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Time-dependent involvement of adult-born dentate granule cells in behavior

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Abstract

Adult-born neurons are continuously generated and incorporated into the circuitry of the hippocampus throughout life in mammals. Cumulative evidence supports a physiological role for adult-born neurons, yet it not clear whether this subset of dentate granule cells makes a unique contribution to hippocampal function. Perturbation or ablation of adult hippocampal neurogenesis leads to deficits in the acquisition of learned associations or memory recall, whereas an increase in adult hippocampal neurogenesis enhances some forms of learning and memory. The observed effects thus far appear to be task-dependent, species-specific, and sensitive to the timing of manipulations. Here, we review the recent evidence correlating adult-born dentate granule cells with hippocampal-dependent behavior and focus on the dynamic properties of this neuronal population that may underlie its function. We further discuss a framework for future investigations of how newly integrated neurons may contribute to hippocampal processing using advanced genetic techniques with enhanced temporal resolution.

Keywords

adult neurogenesis; dentate gyrus; cognitive function; behavior

1. Introduction

Robust adult neurogenesis, the generation of new neurons from neural progenitor cells, is observed throughout life in almost all mammals examined and there is much interest in identifying the functional significance of this phenomenon [1–5]. Two primary sites of adult neurogenesis in mammals are the dentate gyrus of the hippocampal formation and the subventricular zone/olfactory bulb system. Because the hippocampus is believed to mediate various forms of learning and memory [6–8], this region has received the bulk of attention

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from investigators trying to establish a causal link between adult neurogenesis and the maintenance or enhancement of cognitive abilities [9]. In the dentate gyrus of young adult mice, approximately 4,000–7,000 new cells are born each day as measured by pulsing dividing cells with nucleotide analog bromodeoxyuridine (BrdU) and in young adult rats, the rate of neurogenesis is nearly 50% higher [10–12]. In mice, less than a third of the newborn neurons survive and are ultimately integrated into neuronal circuitry in the adult brain [12–14]. Although adult neurogenesis appears to recapitulate embryonic neurodevelopment in many respects, it is unique in that adult-born neurons must incorporate into established circuitry within a functionally mature brain. A fundamental question in this field is whether these comparatively young neurons make a special contribution to information processing mediated by the local circuitry.

To address this question, there have been many attempts to correlate levels of adult neurogenesis with behavior. Suppression of adult neurogenesis in rodents has met with mixed results in that most, but not all, hippocampal-dependent tasks are negatively affected by a decrease in neurogenesis and that the effects can be species-specific and/or temporally graded [9, 15]. Although a consistent function for adult neurogenesis in all forms of hippocampal-dependent learning has not been identified, there may be confounding factors that prevent this observation. First, most manipulations used to arrest adult neurogenesis have some nonspecific effect on the system or local circuitry that could have independent effects on behavior. Second, there has been very little parametric testing to identify the critical age of adult-born neurons within the same testing conditions. Newborn neurons undergo robust changes in morphology, ion channel expression, neurotransmitter response, and other critical intracellular properties over the course of development [16, 17]. All of these factors affect signaling both within and between cells, and interactions between adult-born neurons and the local environment likely depend on the stage of cellular maturation. In this review, we discuss the contribution of adult-born dentate granule cells (DGCs) to behavior as a function of time-dependent intrinsic changes in their properties and consider optimal approaches to evaluate the role of this continually evolving population.

2. Development of newborn dentate granule cells in the adult hippocampus

We focus on young adult mice to discuss functional stages of hippocampal neurogenesis. The progression of developmental stages is well-conserved in different species, although the timeline of neuronal development may be species-specific [18]. For example, adult neurogenesis in rats appears to occur at a faster pace and at a higher rate than in mice [12]. Quantitatively, levels of proliferation and survival are reduced in aged animals. It remains to be fully characterized whether there is a change in the pace of maturation or functional properties of the surviving adult-born neurons in aged animals [19]. We divide the development of newborn DGCs in the adult mouse hippocampus into four stages (Figure 1).

2.1 Proliferation of adult neural progenitors and survival of early neuronal progeny

In the adult dentate gyrus, neural progenitors are located in the subgranular zone (SGZ) at the border between the hilus and the granule cell layer (GCL). $GFAP^+nestin^+$ radial glia-like

cells [20, 21] and $Sox2^+$ non-radial cells [22] are believed to be multipotent adult neural stem cells. These precursors give rise to rapidly dividing transient amplifying cells expressing Tbr2, which in turn generate immature neurons in the dentate gyrus [23]. Following BrdU pulsing to label proliferating cells during the S-phase, most $BrdU^+$ cells differentiate into neurons, but some differentiate into astrocytes [11, 24]. Time course analyses using retrovirus-based lineage tracing showed that proliferating neural progenitors in young adult mice largely commit to a neuronal fate, express the immature neuronal marker DCX within 3 days, and become post-mitotic within 7 days after birth [25]. There is a significant loss of newborn progeny during the first 4 days after birth. A recent study suggests that apoptotic mechanisms trigger cell death and microglia-mediated phagocytosis rapidly clears the affected cells from the SGZ during this early critical period [14].

