Formation and integration of new neurons in the adult hippocampus

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Abstract | Neural stem cells (NSCs) generate new neurons throughout life in the mammalian brain. Adult-born neurons shape brain function, and endogenous NSCs could potentially be harnessed for brain repair. In this Review, focused on hippocampal neurogenesis in rodents, we highlight recent advances in the field based on novel technologies (including single-cell RNA sequencing, intravital imaging and functional observation of newborn cells in behaving mice) and characterize the distinct developmental steps from stem cell activation to the integration of newborn neurons into pre-existing circuits. Further, we review current knowledge of how levels of neurogenesis are regulated, discuss findings regarding survival and maturation of adult-born cells and describe how newborn neurons affect brain function. The evidence arguing for (and against) lifelong neurogenesis in the human hippocampus is briefly summarized. Finally, we provide an outlook of what is needed to improve our understanding of the mechanisms and functional consequences of adult neurogenesis and how the field may move towards more translational relevance in the context of acute and chronic neural injury and stem cell-based brain repair.

Granule cell layer
The granule cell layer consists mainly of excitatory granule cells, the principal neurons of the dentate gyrus.

The long-held belief that neurons are exclusively generated during embryonic and early-postnatal development was challenged in the 1960s by the discovery of newborn neurons in the adult rat brain[1]. After decades of controversy that was overcome only by improved methodology[2], adult neurogenesis has been since investigated from many different angles, including the potential of neural stem cells (NSCs) to produce neurons throughout life, the integration of newborn neurons, its contribution to behavioural output and its potential role in various diseases affecting the adult brain[3,4].

Adult neurogenesis has been reported throughout the animal kingdom, ranging from crustaceans to higher vertebrates, including birds, rodents, primates and humans[2,5–10]. In this Review, we focus mainly on adult neurogenesis in rodents, but the controversy regarding the evidence for human neurogenesis is summarized (BOX 1). Whereas the amount of widespread adult neurogenesis decreases as we progress up the phylogenetic tree, neurogenic areas also become more defined, arguing for an evolutionarily conserved plasticity in those specific regions[11]. In rodents, adult neurogenesis is found mainly in two brain regions. The first is the subventricular zone, which lines the lateral ventricles, where NSCs give rise to cells that tangentially migrate to the olfactory bulb before they differentiate into different types of olfactory neurons[4,12]. The second main neurogenic region is the subgranular zone of the hippocampal dentate gyrus (DG), where NSCs generate excitatory, glutamatergic neurons that integrate into the granule cell layer (GCL)[3] (FIG. 1). Besides these two main neurogenic areas, neurogenesis has been reported in the hypothalamus and the brainstem, and might exist in the neocortex, striatum, amygdala and substantia nigra of rodents and other mammals; however, the existence of neurogenesis in the latter regions, and also within the human brain, as outlined in BOX 1, remains controversial[10,13–15]. Here we will focus on adult neurogenesis in the rodent hippocampal DG.

During the process of neurogenesis, cells undergo dramatic molecular and morphological changes. NSCs divide and generate committed neuronal progenitors, which then differentiate into immature neurons that integrate into the DG circuit, where they mature over the course of several weeks in the rodent brain[16–18]. At each stage, cell-intrinsic signalling and inputs from the surrounding niche regulate the transition to the next cellular state, leading to selection and successful integration into the circuitry, or cell death. In the following sections, we decipher each step from NSC activation to functional integration of newborn neurons, summarize known regulators affecting NSC activity and neuronal survival, and discuss open questions in the field.

Stem cell identity and potential

The source of newborn hippocampal neurons is NSCs, also commonly referred to as neural stem or progenitor cells, owing to the still-controversial long-term self-renewal capacity and in vivo multipotency of individual hippocampal precursors[19–21]. Isolated from

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Box 1 | Neurogenesis in the human hippocampus

On the basis of thymidine analogue labelling, carbon dating and expression analyses of proteins expressed in neural stem cells and immature neurons, several studies have reported neurogenesis to occur throughout the lifespan in the human hippocampus[5–8,14,53,54]. However, others have questioned these findings using immunohistochemical detection of markers expressed in immature neurons, such as doublecortin (DCX)[168]. Not only the presence of immature neurons (indicative of neurogenesis) is debated; there is also controversy regarding the presence of neurogenic progenitor cells and a respective niche in the human hippocampus. Whereas some groups have reported the presence of proliferating cells with characteristics similar to those of neurogenic cells found in the rodent brain, others have not found evidence for neurogenic cell divisions in the human hippocampus[8,53,54,168,169]. Furthermore, labelling cells that express markers of 'immaturity' such as DCX may label cells that are not newborn but that retain such a molecular (and potentially also a functionally immature) profile over extended periods — a cellular phenotype that has been described in a number of cortical areas in different species[170,171]. Together, these findings reignited discussions of whether neurogenesis is of relevance for human physiology and disease, similar to the controversy that existed when neurogenesis in rodents and primates was at the centre of scientific discussions in the 1980s[2,164,165,168,172,173].

One issue in addressing the controversy is the scarcity of healthy human tissues and delays when fixing post-mortem samples for tissue analyses[165]. Furthermore, none of the techniques to detect and visualize newborn cells is perfect and free of experimental error[164,165,172]. For example, one argument is that thymidine analogues may also cause low-frequency labelling of cells that are not newborn, although there is evidence that this may not cause substantial concern, at least in rodents[174].

These are not unique problems for the field of neurogenesis; indeed, the direct translation of rodent-based experimental data to human brain function is by definition challenging and complex. The evidence for and against the birth of new neurons in the human hippocampus has been extensively discussed recently[2,164,165,172]. Previous attempts to use non-invasive imaging approaches to detect neurogenesis certainly require further validation[162,163]. Thus, novel technologies, including single-cell genomics[48], are needed to evaluate the existence of neurogenic neural stem cells in the adult human brain and the potential mechanisms and roles of hippocampal neurogenesis in physiology and disease.

the adult mouse brain and cultured in vitro with the addition of mitogens such as fibroblast growth factor (FGF) and epidermal growth factor (EGF), NSCs can be propagated eternally[22]. On withdrawal of growth factors, cultivated NSCs can differentiate into neurons, astrocytes or oligodendrocytes[22,23], demonstrating the potential for unlimited self-renewal and multipotency of in vitro NSCs derived from the adult mouse hippocampus. However, growth factors might stimulate cellular behaviour that would rarely occur naturally within the endogenous niche; for example, the addition of insulin-like growth factor 1 (IGF1) pushes adult NSCs to differentiate into oligodendrocytes[24].

In vivo, the fate and potency of individual hippocampal NSCs remain controversial. With use of thymidine analogues (such as 5-bromo-2′-deoxyuridine (BrdU)), transgenic labelling or viral labelling, NSCs have been identified in the subgranular zone underlying the GCL[21,25]. In the rodent DG, hippocampal NSCs display certain astrocytic features, such as branched morphology and vascular end-feet, as well as passive current characteristics that are comparable to those of astroglial cells[25,26]. Furthermore, hippocampal NSCs extend radial processes, reminiscent of those of radial glial cells (which are neural progenitors in the embryonic brain)[27], that reach into the GCL and express a distinct set of proteins that are also expressed in classical astrocytes, including the transcription factor SOX2 and glial fibrillary acidic protein (GFAP)[28–30]. On the basis of their morphology, glia-like cellular properties and gene expression profiles, hippocampal NSCs are often referred to as radial glia-like cells (R cells, or type 1 cells) (FIG. 1).

