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Modes of division and differentiation of neural stem cells

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ABSTRACT

Hippocampal neurogenesis presents an unorthodox form of neuronal plasticity and may be relevant for the normal or abnormal functioning of the human and animal brain. As production of new neurons decreases after birth, purposefully activating stem cells to create additional new neurons may augment brain function or slow a disease's progression. Here, we describe current models of hippocampal stem cell maintenance and differentiation, and emphasize key features of neural stem cells' turnover that may define hippocampal neurogenesis enhancement attempts' long-term consequences. We argue that even the basic blueprint of how stem cells are maintained, divide, differentiate, and are eliminated is still contentious, with different models potentially leading to vastly different outcomes in regard to neuronal production and stem cell pool preservation. We propose that to manipulate neurogenesis for a long-term benefit, we must first understand the outline of the neural stem cells' lifecycle.

1. Introduction

There is a growing appreciation for neurogenesis as vital for adult brain's healthy function at all stages of life [1–7]. The hippocampus harbors a large depot of neural stem cells which produce neurons and glia long after this region is fully formed in the perinatal brain. These new neurons, which are born from stem cells in the dentate gyrus of the hippocampus and then migrate locally and integrate into existing neural circuitry, are subject to a plethora of endogenous and exogenous stimuli. In most cases, their production is increased by beneficial stimuli like physical activity, enriched environment, antidepressants, or repeated winning in a social conflict [8–12]; and decreased by detrimental stimuli like chronic stress, social defeat, disease, radiation, and trauma [13–16]. Such dynamic changes in adult neurogenesis extend beyond mere epiphenomena, with augmentation or suppression of neurogenesis eliciting distinct cognitive and behavioral changes in diverse paradigms [17–22].

Postnatal neurogenesis has been convincingly demonstrated not just in numerous animal species [23], but also in humans, as supported by at least four non-overlapping approaches. These include nucleotide analog incorporation, carbon dating, isolation of cells with stem cell capacity from the brain tissue, and post mortem immunocytochemistry [[24–34]; also see a comprehensive review of this issue [5]]. While high level of hippocampal neurogenesis in the child and adolescent human brain is not disputed, its true extent in the adult and aging human brain has recently spurred intense debate, prompted by highly divergent results of immunocytochemical detection of several widely used markers [25,34–38]. This disparity still awaits resolution, and may be due to unusual vulnerability of those markers in the perimortem human brain tissue [5,35,39], different pace of neuronal maturation, or variable kinetics of neurogenesis across rodent and human lifespans [40]. Remarkably, recent corrections indicate that neurogenesis in adult and aging humans may be quite high and exceed the levels extrapolated from the rodent studies [25,31,35,38,40].

These findings, paired with the causative relation between neurogenesis and brain function, are driving efforts to enhance memory, improve mood, and prevent age- or disease-related cognitive deficits by finding agents that would augment the production of new hippocampal neurons [41–43]. Still, these efforts' long-term outcome depends on how generation of new neurons is stimulated, as a look at the complex process of stem cell differentiation reveals.

2. Long-term outcomes of stimulating neurogenesis may be dissimilar

New hippocampal neurons are produced from a pool of dedicated neural stem cells that reside in a narrow zone between the granule cells and the hilus of the dentate gyrus. To be converted into a fully

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differentiated and integrated new neuron, a neural stem cell and its progeny undergoes a protracted cascade of division, differentiation, and elimination events. Each step of this cascade provides a potential target for a particular pro- or anti-neurogenic factor, fueling a quest for finding compounds and signaling pathways that could augment stem cell output. But while in the short-term, diverse pro-neurogenic stimuli might lead to a similar outcome (enhanced neurogenesis), in the long-term, their consequences could vary dramatically based on the cascade step targeted.

For instance, if neural stem cells are excessively activated, neuronal production may temporarily increase, but may be accompanied by accelerated loss of neural stem cells, leading to premature exhaustion of the stem cell pool. If, however, the pool of stem cells is continuously supported by their symmetric divisions or *de novo* generation from other cell types, the loss of stem cells may be counteracted. Likewise, if the pool of stem cells is preserved, but these cells become increasingly quiescent, the production of new neurons will decrease with age; still, this dormant pool may be potentially reactivated by relevant stimuli. So if we seek to improve normal cognitive performance or rejuvenate deteriorating cognitive function in the adult brain, we must first better understand neural stem cells' lifecycle; we argue that it is impossible to predict such a short-term enhancement's long-term consequences, without knowing which subpopulation of stem and progenitor cells is targeted or determining the blueprint that guides their development.