2.2 Migration and initial integration of immature neurons with GABAergic synaptic inputs

Newborn immature neurons migrate only a short distance into the inner granule cell layer after birth and express Prox1, a marker for DGCs [26, 27]. These new neurons lack dendritic processes and display a high membrane resistance due to a low density of somatic ion channels [25, 28, 29]. Nevertheless, within 3 days after birth, these immature neurons already exhibit functional $GABA_A$ receptors that are tonically activated by ambient GABA in the environment [25, 30]. By 7 days, newborn neurons extend dendrites toward the molecular layer and start to receive functional GABAergic synaptic inputs, before any functional glutamatergic inputs can be detected [25, 31, 32]. As in perinatal neuronal development, the classical inhibitory neurotransmitter GABA initially exerts a depolarizing influence on immature neurons [33, 34]. This GABA-mediated depolarization serves as a trophic mechanism to promote differentiation, migration and maturation of immature neurons in the dentate gyrus [25, 35–37]. Immature neurons thus respond in a diametrically opposed way to ambient GABA as compared to mature neurons. Increased GABA levels within the neurogenic region will both inhibit older neurons and promote the growth of recently born neurons. It is not yet clear how this GABA-mediated balance of excitation and inhibition in distinct neuronal subpopulations within the dentate gyrus circuitry may contribute to hippocampal function.

2.3 Activation and synaptic integration of immature neurons with glutamatergic synaptic inputs and outputs

Within 2–3 weeks after birth, newborn DGCs in young adult mice exhibit elaborated dendritic processes and project axons to the CA3 target region [25, 29, 31, 38]. These adult-born neurons also start to receive functional glutamatergic synaptic inputs [25, 31] as their efferent mossy fibers begin to make synaptic contacts with downstream hilar interneurons and CA3 pyramidal neurons [38, 39]. Because the number of efferent (CA3) and afferent (entorhinal cortex) target neurons do not change considerably in the adult brain, newly generated DGCs compete with a population of mature neurons for potential sites of synaptic contact. Dendritic filopodia of new neurons form synaptic contacts with pre-existing axonal boutons [40], whereas the axons of newborn cells initiate early synapse formation primarily on dendritic shafts in CA3 [39]. The time course of presynaptic and postsynaptic targeting appears to be synchronized and both processes are at least partially governed by activity-driven competition [16, 41]. Neuronal activity also regulates the survival of newborn

neurons during this period in an NMDA receptor-dependent fashion [42]. Optogenetic and pharmacological analyses have demonstrated that adult-born DGCs release glutamate once fully mature [39], but it is unknown whether newborn DGCs may also release GABA transiently during development [43, 44]. It is also during this period of time that the polarity of GABAergic responses in newborn neurons switches from excitation to inhibition [25]. The electrophysiological properties of immature DGCs at this stage are strikingly different from their mature counterparts [25, 31, 45]. Despite similar resting potentials, immature DGCs have a higher input resistance and are capable of generating action potentials in response to weaker stimulation than that required for mature DGCs [46].

2.4 Synaptic maturation of afferent and efferent connections and critical period of synaptic plasticity

Between 4–8 weeks after birth, new neurons in young adult mice exhibit increases in dendritic arborization and dendritic spine number, as well as refinement of axon terminals and maturation of mossy fiber boutons [29, 38]. This is also the period when newborn DGCs exhibit unique properties in synaptic plasticity [16, 45, 46]. Long-term potentiation (LTP) exhibits a lower threshold for induction and larger amplitude in these adult-born neurons compared to perinatal- or adult-born neurons at more mature stages. Pharmacological analysis showed that this enhanced plasticity is mediated by NR2B-containing NMDA receptors in adult-born neurons [45, 47]. Furthermore, LTP induction in adult-born neurons during this period is insensitive to GABAergic inhibition, whereas suppression of GABAergic transmission is a requirement for LTP in mature DGCs in the acute slice preparation from adult animals [16, 45, 47]. This transient facilitation for associative plasticity in adult-born neurons could have two consequences; 1) synaptically-connected adult-born neurons make a unique contribution to information processing mediated by the dentate gyrus; and 2) adult-born neurons have an advantage in the competition with mature DGCs for stability of afferent and efferent synaptic connections [40, 42].

2.5 Maintenance of adult-born dentate granule cells

After adult-born DGCs establish stable synapses, they can survive for at least 6–11 months in rodents, and only a small fraction of cells are further eliminated by programmed cell death [26, 48]. Considering the 2–3 year life span of rodents, the evidence suggests that adult-born DGCs remain a part of the mature dentate circuitry throughout life. Whole-cell recording in acute slices prepared from adult animals showed that adult-born neurons, once they reach full maturation, appear to exhibit basic electrophysiological properties indistinguishable from those of DGCs formed in embryonic and early postnatal stages [49, 50]. However, it is possible that there may be structural differences in synaptic patterning between adult and perinatal-born neurons that could alter the input-output relationship in a more physiological context. For example, adult-born neurons may be predisposed toward establishing certain synaptic contacts due to enhanced plasticity and the need to compete with existing neurons during synapse formation. In vivo recording of adult-born DGCs at different developmental stages will be necessary to determine if adult neurogenesis produces a population of dentate granule neurons that become functionally equivalent to the pre-existing population of granule cells.

3. Timing of the functional involvement of adult-born DGCs

The dentate gyrus of the hippocampal formation has been implicated in the regulation of emotion and cognition. Lesions or manipulations of this region in rodents can alter anxiety levels and affective and cognitive-like behaviors [51–53]. Many of the behavioral tests used in functional assays of neurogenesis were designed to measure hippocampal-dependent behavior and general anxiety and depressive-like symptoms (Table 1). Computational and theoretical models of specific functions of the dentate gyrus have primarily focused on the proposed orthogonalization of inputs mediated by this region, which allows for pattern separation and the ability to discriminate between similar events [9]. For example, an expansive population of adult-born neurons could be involved in disambiguating events by providing a non-diminishing pool of dentate cells available for encoding novel experiences. In this way, different patterns could be successfully represented without overlap or distortion by distinct dentate granule cell populations. Recently, it was argued that adult-born neurons could also play a role in the temporal integration of events that occur closely in time and that the enhanced plasticity of young neurons effectively provides a timestamp for experiences [9, 54]. This temporal tagging hypothesis asserts that disambiguation of similar events can result from associating an event with a population of adult-born neurons at a distinct stage of maturation. This would occur during the period of heightened plasticity and result in a neural representation with embedded temporal reference information. The general proposal that the capacity to encode information scales with synaptic plasticity is compelling but it is unknown whether this enhanced plasticity translates into decreased stimulus selectivity and/or more robust or longer-lasting potentiation of effective synapses in the behaving animal. In vitro slice recording data appears to support both interpretations. LTP is both easier to induce and results in a higher amplitude response following high frequency stimulation when newborn cells are 4–6 weeks old [45]. However, the lasting properties of this potentiation are difficult to measure in a slice preparation. Determining how this critical period affects the acquisition and long-term expression of memory is one of the outstanding questions in the field of adult neurogenesis.