R cells in rodents are found mostly in a non-dividing, quiescent state and, once activated, they generate non-radial glia-like progenitor cells (NR cells, or type 2 cells) through cell divisions that divide again and subsequently differentiate into neurons[31–33]. The cellular behaviour of R cells — in particular, their mode of division, process of lineage progression and differentiation within the adult brain — has been assessed by various techniques (TABLE 1). New technologies, such as retroviral labelling to characterize newborn granule cells in acute hippocampal slices, have enabled the field to gain novel insights[18]. However, each technique comes with advantages and disadvantages, leaving grounds for speculations and interpretations (TABLE 1).

Lineage tracing using transgenic reporters to label lineage-related cells has been used to determine the output of activated R cells[19,21,34–37]. However, these approaches led to ambiguous results for key questions in the field, such as the self-renewal potential of individual NSCs: whereas some studies reported extended self-renewal of R cells, including symmetric, duplicating R cell divisions and multipotency in terms of generating neurons and astrocytes, others reported a relatively fast depletion of R cells, with these cells giving rise to a few neurons before terminal differentiation[19,21,35]. The different outcomes might depend on the choice of reporter lines, suggesting considerable heterogeneity of NSCs. The different reports may also depend on whether lineages were studied on the level of individual clones (in which case coexistence of R cells and NR cells would imply self-renewal capacity) or whole cohorts, where sufficient resolution may be lacking. Static, post hoc analyses, which are the only possible analyses when histological sections are used for example after Cre-mediated lineage tracing or genetic ablation of candidate genes, inherently miss cellular events before the final cellular outcome. Therefore, the ability to longitudinally image NSCs in the living mouse brain using two-photon microscopy has allowed the analysis of the neurogenic process over time within the endogenous niche[31]. Intravital imaging has revealed both asymmetric divisions and symmetric divisions of R cells; however, when DG NSCs expressing the stem cell-expressed Achaete–Scute homologue 1 gene (*Ascl1*) were followed in vivo using intravital imaging, most R cells were seen to terminally differentiate within 3 months after activation[31]. Most commonly, R cells generated NR cells that further amplified the pool of dividing cells, revealing a high amount of asymmetric NR cell divisions, generating proliferative NR cells that went through additional rounds of cell divisions and differentiating neuroblasts. In addition, in vivo imaging revealed that R cells can directly generate neurons through asymmetric cell division[31]. Furthermore, intravital imaging of R cells (genetically labelled by targeting neurogenic cells through the regulatory elements of the *Gli1* promoter) identified long-term (more than 100 days) self-renewing

Thymidine analogues
Analogues of the DNA component thymidine that can be injected in animals and are integrated into replicating DNA strands and detected using antibodies.

Vascular end-feet
Terminals of astrocytic processes at the vascular surface that regulate vascular function.

Asymmetric divisions
Divisions generating daughter cells with different fates or properties.

Symmetric divisions
Divisions generating daughter cells with similar or identical fates or properties.

neurogenic cells in the adult mouse DG[38]. Together, intravital imaging studies have identified previously unknown steps in the highly dynamic process of neurogenesis but also confirmed some of the previously held notions regarding lineage relationships that were based on static lineage-tracing experiments.

Therefore, numerous studies using various techniques such as thymidine analogue labelling, genetic

