptotic level. It has been reported that the early phase of training in the water maze has no effect on proliferation, whereas the late asymptotic phase increases cell proliferation and decreases the survival of new cells produced initially [37]. This type of intricate regulation of hippocampal neurogenesis by learning was also observed in the social transmission of the food preference paradigm, a natural form of associative learning dependent on the hippocampus [68]. Therefore, certain BrdU labeling paradigms may not be appropriated for detecting the relationship between learning and neurogenesis. Indeed, BrdU labelling procedures that occur over many days may result in an overall

lack of differences for the number of labelled cells due to both decreases and increases in the number of neurons, depending on the age of the cells at the time of training.

Other possible explanation for the different effects of learning on adult neurogenesis may result from differences in training protocols. Olariu et al. [68] have shown that the amount of training determines whether the effect on neurogenesis is positive or negative. It seems that a few number of training trials have been associated with enhanced cell survival, and a more extensive training has been associated with no effect or decreased survival [35-37,68,72]. However, this is not true for all types of tasks. With trace eyeblink conditioning, exposure to just 200 trials did not enhance cell survival, whereas training with 800 trials did it [16]. Nevertheless, although the overall number of newborn cells was not affected by a shorter training episode, the number of learned responses emitted during the 200 trials was positively correlated with the number of cells that survived. These data suggesting individual differences in early acquisition are predictive of whether new neurons will survive or not. Moreover, it is consistent with findings suggesting that different phases of learning (i.e., acquisition, retention, retrieval) must be taken into account when assessing the effects of learning on adult neurogenesis [37,38].

Finally, even more subtle interactions between neurogenesis and learning can be revealed when the experimental design involves successive tasks that can give rise to possible interference effects. Hence, in a recent study, animals with reduced neurogenesis performed better than controls in a radial-arm maze task when tested under high interference conditions [74].

#### **4. Effects of neurogenesis reduction over learning**

Correlative approaches do not exclude other plastic phenomena in the brain that could participate in the learning process thereby rendering enhanced neurogenesis an epiphenomenon. For instance, synaptic strengthening within CA1 field of the hippocampus is thought to be a prime candidate for encoding spatial information within the hippocampus. Definitive evidence for a requirement of new neurons can only be obtained by demonstrating learning deficits following selective removal of new neurons. Designing experiments to address this question becomes difficult for two main reasons. First, methods to selectively remove new neurons without affecting other aspects of brain function are not yet available. And second, the timing and duration of neuronal removal may be a critical factor in detecting learning deficits.

To date, several experimental methods have been used to decrease or even ablate DG neurogenesis in adult animals. These include (1)

low-dose irradiation of either whole brain or restricted brain regions; (2) systemic treatment with antimetabolic drugs such as methylazoxymetanol acetate (MAM); (3) using aging as a natural process to reduce neurogenesis; (4) using genetically engineered mice to specifically eliminate neural progenitors. Although all these procedures induce non-specific effects on performance or brain function raising the possibility of false positive results, they begin to reveal a causal link between DG neurogenesis and cognition

It is fundamental to bear in mind that new neurons may participate in learning for only a discrete period after their production, and false negative results could occur if neuronal removal is insufficient in length or the interval between neuronal removal and training is inappropriate. Thus, ruling out a possible role for adult-generated neurons in any type of learning would require numerous schedules of neuronal removal along with assessing performances during different phases of the learning process. Additionally, since the hippocampus has been associated to several types of learning with no obvious link (e.g., trace eyeblink and fear conditioning, contextual fear conditioning, spatial navigation, delayed non-matching to sample), detecting and characterizing a role for new neurons in learning mechanisms will require extensive behavioral assessment. With all these methodological considerations, a number of studies have tried to answer the question of whether new neurons are used in the acquisition and/or retention of new memories.

Shors et al. [75] found that MAM-treated rats failed to form conditioned responses in trace eyeblink conditioning or trace fear conditioning but not in contextual fear conditioning (which are all hippocampus-dependent tasks). Similarly, no deficit in contextual fear conditioning was detected in *tlx* conditional knockout mice, in which hippocampal neurogenesis was seriously reduced [76]. In contrast, irradiated rats or mice and ganciclovir-treated *GFAP-tk* mice were impaired in contextual fear conditioning tasks [77,78]. In these studies, only very young cells were targeted by the MAM treatment, whereas in the irradiated animals and *GFAP-tk* mice both young and mature newborn cells were affected. Therefore, it is possible that immature and mature newborn neurons play different roles in learning. An important variant of such experiments is to introduce a delay between the antimetabolic treatment and the behavioral test. During this delay the pre-existing postmitotic neurons can proceed through their normal developmental process so that, effectively, at the end of the delay period only mature neurons are present. One of such studies with MAM-treated animals showed impaired trace-eyeblink conditioning but normal spatial learning when animals were tested 1-2 weeks later [79].