To date, three major experimental approaches have been used to evaluate the role of newborn neurons in hippocampal-dependent behavior. First, after birth-dating dividing cells via BrdU, EdU or GFP-tagged retrovirus injections, functional involvement of labeled DGCs can be identified based on co-labeling with markers for IEGs. IEG expression has been widely used as an index of neuronal activation following controlled exposure to environmental stimuli or direct stimulation of specific brain regions [55] and can provide information concerning the magnitude and timing of the involvement of newborn DGCs in response to a particular experience. Second, the rate of neurogenesis or the survival of newborn neurons can be increased or decreased by several factors, such as exercise, environmental enrichment, antidepressant treatment, aging and stress; and behavioral responses of animals with different levels of adult neurogenesis can then be compared. Third, elimination of adult neurogenesis has been largely achieved through irradiation and methylazoxymethanol acetate (MAM) treatment to target dividing cells or through genetic modifications to target progenitor subtypes [56]. Each of these ablation techniques has advantages and disadvantages, but the shared rationale is that effective removal of adult

neurogenesis from the hippocampus will result in behavioral changes that should be indicative of the function of this population in the intact animal. We discuss the benefits and limitations of each of these approaches in identifying the contribution of newborn dentate granule neurons to behavior.

3.1 IEG expression

IEGs are transiently induced in the adult dentate gyrus for a few hours following electrical and pharmacological stimulation or exposure to a novel environment [57–59]. One of the distinct advantages of using IEG expression as a readout of the involvement of newborn neurons is that activation can be linked to an age-restricted subset of neurons based on coincident birth-dating. In mice, the initiation of a significant IEG response at the population level to behaviorally relevant stimuli does not occur until newborn granule cells are at least 3 weeks old [12, 60]. Although early studies based on IEG quantification had shown that there is a preferential activation of adult-born neurons in some tasks [61], emerging data paints a different picture. Other studies report that adult-born DGCs, once reaching the stage of heightened plasticity, are recruited at the same rate as embryonically-derived DGCs [12, 62]. Once the newborn neurons become responsive, recruitment of newborn neurons in learning a spatial task, such as the Morris water maze (MWM), gradually increases as the new neurons become mature [12]. These data thus suggest that the involvement of newborn neurons in learning and memory may reach asymptotic levels at the same time when plasticity thresholds are lowered, rather than exhibiting a transient peak in activity that correlates with enhanced plasticity.

Retrieval of remote spatial memory was shown to activate neurons that were less than two weeks old at the time of training, demonstrating the involvement of newborn neurons during memory expression, despite having been functionally immature at the time of encoding [63]. This would suggest that DGCs, regardless of developmental origin, are activated at the same rate and that involvement of new DGCs in behavioral tasks is based on functional maturation. Re-exposure to task features may occur when a completely distinct population of newborn cells is in the critical period of enhanced plasticity. If subsets of DGCs are associated with particular stimuli, it is unclear how the mature population that encoded the previously experienced environment would compete with recently born DGCs that are also primed to respond, but as though it were a novel context.

Contrary to these data which minimize the “uniqueness” of the newborn neurons, a recent study made the provocative claim that the most, and perhaps only, functional and behaviorally relevant population of neurons in the dentate gyrus consists of recently born DGCs, as mature cells are phased out and become unresponsive [64]. If most mature DGCs no longer contribute to neural representations mediated by the dentate gyrus, it is unlikely that a particular population of newborn neurons is linked to a specific memory trace for an unbounded time. In this study, IEG expression was quantified following either re-exposure to the same environment at several time points or a series of novel environments, and the analysis showed that only a small number of granule cells are active in any environment, suggestive of a restricted pool of readily available neurons. If young neurons remain associated with particular events as they mature, as predicted by the temporal tagging

hypothesis, then re-exposure would result in more cumulative activation due to the inclusion of additional populations entering the critical period at each time point. The authors instead observed that the total number of active cells was independent of previous exposure to the experimental contexts. The interpretation of these data was that the critical determinant of DGCs involvement in hippocampal-dependent memory is the age of the cell, with new populations being recruited almost exclusively.

3.2 Physiological regulation of neurogenesis

The rate of adult neurogenesis is modulated by various factors: activities such as voluntary wheel running and learning, or prolonged exposure to enriched environment, stress, hormones, antidepressants and neurotransmitters [65, 66]. Up- or down-regulation of adult neurogenesis can, in turn, affect behavioral performance in some tasks. When animals are exposed to an enriched environment or exercise for 3–4 weeks, the numbers of newborn DGCs can increase up to 50% [67–70]. Under these conditions, spatial learning and memory are enhanced in the MWM and radial arm maze. When mice are given accelerating rotarod training for 5 days, adult neurogenesis is increased approximately 40% [71]. Subsequently, instrumental conditioning is enhanced in the pre-trained group, whereas trace eye-blink conditioning, a hippocampal-dependent task, is unaffected. Therefore, not all types of hippocampal-dependent learning are enhanced following exposure to pro-neurogenic stimuli. Although the impact of increased neurogenesis on behavior appears to be task-dependent, there is a consistently positive effect on the survival of newborn neurons born during a restricted time period before exposure to explicit learning protocols, exercise or environmental enrichment. Converging evidence suggests that activity-driven reduction in programmed cell death is most prominent between one and three weeks after the birth of the neurons [60].