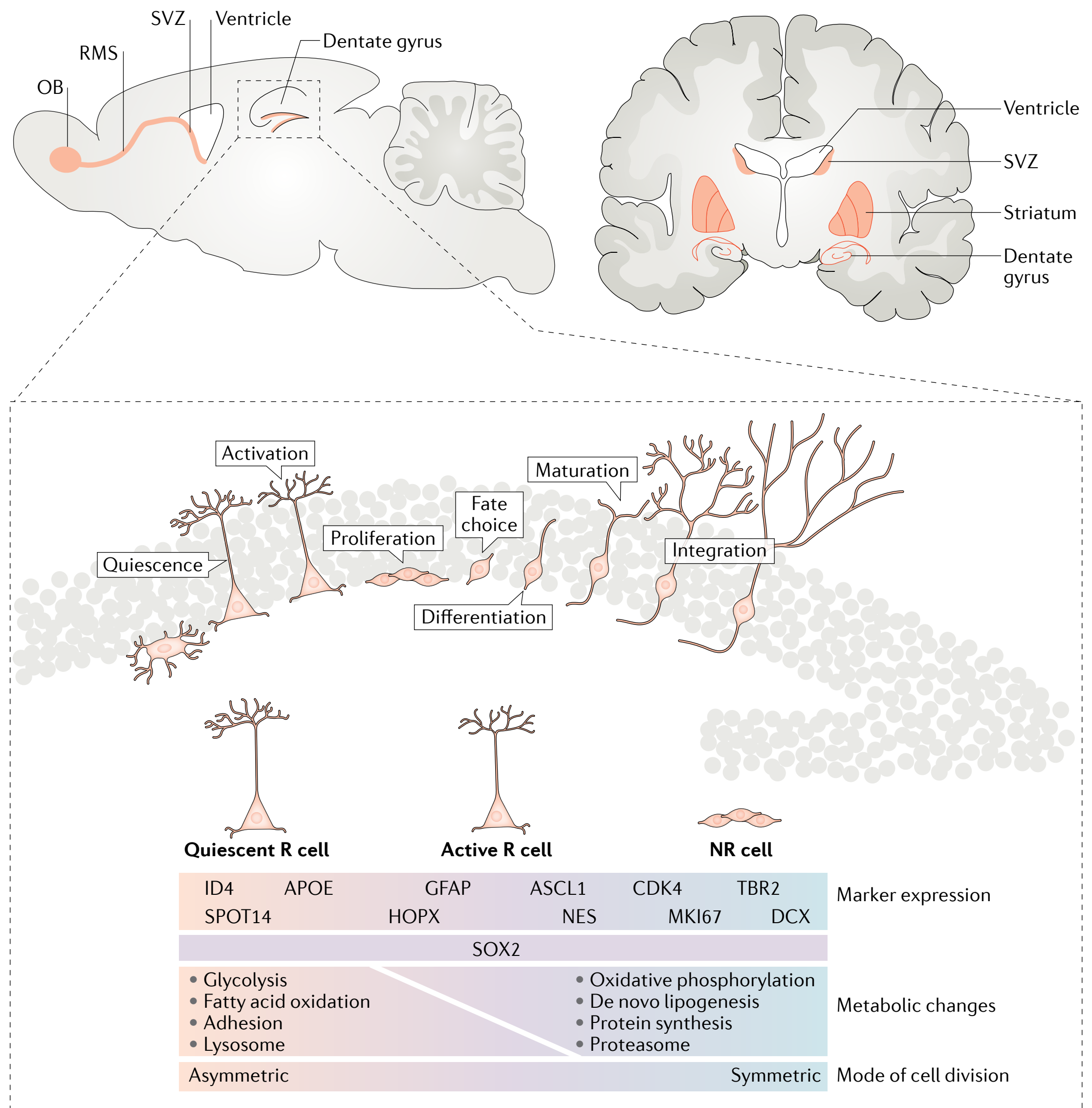


Fig. 1 | **Neurogenesis in the adult hippocampus. a** | Schematized view of a sagittal section through the mouse brain showing the two main neurogenic areas of the adult rodent brain. Neural stem cells (NSCs) reside in the subventricular zone (SVZ) and give rise to newborn cells that migrate via the rostral migratory stream (RMS) towards the olfactory bulb (OB), where newborn cells differentiate into different types of olfactory neurons. The focus of this Review is on neurogenesis in the dentate gyrus (DG) of the hippocampus (boxed). The schematic on the right shows a coronal view of the human brain highlighting areas where neurogenesis has been described in the human brain. For details regarding the controversial evidence of neurogenesis in the human DG (and other brain areas such as the striatum), see BOX 1. **b** | Over the course of several weeks, NSC-derived newborn cells in the rodent DG mature into excitatory, glutamatergic granule cells and integrate into pre-existing hippocampal circuits. NSCs in the DG have a triangular shape with a long radial process extending from the apical part of the cells; thus, they are often referred to as radial glia-like cells (R cells). Most R cells are quiescent in the adult hippocampus and express a distinct set of marker proteins (selected marker proteins are indicated). Owing to extrinsic or intrinsic regulators, quiescent R cells become activated and make first fate decisions: they symmetrically duplicate, leading to expansion of the R cell pool; asymmetrically divide to give rise to a self-renewed R cell and a non-radial glia-like progenitor cell (NR cell) (by far the most common fate of R cells); generate two NR cells; directly give rise to neurons; or, rarely, generate astrocytes[31]. R cell activation is accompanied by profound changes in gene expression and cellular metabolism, as indicated[32,42,166]. Whether R cells exist in distinct states (for example, prone towards differentiation versus self-renewal) or there are different R cell populations remains poorly understood but may be the case given the heterogeneous behaviour of R cells in the adult DG[19,21,31,38]. APOE, apolipoprotein E; ASCL1, Achaete–Scute homologue 1; CDK4, cyclin-dependent kinase 4; DCX, doublecortin; GFAP, glial fibrillary acidic protein; HOPX, homeodomain-only protein; ID4, inhibitor of DNA binding 4; MKI67, marker of proliferation Ki-67; NES, nestin; SPOT14, thyroid hormone responsive protein; TBR2, T-box brain protein 2.

Table 1 | **Technical approaches to analysing adult neurogenesis**

Approach	Description	Advantages	Disadvantages	Refs
Thymidine analogues	Incorporation of thymidine or its analogues (tritiated thymidine, BrdU, EdU, IdU or CldU) during S phase	Easy method No special equipment needed Human tissue available	Potential toxicity of analogues Label is diluted by divisions Visualizes only the nucleus (DNA)	6,60
Antibody labelling	Detection of markers selectively expressed in NSCs or their progeny (for example, nestin, DCX, NEUROD1 or PSA-NCAM)	Easy method No special equipment needed Human tissue available	Markers may be species specific Tissue fixation important	25,33
Retroviral labelling	Specific labelling of proliferating cells using Moloney murine leukaemia retrovirus	Fast method for tissue-specific manipulation of gene expression Enables live analyses in sections	Invasive method (stereotactic injections of virus) Labels only small cohorts of cells	18
Transgenic markers	Transgenic expression of fluorescent label under control of cell type-selective promoters	Labels large cohorts	Potential unspecificity of promoters used (for example GLAST and nestin)	75,161
Lineage tracing	Transgenic expression of recombinase (such as Cre) under control of cell type-selective promoters	Can label large cohorts but also trace clonal lineages	Potential unspecificity of promoters used (for example, GLAST and nestin)	21,36
scRNA-seq	Cell type-selective isolation and subsequent RNA expression analyses at the single-cell level	Provides extensive molecular information Analyses of neurogenic cells and niche possible Possible in humans	Morphological information is lost Potential alteration in gene expression due to single-cell isolation procedures	42,46
Intravital imaging	Prolonged intravital imaging using microscopy	Captures real-time dynamics of the neurogenic process	Invasive method Special equipment needed	31,154
Non-invasive imaging	Based on selective peaks in magnetic resonance spectrum or changes in blood volume associated with neurogenesis	May be used to measure neurogenesis in living humans	Specificity remains controversial No broadly accepted approach available	5,14,162,163
Carbon dating	Measures ^{14}C content in individual cell populations	Measures real age of human brain cells or tissues	Challenging method with limited availability (owing to dilution of ^{14}C in biosphere)	5,14

Advantages and disadvantages do not necessarily apply to all species analysed. For more detailed technical considerations in the context of detecting neurogenesis, especially in the human hippocampus, see BOX 1 (REFS[164,165]). BrdU, 5-bromo-2′-deoxyuridine; CldU, 5-chloro-2′-deoxyuridine; DCX, doublecortin; EdU, 5-ethynyl-2′-deoxyuridine; GLAST, excitatory amino acid transporter 1; IdU, 5-iodo-2′-deoxyuridine; NEUROD1, neurogenic differentiation factor 1; NSC, neural stem cell; PSA-NCAM, polysialylated neuronal cell adhesion molecule; scRNA-seq, single-cell RNA sequencing.

lineage tracing and intravital imaging have described the generation of neurons and astrocytes from R cells in the mouse DG. Furthermore, there is evidence that neurogenic cells in the DG are intrinsically able to generate oligodendrocytes but under normal conditions are prevented from doing so by microRNAs or the transcription factor neurofibromatosis 1 (NF1)[39–41]. Thus, NSCs in the adult DG possess bona fide multipotency, but may not use it to full capacity under physiologic conditions.

Single-cell RNA sequencing (scRNA-seq) has recently provided novel insights as it not only provides important information about the transcription in individual cells but also enables the reconstruction of developmental hierarchies by ‘lining up’ cells on the basis of their transcriptional similarity. One scRNA-seq study demonstrated that similarities in gene expression profiles could be used to reconstitute trajectories in the context of NSC activation[42]. This trajectory revealed a change in expression of transcription factors associated with NSC activation, confirming previously known regulators but also identifying novel players in this step. A molecular switch from active niche signalling and glycolysis towards oxidative phosphorylation, ribosome biogenesis and cell cycle progression was observed[42]. Furthermore, scRNA-seq may be useful to identify the molecular mechanisms underlying the heterogeneous behaviour of neurogenic cells in the DG — for example, in the context of self-renewal[19,21,38]. Hence, several studies using scRNA-seq have provided important insights into different cellular states during adult NSC activation that are accompanied by changes in gene expression, translation and metabolism[42–46].

Similarly, a combination of single-nucleus RNA sequencing and 5-ethynyl-2′-deoxyuridine labelling has

shed light on NSC transcriptional dynamics, including a shift from the expression of genes involved in proliferation to neuronal differentiation and maturation between activated NSCs and newborn neurons within the hippocampus[44,47]. Thus, although scRNA-seq is a snapshot-based approach, it can be used to examine a dynamic process such as hippocampal neurogenesis, even in the human brain[48].

Although scRNA-seq-mediated lineage trajectories have shed light on different cellular states during this process and identified cell type-specific genes, they inherently miss information on cellular behaviour within each state. A combination of lineage tracing or imaging and scRNA-seq will help us to better understand the differentiation process from quiescent NSCs to newborn neurons in the adult DG[38]. Furthermore, novel techniques enabling the investigation of epigenetic landscapes and single-cell proteomes will complement the insights gained by scRNA-seq[49,50]. Together, the approaches used thus far allow us to conclude that NSCs, although mostly found in a quiescent state, can be activated to divide and to generate new neurons and astrocytes in the adult hippocampal niche.