Localized irradiation to remove the population of newly neurons has some advantages over systemic administration of cytostatic agents. Thus, irradiation completely removes the population of newly generated cells instead of reducing their number, but without inducing weight loss or other health problems. Moreover, a complete elimination of neurons becomes more convincing regarding the involvement of neurogenesis in learning. Irradiated animals tested 4 weeks after the treatment showed impaired contextual fear learning and impaired delayed nonmatching-to-sample learning [78] but apparently normal spatial learning [72,77]. Similar results have been reported recently by Wojtowicz et al. [80]. According to these authors, rats exposed to irradiation showed impaired contextual fear conditioning but normal spatial learning in the water maze. This study is related with the notion that some, but not necessarily all, hippocampal tasks require new neurons [69]. In this line, X-ray irradiation of 2-month-old mice impaired spatial learning and memory in the Barnes maze but not in the water maze [81]. By contrast, X-ray irradiation of 3-week-old mice lead to opposite results: impaired spatial learning and memory in the water maze but not in the Barnes maze [82]. Additionally, other studies have been unable to find any deficit in spatial learning in animals with reduced or abolished neurogenesis [34,72,77,79,83], despite the fact that impaired long-term retention of memories was observed in irradiated rats and in *tlx* mutant mice [72,76]. A possible reason for the lack of effect on spatial learning is a functional compensation by other hippocampal structures like CA1, the remaining young neurons or mature neurons in the DG. Thus, under normal circumstances the young neurons are critically involved in hippocampal functions but various forms of compensation could partially mask the effects of neuronal loss. Finally, in the same way as with the antimetabolic agents, there are some possible side effects of using irradiation, which could inadvertently affect performance. Most notably, irradiation can induce inflammatory responses, which can affect performance during learning [82,84].

These discrepancies are probably due to differences in animal species and strains, the detailed behavioral procedures and the different knockdown strategies. Furthermore, all current studies rely on experimental paradigms based on lesion models where the whole hippocampus is affected. One interpretation of these results is that newly born cells are not required in the same degree for all the tasks. Moreover, these learning tasks may not be sufficiently sensitive to the loss of newly born cells in DG. Other authors have proposed that some tasks only need a low number of new cells to sustain performance [41,56,79,85]. As with the effects of learning on the number of new neurons, it may be important to distinguish among different phases of the learning process. Thus,

Snyder et al. [72] suggest that adult-generated cells may not be important for acquiring spatial information in the water maze, but are required for long-term spatial memories. Zhao et al. [86] conclude that to definitively demonstrate the functions of DG neurogenesis, selective ablation approaches with few side effects and specific behavioral tests need to be developed in the future.

## 5 Discussion

To the present moment, there are not conclusive evidences linking adult neurogenesis and learning, and a number of fundamental questions still remain. For instance, what mechanisms of cell production in the DG are regulated during learning? Does learning induce different patterns of connectivity in these neurons? Does learning affect gene expression in these neurons and does it differ from that in mature neurons? At the functional level, how does a new population of neurons interact with those that were generated during development and how do these interactions lead to the formation of new memories without destroying old memories?

It has been suggested that the production of new cells in the DG increases the opportunity for learning in the future by providing more cells that can be recruited into existing circuits [38]. However, it could be possible that newly born cells influence learning processes even before their maturation. Certain characteristics of synaptic plasticity are enhanced in adult-generated neurons and may make them particularly useful for the processing of new associations. For instance, it has been postulated that newborn cells could be involved in detection or processing of novel stimuli [38,87,88].

The functional relevance of immature adult-generated neurons seems to rely on the particular physiological properties they have, making them particularly inclined to undergo activity dependent plasticity. Some studies have shown the high excitability and sensitivity of newly born neurons to synaptic plasticity. Thus, young neurons in the DG have a lower threshold for LTP induction and produce stable LTP more readily than older neurons [77,89]. This is presumably due to the specific membrane properties of 1-to 3-week old neurons, greater NMDA receptor sensitivity and calcium entry upon synaptic activation [90]. Moreover, newly generated naive neurons appear to be particularly suited to respond to, and integrate information coming from cortical afferents to hippocampus, providing a cell population well adapted for recruitment of new memories. Overall, these findings strongly suggest that the addition of new neurons translates into changes in network properties, increasing the computational capacity of circuits in which they are embedded [91].

Some authors have postulated that newborn neurons may be related to temporary storage of information [2,92]. The hippocampus plays a time-

limited role in memory storage, so its lesion becomes less effective at disrupting task performance as more time elapses between acquisition and memory recall [93-95]. Gould, Tanapat et al. [92] have postulated that a rapidly changing population of new adult-generated neurons may provide a substrate for maintaining memories over relatively short periods of time. Therefore, the lifespan of a new neuron would correspond to the duration of the memory that it supports. However, this is not necessarily the case, for it has been reported that learning increases the survival of new hippocampal neurons and they remain there for at least two months after training [16], which is beyond the time that the hippocampus is necessary for the retention of those memories [93,95]. Some authors have shown that the recruitment of newborn neurons does not occur until they are at least 4 weeks old [44]. Therefore, if the neurons whose survival is increased by learning are not recruited by the ongoing behavior, they might support a future learning experience. Moreover, the addition of these new circuits could encode the time of new memories [96]. Nevertheless, additional research is required to establish whether adult-born neurons exert a functional role in memory formation before or after reaching complete maturity.

A definitive link between adult neurogenesis in the hippocampus and learning remains an open question. Whether or not a role for adult-generated neurons in learning and memory is ultimately ruled out, alternative functions should be considered. One possibility is that adult neurogenesis is a vestige of development, without functional significance in adulthood. Alternatively, new neurons may contribute to other functions of the hippocampus, such as anxiety and stress regulation, or as a latent mechanism for endogenous repair of this brain region, known for its susceptibility to ischemia and seizures. However, the increasing interest of researchers to solve the questions about adult neurogenesis will provide definitive answers in the future.

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