A decline in neurogenesis of 32–70% can be induced by physiological insults, such as restraint stress for 21 days or sleep disruption [72, 73]. Functional consequences include impairments in spatial reference memory in the Barnes maze and a radial arm maze. Although sleep deprivation appears to have a consistently negative effect on neurogenesis and behavior, the behavioral effects of stress depend on the species, induction protocol, and duration of exposure [60, 74]. The data thus far suggests that stress and sleep disruption are most detrimental to the proliferation and survival of recently born neurons. Whether these conditions also alter the likelihood of newly matured neurons to be recruited during the formation of hippocampal-dependent memory remains to be determined.

One challenge in identifying the functional role of these exogenous regulators is that many of these treatments affect many physiological processes that have independent effects on learning and memory. In addition, these treatments have the most pronounced effect when administered chronically and thus it is difficult to target an age-constrained population of adult-born cells. It is therefore difficult to argue for a causal link between a specific effect on neurogenesis and changes in behavior without dissociating the neurogenic and systemic effects of the manipulation. Recent studies to explicitly test this relationship have shown that environmental enrichment can enhance learning and decrease anxiety-related behavior in the absence of adult neurogenesis [75], and conversely, that enhanced neurogenesis is not

sufficient to induce anxiolytic-like behavior [76]. However, it has also been reported that suppressing neurogenesis during exposure to an enriched environment blocked long term memory enhancement [77]. More data is needed to resolve whether the impact on neurogenesis mediates the neural changes that underlie exogenous modulation of cognitive and affective behaviors.

3.3 Targeted ablation and increase of adult-born dentate granule cells

Because the population of newborn granule neurons is distributed throughout the dentate gyrus, it is impossible to selectively target these cells using traditional lesion and inactivation methods (Table 1). Instead, ablation techniques have been employed that take advantage of one of the unique properties of this population, i.e. cell division. MAM, an anti-mitotic agent, was first used to induce a targeted ablation of adult-born neurons [78–80]. After 2 weeks of treatment, the population of newly born DGCs is reduced over 75%. In rats treated with these drugs, hippocampal-dependent trace eye-blink and fear conditioning are impaired. Other approaches include injection of the anti-neoplastic agent cyclophosphamide, or neurotoxin 192 IgG-saporinin, which also leads to a reduction in the number of proliferating cells by 50–80% [81–83]. Following these treatments, spatial memory in the water maze, fear conditioning in the passive avoidance test, and object memory are severely impaired.

Because of the detrimental systemic effects of all of these drug treatments, cranial irradiation has been recently become the most prevalent means of neurogenic ablation. Actively dividing cells are sensitive to irradiation and undergo apoptosis, so it is possible to selectively ablate proliferating neural progenitors and neuroblasts with minimal damage to nearby mature neurons, glia or endothelial cells [84–88]. After irradiation, proliferating cells are reduced 70–95% and the number decreases further for another 2–3 months [78, 89]. Thus, this manipulation allows investigation of the cumulative contribution of adult-born DGCs at various time intervals from the onset of ablation. Interestingly, 2–4 weeks following either whole or focal brain irradiation in rats, contextual fear conditioning and place memory are selectively impaired, but spatial and object memory are virtually intact [90–94]. In mice, however, the most severe impairments of learning and memory have been reported to occur 2–3 months after irradiation. In the absence of adult neurogenesis for 2 months, spatial pattern separation is selectively impaired in a delayed non-matching to place task [95].

Recent evidence suggests that newly generated DGCs may play a role in the gradual decay of hippocampal-dependence of recently formed memory traces [89]. In the absence of adult neurogenesis for 5 weeks following irradiation, at a time-point when the memory trace is thought to rely on extra-hippocampal cortical structures, recall of the remote memory was still dependent on the hippocampus [89]. This result suggests that new neurons can regulate the transfer of memory to a reliance on extra-hippocampal structures, presumably to maintain the online storage capacity of hippocampus. Consistent with this hypothesis, enhanced neurogenesis speeds up the decay (clearance) rate of memory from hippocampus. Although 5 week-old new neurons express mature neuronal markers, synaptic plasticity is still higher than in the pre-existing mature neurons [45]. Taken together, we could

hypothesize that if new neurons are selectively involved in the encoding of individual events and also the effective reorganization of memory traces, then both processes may due to enhanced synaptic plasticity of newborn neurons. Because new DGCs are more excitable, they could be primed to respond to new information. Furthermore, because they actively invade and incorporate into pre-existing circuits during competitive synapse formation, new neurons could also interfere with the efficacy of previously formed synapses. Thus, this population of developing neurons may be involved in both memory formation and decay. Because the memory traces are maintained by other neural systems, the decay of memory in the hippocampus should be viewed in terms of a homeostatic process that allows for the acquisition of new information through reorganization of more remotely acquired memories, rather than complete elimination of the memory trace. It is possible that the newborn neurons are promoting hippocampal independence through an active mechanism that also ensures the fidelity of the original memory. What does seem to be clear from the irradiation studies in mice is that there may be a cumulative effect such that prolonged training intervals from the time of irradiation may reveal additional deficits. By more carefully teasing apart the time-dependence of irradiation effects, we can get a better picture of how neuronal age determines the extent of behavioral impairments.