Regulation of neurogenesis

Numerous different conditions, factors and genetic manipulations have been found to influence adult neurogenesis (FIG. 2). Whether they affect NSCs intrinsically or act through the hippocampal niche is often difficult to entangle[3]. Decreases in neurogenesis have been observed to be driven by diverse genetic aberrations as well as extrinsic factors such as drugs, stress and inflammation[3,51,52]. Furthermore, altered neurogenesis has been observed in animal models of, and also partly in tissues from humans with, various neurological and psychiatric disorders, including major depression, Alzheimer disease, Parkinson disease, ischaemic stroke and medial temporal lobe epilepsy[8,53–56]. Restoring or normalizing levels of hippocampal neurogenesis through pharmacological or environmental interventions may be sufficient to partly prevent the onset of or ameliorate symptoms in certain models of disease, ranging from epilepsy to neurodegenerative diseases and depression[56–59]. Thus, targeting changes in neurogenesis may potentially hold promise for novel therapeutic approaches, as discussed later. Robust evidence across different species indicates a dramatic decrease of neurogenesis with advancing age[8,53,54,60,61] (BOX 2). The studies observing changes in neurogenesis, whether on the level of NSC proliferation, neuronal differentiation or cell survival, suggest that adult neurogenesis is regulated at multiple levels, and that any disturbance of the balance between activation and quiescence and between differentiation and survival can have detrimental effects on outcome.

Various factors that stimulate NSC proliferation and neurogenesis have been identified (FIG. 2). In rodents, voluntary exercise increases NSC proliferation, and environmental enrichment enhances the survival of newborn cells, promoting both memory and learning[62–64]. How increased NSC activation affects the long-term maintenance of the stem cell pool remains only partly understood[32]. Quiescence is maintained by niche factors, such as GABA released by parvalbumin-positive interneurons, as well as intracellular factors, such as bone morphogenetic proteins[65–67] and the E3 ubiquitin ligase HUWE1, which destabilizes the stem cell-associated transcription factor ASCL1 (REF.[20]). Furthermore, other

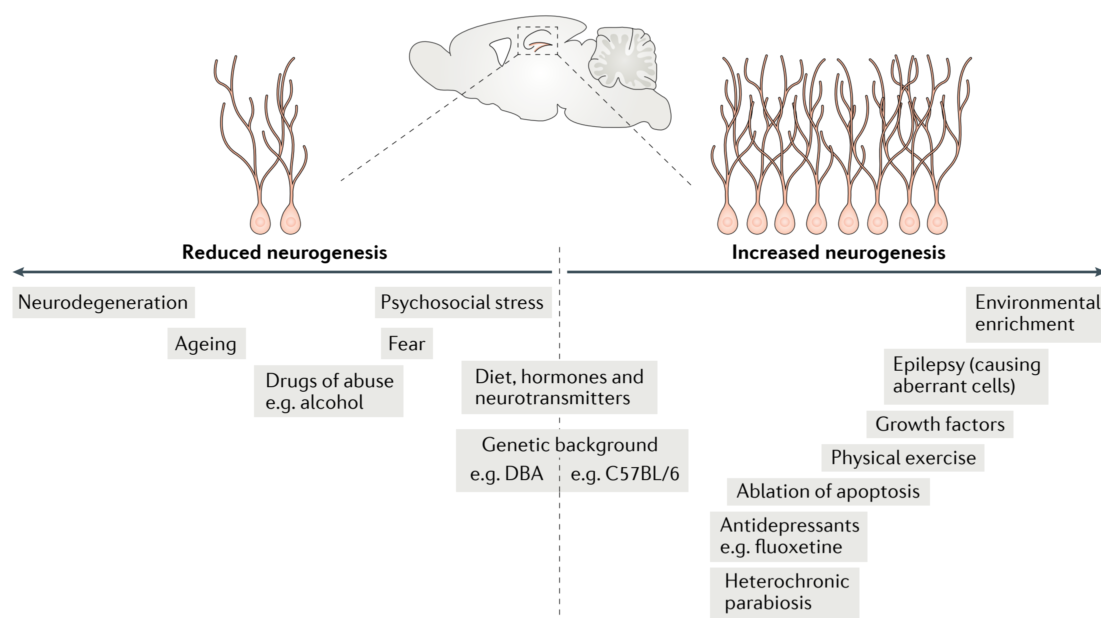


Fig. 2 | **Systemic regulators of adult hippocampal neurogenesis.** Shown are key regulators of hippocampal neurogenesis that have been demonstrated to be associated with either an increase or a decrease of the levels of newborn neurons in the adult dentate gyrus. Systemic regulators can affect the neurogenic process in the adult dentate gyrus at each step, from activating quiescent neural stem cells, to enhancing or impairing the proliferation of radial glia-like cells (R cells) and non-radial glia-like progenitor cells (NR cells), to altering the survival and integration of newborn granule cells.

Box 2 | **Age-dependent dynamics of neurogenesis**

The amount of neurogenesis decreases substantially with advancing age both in rodents and in humans[7,8,53,60,61]. In rodents, the number of proliferating neural stem cells (NSCs) and newborn neurons decreases exponentially; however, the neurogenic output of NSCs then seems to reach a plateau at around 7–10 months of age, resulting in low but detectable amounts of neurogenesis throughout life[60,61]. As the survival of newborn cells seems to be largely independent of the age of the animal, the major contribution to decreased neurogenesis seems to be the reduced proliferation of NSCs[175]. During ageing, neurogenic niches in the dentate gyrus but also in the subventricular zone undergo substantial changes, such as increased inflammation, transcriptional changes and changes in cellular composition, including infiltration of T cells in the subventricular zone[176–178]. Thus, various extrinsic factors that change with age, many of which might affect NSC proliferation, can be modulated by dietary interventions and exercise[179]. Infusions of young blood plasma or heterochronic parabiosis is a systemic manipulation that can reverse or reduce the age-dependent decline in neurogenesis and enhance synaptic plasticity and cognitive function in rodents[180–184]. Thus, studying the alterations that lead to decreased neurogenesis is important to understand how the process is regulated, and emerging manipulations that promote neurogenesis are of particular interest, given the possibility of alleviating age-dependent decreases and the potential benefits for memory and learning[185,186].

On a cellular level, NSCs from aged animals show lysosomal defects and increased amounts of aggregated proteins, concomitant with decreased levels of the chaperonin T-complex protein ring complex (TRIC; also known as chaperone-containing TCP1)[187,188]. Accordingly, activation of lysosomal activity by forced expression of transcription factor EB (TFEB), a master regulator of lysosome biogenesis and autophagy, enhances NSC proliferation in aged mice[188]. Furthermore, vimentin has a crucial role in recruiting the proteasome and protein aggregates to the aggresome, leading to their asymmetric segregation during NSC divisions[189].

In aged NSCs, a weakened diffusion barrier leads to impaired asymmetric segregation of damaged proteins[190]. Whereas the fidelity to segregate damaged proteins asymmetrically declines with age, it remains unclear whether in young animals such damaged proteins are inherited by the stem cell or the differentiating cell, whether they accumulate with increased numbers of divisions and what their consequences are for the inheriting cell. Thus, the effect of age on neurogenesis, and NSCs in particular, is a field of emerging interest.

key pathways such as the forkhead box protein O (FOXO), Notch, sonic hedgehog (SHH) and canonical WNT signalling pathways are critically involved in regulating NSC activation and maintenance[32]. Decreased Notch and FOXO signalling was reported to enhance proliferation, leading to rapid stem cell exhaustion, whereas impaired signalling of these pathways eventually leads to decreased neurogenesis[68–70]. In addition, other neurotransmitters besides GABA (such as serotonin), hormones and growth factors affect the levels of neurogenesis[3,32,71,72]. Indeed, some hormones, such as corticosteroids, reduce proliferation and neurogenesis, whereas others, such as oestrogens and male pheromones, seem to stimulate neurogenesis. Other growth factors, such as EGF, FGF2, brain-derived growth factor (BDNF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF) and transforming growth factor (TGF), have been associated with increased neurogenesis[3,73].

Once activated, NSCs divide, differentiate and thereby deplete themselves, or return to an inactive phase, the so-called resting state, which might be a truly quiescent state (corresponding to G0) or a prolonged cell cycle phase (corresponding to G1)[31,32]. The transition from NSC quiescence to NSC activation is accompanied by decreased intercellular interactions, increased ribosomal biogenesis and switches from lysosomal to proteasomal activity, from fatty acid oxidation to de novo lipogenesis and from glycolysis to oxidative phosphorylation — all changes that are regulated by genetic and epigenetic factors[42,74–78]. Furthermore, there is evidence, mostly based on gene expression data, that NSC quiescence may be a rather 'active' state, characterized by many niche signalling interactions although also by low protein translation capacity[32,42]. Recent data from *Drosophila melanogaster* suggest that NSCs can also rest in G2 during quiescence, rendering them more rapidly activated than cells in G0 (REF.[79]). Whether the same holds true in mammalian NSCs remains unclear.

It seems plausible that there is heterogeneity among different quiescent and activated stem cell states, with some NSCs more prone to rapidly divide and deplete themselves and others more likely to divide slowly and even return to true, long-lasting quiescence[32,38]. Whether these are different states of the same starting stem cells, or whether different pools of stem cells exist, remains an open question[32,35]. Genetic approaches such as intersectional genetics may enable the dissection of the exact lineage relationships of neurogenic cells in the adult DG. In addition, the fate and behaviour of individual NSCs may depend on their previous cellular biography, such as previous cell division histories of mother cells[4,80,81].

Integration of newborn neurons

Survival and stability of newborn cells. The number of cells generated via NSC divisions is delicately regulated by various extrinsic and intrinsic molecular cues. However, the birth of new cells is just the first step in a process that will eventually lead to the stable addition of newborn neurons into the adult DG circuitry.

Indeed, a substantial portion of newborn cells in the hippocampus of young adult rodents die within 3 weeks of their birth[82–84]. Cell death occurs in two distinct waves. On the basis of thymidine labelling and intravital imaging in rodents, a large number of cells (approximately 60% of all cells undergoing cell death) were observed to die within the first 24–48 hours after progenitor cell division[31,83,85]. The underlying cause for this early phase of cell death, which seems to be mediated by BAX-dependent apoptosis, is unknown[86,87]. Furthermore, it remains to be determined whether the mode of mother cell division (for example, symmetric versus asymmetric) may be predictive of subsequent cell death. Given the previously described high degree of genetic mosaicism in the adult brain, and especially in the DG, one hypothesis is that a subset of cells is removed due to deleterious or hazardous genetic alterations[88,89]. Future experiments isolating and molecularly characterizing this population of dying cells will aim to address this hypothesis.

The early phase of cell death is followed by a second wave approximately 12–16 days after the birth of the cells[31,82,90]. This wave of cell death coincides with the start of synaptic integration of newborn cells (see below), and previous work demonstrated that the successful survival at this maturational stage is input dependent: newborn neurons must receive NMDA receptor-dependent input or they will be removed from the circuit[90]. Thus, newborn cells have to pass through two distinct selection

Heterochronic parabiosis
Cross-circulation of humoral factors via shared blood circulation, most commonly used by connecting the vasculature (and thus blood circulation) of young and aged mice.

Vimentin
A type III intermediate filament protein that is a cytoskeletal component in various cell types.

Intersectional genetics
Approaches that increase the accuracy of genetic access to cells by combining two or more regulatory elements for a single synthetic output.

Genetic mosaicism
The presence of different genotypes in individual cells arising from a single zygote within an individual.

processes before they are stably integrated into the DG circuit.

Once newborn cells survive these early selection processes, they seem to become stable members of the DG circuitry[82], indicating that once newborn cells survive, they last throughout the animal's lifespan. However, the continuous birth of new neurons (and their stable integration into the DG, albeit only at relatively low frequency) predicts that either the DG should grow with advancing age or that embryonically or early postnatally generated cells should die to make space for adult-born neurons. Indeed, the DG in a mouse brain grows in volume until approximately 6 months of age, and in primates this growth period may be even longer[11,91,92]. Furthermore, a substantial fraction of early postnatally generated granule cells have been shown to die even after reaching full maturity[93]. However, Cre-mediated population labelling with an extended chase of labelled adult hippocampal NSCs indicated that the fraction of labelled cells increases in the mouse DG within the first 3 months after labelling but then reaches a plateau, suggesting that adult-born cells tend to replace adult-born sister cells[36,37,94]. Nevertheless, the generation of new neurons in the DG beyond 9 months of age in the mouse brain is considerably reduced (BOX 2), making it difficult to measure increases in cell numbers experimentally. Thus, whether adult-born cells replace each other (and thus represent a population with cellular turnover in itself), whether newborn neurons replace embryonically or early postnatally generated cells, or whether there is true competition and stochastic replacement of embryonically but also adult-born cells remains incompletely understood. In contrast to the olfactory bulb, where tissue deteriorates on depletion of adult NSCs in the subventricular zone, the DG remains relatively unaffected in terms of size and neuron numbers after depletion of adult NSCs[94,95]. Longitudinal observations using intravital imaging of individual cohorts of cells labelled embryonically or early postnatally and also of cells generated in the adult DG within the same rodent brain may be a suitable approach to understanding the population dynamics of adult-born DG neurons.

Morphological maturation and integration of newborn neurons. While experiencing the stages of selection and subsequent survival, newborn cells undergo dramatic morphological changes (FIG. 3). Initially, newborn cells tangentially migrate away from their mother progenitor cells, guided at least partly by DG vasculature, before they stop tangential migration and move radially into the GCL[16,96]. What causes the switch from tangential to radial migration is largely unknown. Furthermore, the temporal stability and the dynamics underlying the extension of neurites growing from newborn cells remain relatively unclear, although in vivo imaging data have started to address that question[97,98].

Newborn DG neurons undergo extensive neurite growth, during which their dendrites extend through the GCL and axons grow towards area CA3 of the hippocampus[16,17,99–101]. During the early phase of maturation, newborn cells receive largely extrasynaptic GABAergic input[102–104]. Approximately 12–14 days after cell birth, the first dendritic synapses are formed and the cells thus receive extrasynaptic and synaptic GABAergic and glutamatergic inputs[103,104] (FIG. 3). Newborn granule cells receive synaptic inputs initially from local neurons (largely interneurons) before they receive synaptic inputs from long-range projection neurons[105–107] (FIG. 4a). When the first synapses are formed onto newborn neurons in the molecular layer, these neurons also form axonal synaptic connections with hilar cells and pyramidal cells in area CA3[99,108] (FIG. 4a). Furthermore, newborn granule cells may directly connect monosynaptically with mature granule cells to affect DG network activity (see later)[109].