Genetic targeting, including the expression of toxins or pro-apoptotic genes under control of neural progenitor-specific promoters, allows for the specific ablation of adult-born DGCs with minimal confounds. In addition, inducible genetic manipulations increase temporal precision, which is critical for understanding the role of adult-born DGCs in hippocampal-dependent behavior. Four lines of transgenic (Tg) mice have been developed to explore the functional involvement of adult-born DGCs. One line expresses herpes virus thymidine kinase (TK) under the regulation of the mouse GFAP promoter. Proliferating cells are reduced by 75% following chronic delivery of the antiviral pro-drug ganciclovir (GCV) for 6–10 weeks. In these mice, working memory and contextual fear conditioning are impaired, but after 10 weeks recovery in the absence of GCV, working memory has fully recovered [96, 97]. This result suggests that new neurons, under 10 weeks of age, are involved in working memory processes. When TK is expressed under the nestin promoter and enhancer, only 2 weeks of treatment with GCV results in a 50% reduction in neurogenesis and deficits in both spatial memory and contextual fear memory extinction. However, after 4–9 weeks of recovery in the absence of the drug, the behavioral impairments fully recovered [98]. Similarly, using a Tet-On inducible system, the pro-apoptotic gene, Bax, is expressed under the regulation of the nestin promoter to ablate new born neurons. Following 6 weeks of treatment with doxycycline (Dox), there is a 60% reduction in proliferating cells and spatial learning is impaired, although contextual fear conditioning remains intact [99]. Much of the data suggests that 6–10 week old new neurons are critically involved in the functional deficits following transient reduction of neurogenesis, similar to the time course of involvement shown by IEG expression. Importantly, these studies using inducible techniques also demonstrate that impaired functions can be recovered when neurogenesis is restored, thus providing a link between adult neurogenesis and specific functions. A recent study employed a targeted genetic approach to suppress endogenous expression of Bax and increase adult neurogenesis in a gain-of-function experiment. Although novel object recognition and spatial memory were unaffected, the manipulation did result in a significant

improvement in the ability of the mice to discriminate between similar contexts, suggesting that adult neurogenesis plays a role in pattern separation [76]. Despite the preponderance of evidence indicating that adult-born neurons are active between 1 and 3 months after birth, it is still difficult to identify a critical period during which these neurons play a distinctive role in hippocampal-dependent behavior from these studies. Even targeted treatments create a transient inflammatory response with potential functional consequences. Moreover, many of the ablation methods are irreversible and temporal control is limited. To cope with these limitations, new approaches are necessary to clarify the optimal timing for functional involvement and the specific role of adult born DGCs.

4. Newly advanced approach: optogenetics

Optogenetic techniques have emerged as an extremely effective and specific tool to answer some of the fundamental questions regarding the temporal involvement of adult-born neurons in behavior [100]. Through genetically controlled introduction of an opsin gene, we can control the activity of specific populations in the neurogenic regions through light-driven activation or suppression of targeted cells. Briefly, channelrhodopsin-2 (ChR2) is a light-gated, cation-permeable channel derived from *Chlamydomonas reinhardtii*, which can be activated by blue light at 470 nm. Halorhodopsin (NpHR), derived from *Natronomonas pharaonis*, is a chloride pump that responds to yellow light at 589 nm, which effectively silences NpHR expressing neurons. If both ChR2 and NpHR are simultaneously expressed in the same neurons, we can bidirectionally control the activity though two different wavelengths of light (Figure 2). This technique holds significant promise for experimental tractability in understanding how newborn neurons contribute to behavior. It is now theoretically possible to disrupt the activity of this population acutely during episodes of encoding and recall to determine how this dynamic granule cell population contributes to hippocampus-dependent information processing. In addition to examining behavioral effects of disrupted signaling in adult-born cells, we can also begin to address the downstream effects on neural processing in an intact system. By recording from adjacent areas and efferent targets of the hippocampal formation, we can monitor whether activation or suppression of this group of cells may impact population activity, long-range synchronous responses and oscillatory phase-dependent firing within the hippocampus. We anticipate much progress in the effort to understand why new neurons are necessary, when they become involved in hippocampal function, and how they contribute to specific forms of learning and memory. One potential drawback of this technique is that chronic disruption of newborn granule cell activity will be more technically challenging to achieve. Although the temporal resolution is sufficient to perturb activity on a millisecond scale and therefore ideal for acute investigations during task performance, it does not easily allow for ongoing manipulation outside the experimental setting. This will be critical to evaluate the involvement of adult-born neurons in systems-level consolidation or offline memory reorganization. Another serious challenge will be to ensure that a sufficient portion of the population of newborn neurons is targeted through acute viral injections. The appeal of in vivo studies of function is largely derived from the fact that neuronal networks remain intact, which makes it possible to investigate mechanisms in a physiological context. But if a virally-mediated manipulation affects only a subset of targeted cells, then there could be

consequences owing to a perturbation of the network properties that do not reflect the endogenous function of the population as a whole. The most informative aspect of optogenetic manipulation of newborn neurons may be to identify how single cells respond to activity and environmental demands. Understanding the most basic properties of adult-born neurons, even in a cell-autonomous manner, could lead to new hypotheses of how this dynamic population could impact hippocampal-dependent behavior.