A plethora of pathways and metabolic adaptations have been identified that are crucial for early migration, neurite extension and spine formation, including small Rho GTPases, shifts in mitochondrial metabolism and the protein DISC1(disrupted in schizophrenia 1 protein)[3,78,110–113]. Furthermore, astrocytes in the DG have been shown to be important for the proper maturation and integration of newborn granule cells[114]. Blockade of vesicular release specifically from astrocytes led to decreased synaptic input to and spine density on newborn, but not mature, granule cells. However, most previous results were derived from static snapshot observations following genetic or pharmacological manipulations. Thus, the exact involvement of certain genes or pathways in distinct developmental steps underlying the dynamic processes of migration, neurite formation and synaptic integration remains largely unknown. Imaging-based approaches will help to gain further insights into previously observed phenotypes[97,98].

Over the course of 6–8 weeks, a continuing maturation process occurs, including extensive dendritic remodelling, as observed by prolonged intravital imaging[98]. During this time, newborn neurons also dramatically change their intrinsic properties: whereas young granule cells are characterized by inverted chloride potential due to high expression of the Na^+–K^+–Cl^- transporter NKCC1 (causing GABA to have initially a depolarizing effect on newborn neurons' membrane potential), they eventually switch towards mature chloride gradients by expression of KCC2 and subsequently lose their high input resistance[103,104,115–117]. These changes do not occur uniformly and gradually for all cells born at a similar time, but rather show heterogeneity among cells labelled at the same time[118]. From approximately 8 weeks of age, the electrophysiological properties and morphological measures (such as dendritic complexity and spine density) of adult-born cells become highly similar to those of cells that were born embryonically or early postnatally, although adult-born granule cells continue to mature over several months[102,119,120].

It is hypothesized that new neurons must compete for synaptic inputs to enable their survival and integration. This hypothesis is based on the observation that, early on, newborn granule cells largely contact axons in the molecular layer via multiple-synapse boutons, suggesting that they preferentially contact axonal synapses that are already occupied by other neurons; by contrast, in more mature stages, the most prevalent synaptic contact form is single-synapse boutons[17,121] (FIG. 4a). Future

Na^+–K^+–Cl^- transporter NKCC1
A co-transporter that regulates the transport of sodium, potassium and chloride through cellular membranes.

Multiple-synapse boutons
Synapses with two or more postsynaptic terminals on a single presynaptic terminal.

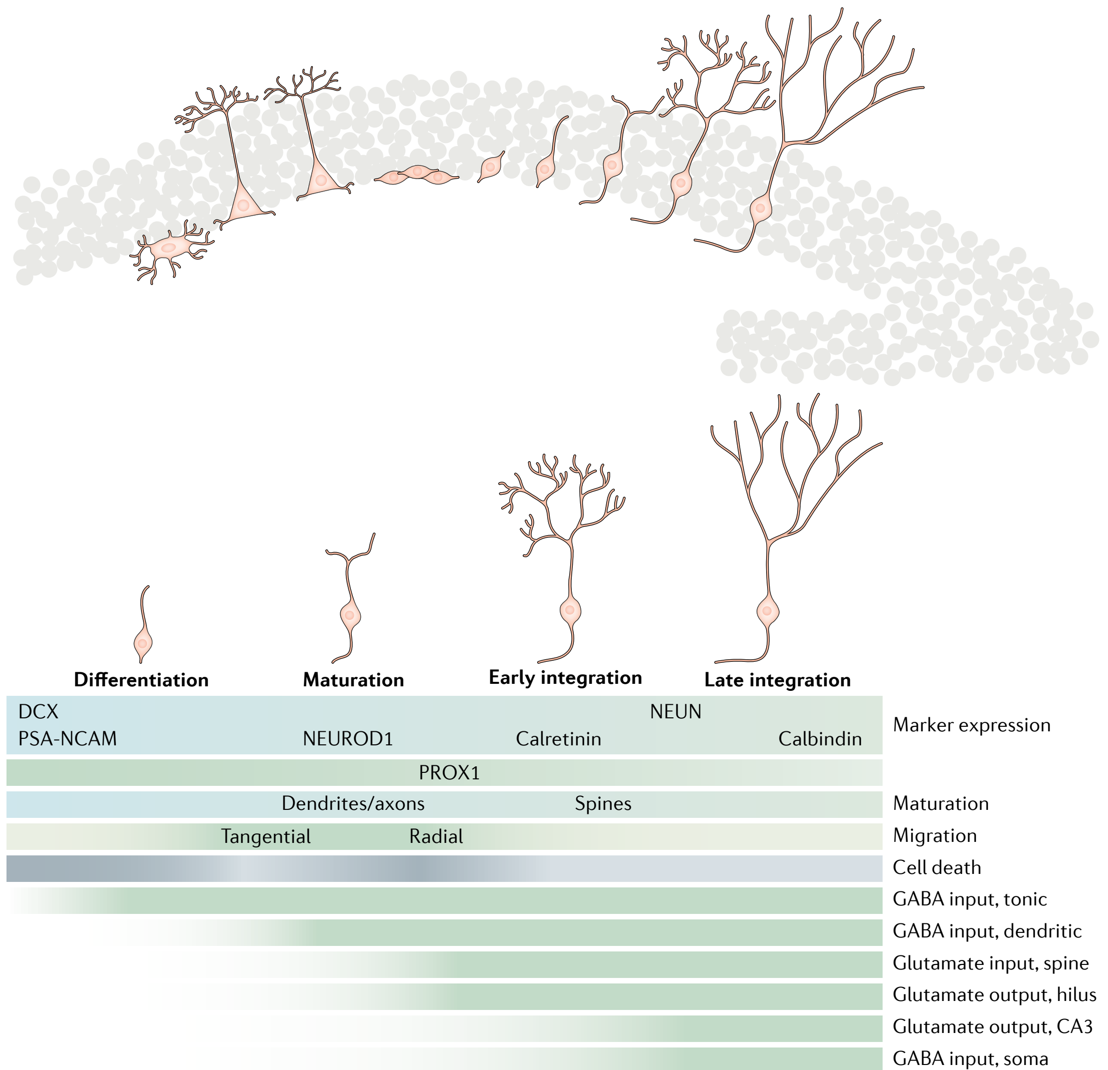


Fig. 3 | **Maturation of adult-born neurons in the hippocampus.** New neurons are either generated via symmetric or asymmetric divisions of non-radial glia-like progenitor cells (NR cells) or through direct neurogenesis from radial glia-like cells (R cells). Directly after birth, new neurons tangentially migrate from their mother cells, start to extend neurites, and radially migrate into the granule cell layer, form dendritic spines and synapses, and extend functionally connected axons[17,18,96–98,108]. However, the exact dynamics of each of those maturational steps on a single-cell level remain partly unknown given the lack of long-term in vivo observations of individual maturing neurons over extended time periods. During each developmental stage, newborn neurons express a set of marker proteins (a selection is shown) and go through distinct phases of physiological maturation, receiving different types of inputs and generating different types of outputs, as indicated[33,102–104,109,150]. DCX, doublecortin; NEUN, neuronal nuclei; NEUROD1, neurogenic differentiation factor 1; PROX1, prospero homeobox protein 1; PSA-NCAM, polysialylated neuronal cell adhesion molecule.

experiments that follow the extension and maturation of single dendritic spines or axonal boutons in vivo may address the dynamics of how newborn granule cells establish synaptic input and output connections.

Functional role of adult-born neurons

Functional importance of newborn neurons for DG-dependent behaviour. The findings that new neurons are added to the adult mammalian DG prompts an obvious question: what are they good for? What is the contribution of hippocampal neurogenesis to hippocampal function?

Early experimental evidence of the functional relevance of neurogenesis for hippocampus-dependent behaviour was largely based on correlative data: increased neurogenesis seemed to be associated with improved hippocampus-dependent behaviour (for example, in the Morris water maze), whereas reduced neurogenesis was associated with impairments in hippocampus-dependent learning tasks[62,63,122]. However, more mechanistic or causal experimental approaches that selectively disrupted neurogenesis without affecting other neural structures were technically challenging. Most, if not all, strategies to impair NSC function, to reduce neuronal output or to kill newborn neurons (for example, by using pharmacological, transgenic, viral or irradiation-based approaches) showed potential off-target effects, such as non-specific targeting of proliferating cells outside the

Morris water maze
A behavioural, spatial navigational task, mostly used in laboratory rodents, to study spatial learning and memory.

DG or the brain, non-specific inflammation or the sheer presence of dead or dying cells within the DG[95,123–127]. Thus, numerous studies were published showing consistent effects but also some opposing effects on hippocampus-dependent tasks, including contextual fear conditioning and spatial learning[128].

Similarly, probing for the functional relevance of adult hippocampal neurogenesis continued to produce partly controversial findings when the field moved towards behavioural tasks that may challenge the function of the DG more specifically. Previous experimental work and computational modelling data indicated that the DG is involved in behavioural pattern separation, the distinct encoding of highly similar experiences or inputs[129,130] (FIG. 4b), not only in rodents but also in humans[131]. Indeed, work from different laboratories using various tasks to probe for behavioural pattern separation, and using both gain-of-function and loss-of-function approaches, identified a critical role for newborn granule cells in DG-dependent pattern separation in mice[86,132,133]. However, others could not confirm these findings[134,135]. In addition, there is evidence that new neurons may be important for hippocampus-dependent behavioural flexibility (for example, when a mouse is required to learn a novel position in the Morris water maze)[44,136]. Notably, new neurons seem to be important not only for encoding novel experiences but also for forgetting previously learned experiences and memory traces[137,138].

Importantly, the DG, and therefore hippocampal neurogenesis, is crucial not only for learning and memory but also for various other behaviours, such as addiction, reward-related behaviour, social behaviour and emotional control[139–141]. Ventral parts of the DG are engaged in mood control, and previous work has linked mood, stress resilience and the efficacy of certain antidepressants to the birth and integration of newborn granule cells in rodents[142,52,59]. Clearly, not all functions of antidepressants rely on hippocampal neurogenesis[143]. Nevertheless, experimentally enhanced neurogenesis has certain antidepressant effects in mice treated with corticosterone or experiencing chronic social defeat stress (models of depression), such as reducing signs of anxiety in an elevated plus maze or forced swim test[144,145].

How do new neurons exert their function on these features of hippocampus-dependent behaviour? As outlined earlier, newborn granule cells show high excitability and a low threshold to induce plasticity-inducing potentiation such as long-term potentiation 4–6 weeks after their birth[115–117]. At this maturational stage, new granule cells show a gradual increase in levels of homosynaptic long-term potentiation and heterosynaptic long-term depression[146]. Furthermore, newborn granule cells also display an increasing molecular responsiveness to stimulation with increasing maturation[147–149]. Weak afferent activity is sufficient to activate newborn cells during this maturational stage, albeit with relatively low input specificity[150,151]. However, when cells mature beyond 6 weeks of age, their threshold for activation increases and their input responses become more specific. Specific manipulations in slices and using in vivo optogenetic experiments have suggested that the activation of adult-born granule cells approximately 4–6 weeks after their birth overall inhibits DG network activity, although this effect seems to be input dependent and a highly complex process[152,153].

Over the past few years, the field has moved towards using more dynamic approaches, such as functional in vivo imaging of newborn neurons while animals learn a task or express a memory, to examine how new neurons affect DG connectivity and network activity[154]. Both head-fixed approaches using two-photon imaging and gradient-index lens-based approaches in freely behaving animals have been used[109,154,155]. Initial evidence gathered with use of these technologies suggests that new neurons show distinct responsiveness to certain cues. Specifically, increasing neurogenesis (by deleting the proapoptotic gene *Bax* from adult NSCs) inhibits the ventral DG, reduces the activity of stress-responsive cells and thereby seems to promote stress resilience in mice[52,155]. Furthermore, distinct effects of newborn granule cell activation via the lateral entorhinal cortex (preferentially mediating contextual information) versus the medial entorhinal cortex (preferentially mediating spatial information) have been described. Activation of the lateral entorhinal cortex causes adult-born granule cells to monosynaptically inhibit mature granule cells, whereas stimulation of the medial entorhinal cortex instead leads to the excitation of mature neurons by newborn granule cells[109] (FIG. 4a). Future work will need to analyse how this diverse responsiveness and transmission of incoming inputs of new granule cells affects activity and the encoding of information in downstream hippocampal structures such as CA3 during natural (that is, behavioural) activation[156]. Understanding how neurogenesis affects local DG circuits at different maturational stages and affects the activity of DG networks connecting to pyramidal cells in the main output area of CA3 — for example, by dynamic imaging approaches — will help to further elucidate the functional relevance of adult-born granule cells in the context of hippocampal connectivity and function.

Conclusions and perspectives

The finding that new neurons are born throughout life in the mammalian hippocampus has had a profound impact on previously held concepts regarding plastic changes and dynamic adaptations in response to experience in the adult brain. However, the translation of experimental data obtained largely in mice to the human brain has been complicated, given the difficulty in accessing healthy human hippocampal tissues and the continuing lack of robustly validated approaches to detect neurogenesis in live human brains (BOX 1). Thus, the extent of lifelong neurogenesis in humans and any roles or changes it may show in human disease remain controversial. Irrespective of a potential translational relevance, the finding that new neurons are born and can integrate successfully into mature neuronal networks, as doubtlessly occurs in the rodent hippocampus, represents a unique entry point to analyse and eventually understand all the required steps, from stem cell division to cell selection and neuronal maturation

Behavioural flexibility
Adaptive changes in the behaviour of an animal in response to changes of the external or internal environment.

Memory traces
Units of cognitive information in the brain that may cause structural or biochemical alterations allowing the storage of memory.