5. Conclusion and perspective

Adult neurogenesis recapitulates embryonic and early postnatal neurodevelopment and shares many underlying mechanisms, but the functional significance of this phenomenon in the mature brain is not well understood. In terms of hippocampal neurogenesis, in particular, the challenge to identify its functional role is further complicated by our limited understanding of how the dentate gyrus itself contributes to cognitive and affective-like behaviors. Numerous studies support the notion that adult neurogenesis positively correlates with many aspects of learning and memory and that disrupting this phenomenon can lead to selective deficits in some forms of hippocampal-dependent memory. Beyond a desire to understand how adult neurogenesis contributes to the processing capacity of the dentate granule cell network, a mechanistic description of how newly introduced neurons are incorporated into the local circuitry may be generalizable to stem cell-mediated therapeutic strategies for neuronal replacement. But one of the most critical questions that remains to be addressed is how these two populations – the dynamic, regenerative, adult-born neurons and the fully integrated, mature, perinatal-born neurons – interact to enhance, or regulate, hippocampal function. The continuous birth of new neurons in the dentate gyrus results in a strikingly plastic structure that is rare in the adult mammalian brain in the absence of pathology. At any given moment, this region is comprised of DGCs that cover an entire spectrum of ages as old as the organism itself and as young as a few hours old. It seems that the unique intrinsic features of newborn DGCs such as an initial phase of atypical GABAergic depolarization compared to surrounding cells, competition for synaptic integration with mature neurons, and finally, a significant period of enhanced plasticity, are designed to maximize the likelihood of survival of newborn neurons in a potentially less hospitable developmental environment in the adult brain. What we still need to understand is why this particular region is so highly neurogenic and how these features of young neurons can be co-opted to enhance memory formation and behavioral modification. It is precisely this juxtaposition of continuously evolving neuronal populations against a background of a structurally stable dentate gyrus that suggests discrete time-limited and age-dependent roles of DGCs. Only by transiently, and reversibly, perturbing the intercellular communication between these populations in a systematic way, can we isolate the impact of one group of cells on the rest of the circuitry in real-time. At that point, we can begin to unravel the fine-grained interactions between mature and newborn neurons and begin to build a comprehensive picture of how these populations may interact to optimize the hippocampal function.

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- Adult neurogenesis continues throughout life but its physiological role is still uncertain
- Contribution of newborn neurons to behavior is regulated by dynamic intracellular environment
- Increased temporal resolution is required to assess role of adult-born cells in learned behavior
- Determining process of functional integration can inform research of stem cell therapeutics

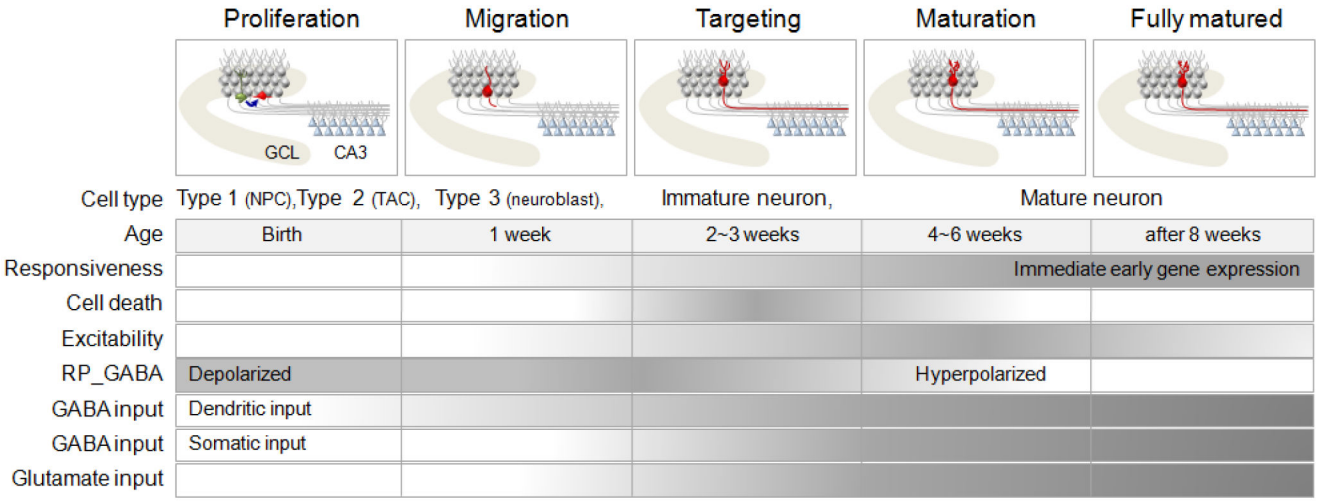


Figure 1. Development of adult-born dentate granule cells.

Top, Morphological maturation of adult-born DGC (red) after birth from neural precursors (green, left). Bottom, Newborn neurons migrate and integrate into the dentate circuit. Axons are elongated and contact the pyramidal cells of CA3 after 1–2 weeks and spines start to appear after 2 weeks. During synaptogenesis, new neurons compete to survive and many are eliminated by programmed cell death. The physiological properties of new neurons reflect a gradual change in the expression of chloride transporters during the maturation process. Immature neurons are initially depolarized by GABA and then hyperpolarized after maturation. These GABAergic inputs are initially dendritic but upon maturation and synapse refinement from afferent pathways, functional perisomatic inputs develop. Lastly, following the expression of NMDA receptors, new DGCs become activated by glutamatergic inputs. NPC: neural progenitor cell, TAC: transient amplifying cell, GCL: granule cell layer, RP-GABA: reversal potential of GABA.

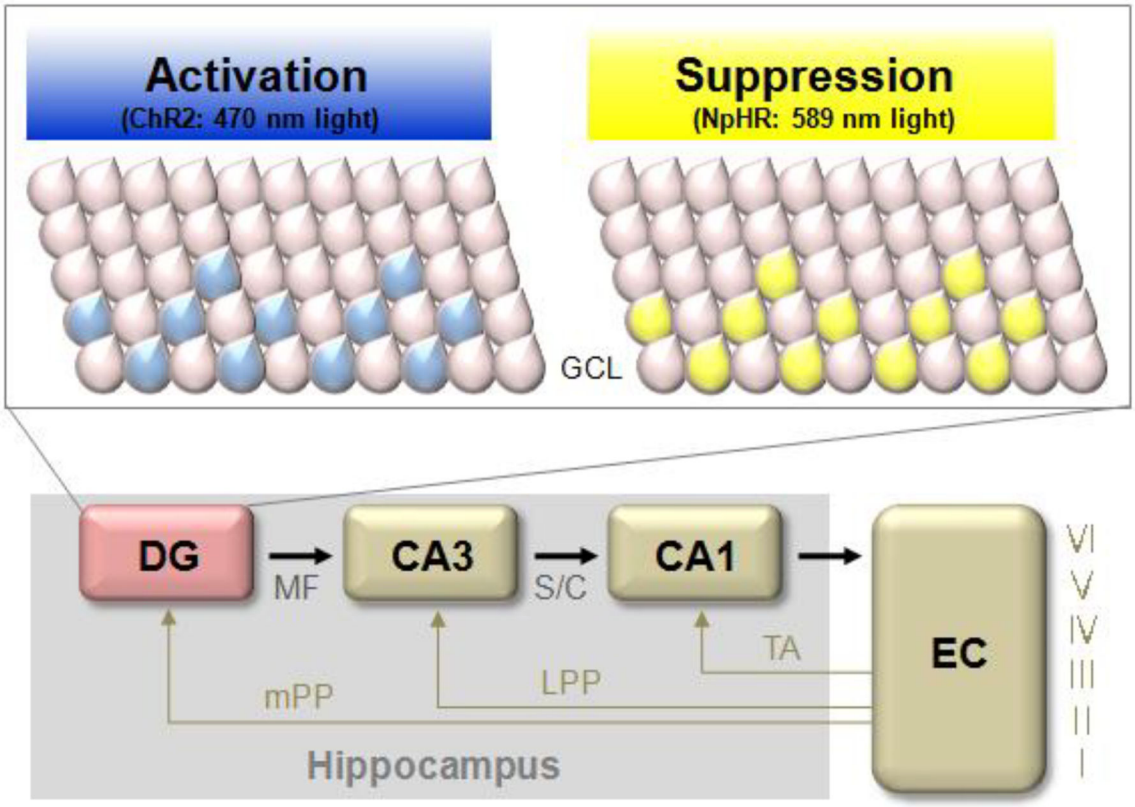


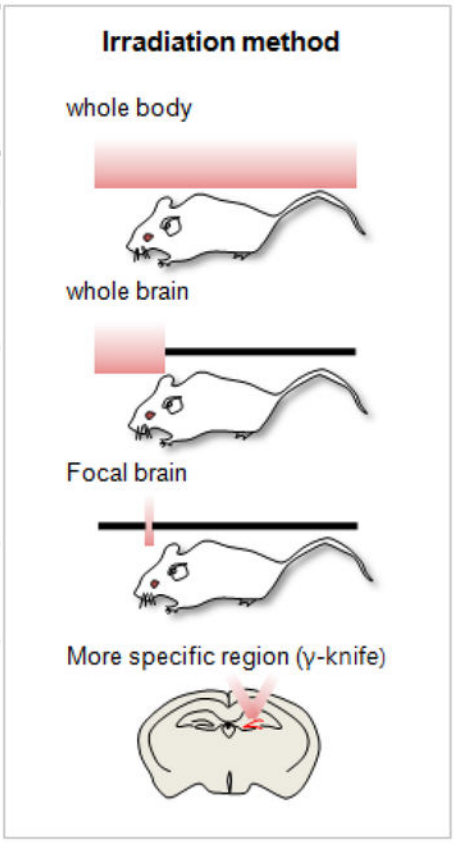
Figure 2. Optogenetic approach to clarify the timing for functional involvement of adult-born DGCs.

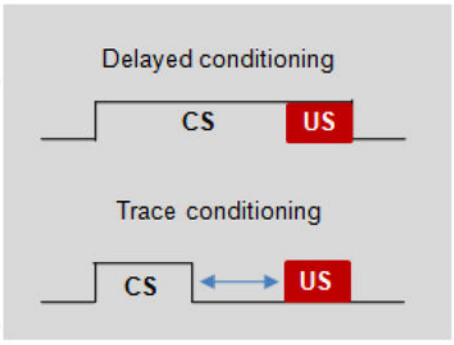
Top, Optical manipulation of neuronal activity with light-sensitive rhodopsins: Blue light (470 nm wavelength)-induced neuronal activation by channelrhodopsin-2 (ChR2), a cation channel (left). Yellow light (589 nm)-induced neuronal suppression by halorhodopsin (NpHR), a chloride pump, derived from *Natronomonas pharaonis* (right). GCL: granule cell layer. Bottom, Hippocampal circuitry and afferent connections from the entorhinal cortex (EC). Granule cells of the dentate gyrus (DG) project their axons, mossy fibers (MF), to the pyramidal cells of CA3. CA3 neurons target CA1 pyramidal neurons via the Schaffer collateral pathway (S/C). CA1 neurons also project back to the EC. The EC sends cortical information and hippocampal feedback to the DG through the medial perforant pathway (mPP), to CA3 through lateral the perforant pathway (LPP) and directly to CA1 through the temporoammonic pathway (TA). Some memories appear to have a temporary dependence on the hippocampus before cortical structures are capable of mediating the long-term maintenance of the memory trace.

Table 1.