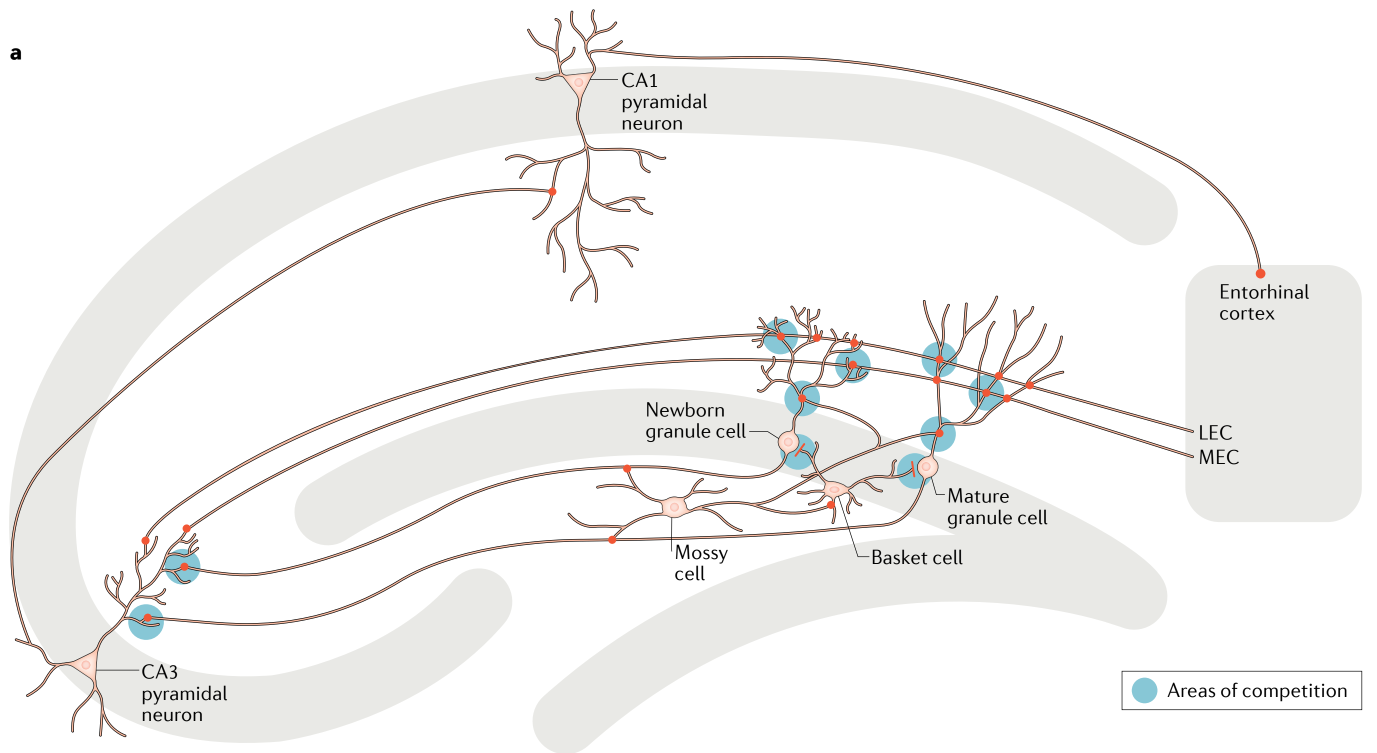
Homosynaptic long-term potentiation
Changes in synaptic strength that are specific for postsynaptic targets that are specifically stimulated by presynaptic cells.

Heterosynaptic long-term depression
A reduction in synaptic strength at unactivated synaptic connections that are input nonspecific.

Gradient-index lens
A lens that makes use of a gradient of the refractive index of a material, allowing a lens with a flat surface or that does not have aberrations of traditional spherical lenses.

Lateral entorhinal cortex
Part of the medial temporal lobe; it projects via the lateral perforant path into the dentate gyrus.

Medial entorhinal cortex
Part of the medial temporal lobe; it projects via the medial perforant path into the dentate gyrus.



b

Context A

Context B

No neurogenesis

Overlap of activation of mature granule cells without neurogenesis/low pattern separation

With neurogenesis

Reduced but more precise activation of mature granule cells due to indirect inhibition of the DG via newborn granule cells/high pattern separation

Silent granule cell

Granule cell activated in context A or context B

Granule cell activated in context A and context B

Newborn granule cell activated in context A or context B

◀ Fig. 4 | **Circuit function of newborn granule cells in the adult hippocampus.** **a** | Newborn granule cells in the dentate gyrus (DG) receive stronger input from the lateral entorhinal cortex (LEC) than from axons arising from the medial entorhinal cortex (MEC). Immature granule cells may affect DG circuit activity by directly exciting mature granule cells or by indirectly inhibiting them via activation of inhibitory interneurons (such as parvalbumin-positive basket cells) or hilar mossy cells. With maturation, granule cells receive strong perisomatic inhibition and form large boutons onto CA3 pyramidal cells, thus modulating output onto area CA3 and area CA1, from where information flows back to the entorhinal cortex and other association cortices. The exact effects of newborn neurons at distinct maturational stages on activity and information flow of the DG circuit and its output into area CA3 will need to be further analysed by in vitro and in vivo imaging and electrophysiological recordings. Blue shaded areas indicate sites where new neurons may have to compete with pre-existing cells for presynaptic and postsynaptic partners. **b** | Newborn granule cells seem to contribute to DG-dependent behavioural pattern separation, which is the distinct encoding of highly similar inputs or experiences, in rodents. Without neurogenesis (upper row), activation of mature granule cells (blue) may activate overlapping cells owing to the similarity of the experience of being in context A and the experience of being in context B (illustrated by a highly similar object arrangement in an arena), and thus the output to CA3 may overlap for similar inputs (reactivating cells are labelled in light blue with a blue outline). When neurogenesis is present (lower row), newborn granule cells (purple) are more easily activated than existing granule cells, but overall reduce the activity of the DG, possibly by inhibiting the activity of mature granule cells (blue). Thus, coding becomes sparser, and the overlap of activated mature granule cells is reduced, enabling enhanced behavioural pattern separation of highly similar inputs. Additional considerations of how new neurons contribute to and influence DG coding are more extensively discussed elsewhere[142,167].

Spatial transcriptomics
The characterization of mRNA composition in individual cells while maintaining information regarding their spatial position within complex tissues.

and integration. Understanding these steps may not only be instructive for future attempts to harness the regenerative potential of endogenous neurogenesis for brain repair but may also guide therapeutic interventions that are based on the transplantation of exogenous cells — for example, in the context of neurodegeneration. For example, what is required for the functional and meaningful integration of new neurons into mature brain networks? And how can we activate endogenous neurogenic precursors to enhance regeneration?

The neurogenic hippocampal niche may hold the answer to those questions, and substantial progress has been made to understand these processes on a molecular and cellular level. However, to make a true impact on the causes and eventual therapeutic amelioration of acute and chronic neuropsychiatric disease, the field will have to push towards a better understanding of exactly how neurogenesis affects functional connectivity and hippocampus-dependent behaviour. Novel ideas are needed to translate findings obtained in rodents to neurogenesis that might occur in human tissues — for example, using human cellular models of hippocampal development and maturation, or ideally by improving current approaches of working directly with human hippocampal tissues. In addition, novel technologies with high cellular or single-cell resolution, such as single-cell genomics and epigenomics, proteomics and spatial transcriptomics, will soon be used to define the molecular mechanisms that underlie neurogenesis in the adult brain[48,157–160]. Furthering our knowledge of lifelong neurogenesis in health and disease may open novel avenues to understand and treat brain diseases affecting the hippocampal formation.

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