Common behavioral tests used to evaluate the function of adult neurogenesis in rodents

Target function		Test
Anxiety		Open field Elevated plus maze Light-dark box
Affective state		Forced swim Tail-suspension Novelty suppressed feeding
Activity/Locomotion		Open field Home cage activity
Associative learning		Delay eyeblink conditioning Passive/active avoidance Contextual discrimination
Hippocampal-dependent	Spatial	Morris water maze Barnes maze Radial maze Object-location recognition
	Trace	Eyeblink conditioning Fear conditioning





CS: conditioned stimulus;
US: unconditioned stimulus

Table 2.

Timing of functional involvement of adult-born neurons in behavior

State	%	SP	Treatment	Time interval	Impaired	Enhanced	No effect	Ref
Increased NG	40	mice	Physical enrichment (36rpm/5days)	**5 d**		Instrumental	Trace eyeblink	(72)
	42	mice	NR2B antagonist (Ro25–6981) ip.	**1 mo**		WM		(100)
	NM	mice	Enriched environment (60 days)	**4 mos**		WM		(71)
	30	mice	Enriched environment (3 wks)	**3 wks EE**		WM, RAM, PA		(68)
	60	mice	Voluntary wheel running	**42 d**		WM		(101)
	47	rats	Enriched environment (4 wks)	**4 wks EE**		WM		(69)
Decreased NG	50	mice	Cyclophosphamide	**12 hrs**	PA, Object			(82)
	50	mice	Cyclophosphamide	**10 d**			PA, Object	(82)
	27	mice	NR2A-containing NMDA receptors inhibitor (NVP-AAM077), (34 days)	**34 d**	WM			(80)
	32	rats	Sleep fragmentation (12 days)	**1 mo**	Barnes			(74)
	70	rats	Ozone exposure (4 hr/90 days)	**3 mos**	PA, Context fear			(102)
	30	rats	Olfactory bulbectomy	**1.5 mos**	PA			(103)
	70	rats	Restraint stress (6 hr/day, 21 days)	**21 d**	RAM			(75)
	80	rats	Neurotoxin (192 IgG-saporin)	**1 mo**	WM			(81)
	74	rats	Dominant-negative WNT Lentivirus	**2 mos**	WM, Object			(104)
Ablation by irradiation	74	mice	Whole-body (2 Gy), single	**1 d**	PA, Object		Open	(105)
	74	mice	Whole-body (2 Gy), single	**3 d**	Object		PA	(105)
	74	mice	Whole-body (2 Gy), single	**5 d**			PA	(105)
	77	mice	Whole brain (5 Gy), single	**3 mos**	WM		Barnes, Object	(106)
	68	rats	Whole brain (7.5 Gy), 2 / 2 days	**2 wks**			T-maze	(89)
	90	rats	Whole brain (7.5 Gy), 10 min/2 days	**4 wks**	Context fear			(90)
	50	rats	Whole brain (8Gy), then running	**5 wks**	Context fear		WM	(91)
	95	rats	Whole brain (10 Gy), 10 min/2 days	**4 wks**			WM	(92)
	75	gerbils	Focal brain(10 Gy,1 M), EE (2 M)	**3 mos**	WM, then recover			(107)
	95	mice	Focal brain, (5 Gy), 3 times	**2 mos**	RAM: spatial separation			(94)
	60	mice	Focal brain (5 Gy), 3 times	**3 mos**		RAM: working		(95)

State	%	SP	Treatment	Time interval	Impaired	Enhanced	No effect	Ref
	78	mice	Focal brain (5 Gy), 3 times, (after 2 months) 54 days running	**2 mos +54d**	WM		Context fear	(108)
	85	mice	Focal brain (5 Gy), 3 times	**3 mos**	Context fear		WM,Y-maze	(96)
	80	mice	Focal brain (10Gy), single	**3 mos**			Fear extinct.	(77)
	88	mice	Focal brain (20Gy), single	**3 mos**	Fear retention		Fear extinct.	(77)
	43	rats	Focal brain (7.5 Gy), 2 times/2 days	**9 wks**	Context fear			(89)
	95	rats	Focal brain (4.58 Gy), 8 days	**3 wks**	Place			(93)
	95	rats	Focal brain (4.58 Gy), 8 days	**2 wks**			WM, Object	(93)
	95	rats	Focal brain (4.58 Gy), 8 days	**7 wks**			Place	(93)
Ablation by MAM	75	rats	MAM (2 wks)	**2 wks**	Trace fear (30 s)		Delayed fear	(79)
	75	rats	MAM (2 wks)	**2 wks**			WM	(79)
	75	mice	MAM (2 wks)	**2 wks**			Fear	(77)
Ablation by transgenic expression of toxins	75	mice	GFAP-TK	**GCV: 6 wks**	Context fear		WM,Y-maze	(96)
	75	mice	GFAP-TK	**GCV: 10 wks**		RAM: working		(95)
	75	mice	GFAP-TK (GCV:10 wks)	**10 wks recover**			RAM: working	(95)
	50	mice	Nestin-TK (GCV:2 wks)	**1 wk**	WM, Fear extinct.			(97)
	50	mice	Nestin-TK (GCV:2 wks)	**3.5, 9 wks**			WM (1 wk)	(97)
	50	mice	Nestin-TK (GCV:2 wks)	**5 wks**			Fear extinct.	(97)
	60	mice	Nestin-rtTA/TRE-BAX	**Dox: 6wks**	WM		Context fear	(98)
	90	mice	Nestin-CreER/NSE-DTA (TM:4 days)	**1.5 mos**	Fear, Barnes			(109)

NG: neurogenesis, SP: species, EE: enriched environment, WM: water maze, PA: passive avoidance, RAM: radial arm maze, Fear extinct: fear extinction, Gy: gray, MAM: methylazoxymethanol acetate, GCV: Ganciclovir, Dox: Doxycycline, TM: Tamoxifen, NM: not measured