3. Current models of stem cell life cycle are not reconciled

Seeking this blueprint, various models of stem cell maintenance, division and differentiation have been proposed. They can be described by several basic scenarios (Fig. 1); note that the reports indicated below are selected as prototypical, do not necessarily describe each feature of a particular scheme, and are cited mainly to highlight the differences and similarities between the schemes. Specific features pertaining to these schemes are compared in more detail in Table 1.

(a) A predominant fraction of hippocampal neural stem cells, referred here as radial glia-like (RGL) cells, undergo symmetric or self-renewing asymmetric divisions; their pool declines with age, but not significantly; decreased neurogenesis is caused by the diminishing propensity of stem cells to produce new neurons (prototypes of this scenario: [44–46]).
(b) The neural stem cell pool is supported by progeny that revert to stem cells or engage in long-term self-renewal, thus acting as *de facto* long-term stem cells (prototypes: [47–49]); this model may potentially include as-yet-unidentified cells which generate the conventional RGL stem cells.
(c) A subpopulation of quiescent neural stem cells forms a transient pool of actively dividing and self-renewing stem cells, which eventually disappear through differentiation or death (prototype: [50]).
(d) Relevant to all depicted scenarios, astrocytes and neurons may arise from either the same type of stem cells or from different subsets of lineage-committed stem cells (prototypes: [44,47,51].
(e) Stem cells mainly engage in asymmetric divisions with highly limited self-renewing potential, and the vast majority of these cells disappears through elimination or division-coupled conversion into astrocytes; such stem-cell-pool depletion is the main driver of age-related decline in hippocampal neurogenesis (prototypes: [47,52,53]). This model is described in more detail below, in part to illustrate the intricacies and caveats of tracing stem cells' lifecycle.

4. A model of a limited stem cell life cycle

We previously generated a genetic toolbox with reporter transgenic mouse lines and their numerous derivatives [52,54–57]. We then combined those reporters with single- and double-DNA tagging and genetic tracing techniques to propose a new model of adult hippocampal neurogenesis where neural stem cells undergo division-coupled differentiation after a limited number of fast sequential asymmetric divisions [52] (Fig. 2). The key claims and features of this model were:

(a) Hippocampal stem cells, presented by RGLs, are dormant in the adult brain and, when activated, undergo a rapid series of asymmetric divisions, with the non-radial progeny giving rise to new neurons.
(b) Rapid division of RGLs is linked to their astrocytic differentiation and loss of stem properties; thus, these cells act in the adult brain as essentially "single use" or "disposable" stem cells.
(c) Age-related decrease in production of new neurons is driven by the loss of stem cells through astrocytic differentiation.
(d) The rate of activation of stem cells decreases with age but their specific output (production of progeny per cell) increases.

This model made several well-defined statements and predictions:

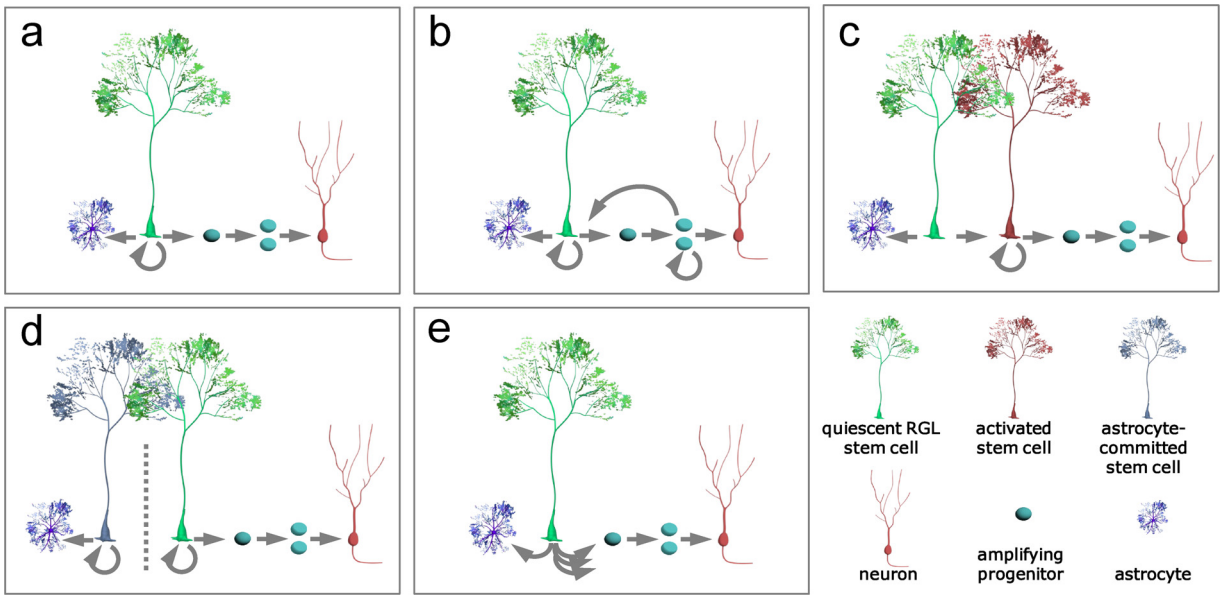

Fig. 1. Schematics of the current models of neural stem cell maintenance, division, and differentiation (a–e), as described in the text.

Table 1
Attributes and predictions of various models of the neural stem cell lifecycle.

	Self-renewing RGLs scenarios			Single-use RGLs scenarios	
	(a)	(b)	(d)	(c)	(e)
source of new neural stem cells in the adult brain	self-renewing RGLs	self-renewing RGLs, RGL progeny, unidentified precursor(s)	self-renewing RGLs both for neural and astrocytic lineages	perinatally developed RGLs, no adult replenishment	perinatally developed RGLs, no adult replenishment
predominant mode of RGL division	symmetric and asymmetric	symmetric and asymmetric	symmetric and asymmetric	symmetric and asymmetric	asymmetric
self-renewal of RGLs	predominant	predominant	predominant	limited	no or highly limited
progeny cell reversion to stem cell status	no	yes	no	no	no
recurrent quiescence	yes	yes	yes	yes	no
astrocytic differentiation of RGLs	stochastic	stochastic	lineage-committed astrocytic differentiation	stochastic	division-coupled astrocytic differentiation
age-related depletion of the RGL pool	moderate or no	moderate or no	moderate or no	moderate or strong	strong
age-related division potential of RGLs	decline	decline	decline	?	no changes or increase
potential to expand the RGL pool	high	high	high	no or low	no
age-related RGL output	decline	decline	decline	?	increase

(a)–(e) Columns correspond to the scenarios depicted in Fig. 1; note that as in Fig. 1, the presented attributes may combine selected features of the prototypical scenarios.

(a) Once activated, stem cells undergo a burst of neurogenic divisions and do not return to the quiescent state.
(b) Asymmetric divisions are the predominant mode of division of the activated hippocampal stem cells.
(c) The birth of new neurons is inherently linked to the disappearance of stem cells.
(d) Age-related decrease in neurogenesis is primarily due to the diminished reserve of stem cells, rather than their diminished propensity for division.
(e) Overstimulation of stem cells' division may lead to their premature exhaustion, even if accompanied by the initial spike in neurogenesis.

Within recent years, several findings have been published which support our original (and at that time controversial [58]) model. For instance, it was shown that both epileptiform activity and sustained seizures lead to stem-cell overactivation and premature depletion [59]. It was also shown that long-range GABAergic projections from the medial septum control stem cells through depolarizing GABA signaling onto local parvalbumin interneurons, such that their ablation leads to excessive activation of stem cells followed by the depletion of the stem cell pool [60]. Moreover, the disease's progression in a mouse Alzheimer's disease model is accompanied by epileptoform spikes and later, seizures, which stimulates divisions of neural stem cells above the norm at early stages but leads to decreased neurogenesis and diminished stem cell pool at later stages [61]. Recently, live tracing of the fate of Ascl1-CreER-activated hippocampal stem cells confirmed some of our model's key conclusions and predictions, such as a burst of asymmetric divisions of the activated stem cells; no return to the quiescent state after activation; and limited number of divisions of both stem cells and their amplifying progeny [47]. Importantly, the latter study followed the fate of single activated stem cells for a long time in vivo and found the same general scheme of stem cell activation (and even specific parameters, such as the rounds of successive divisions of stem cells and their progeny) that we have proposed. This live analysis, on the level of individual stem cells, thus confirmed what was predicted in our model via static analysis, on the level of stem cell populations.

The key conclusion from the comparison of this and other models (Fig. 1 and Table 1) is that in attempts to bolster neurogenesis, the underlying scenarios may lead to very different long-term outcomes, including premature depletion of the stem cell pool. These models have not yet been reconciled, despite their relevance to stem cell biology and the prospects of brain rejuvenation through augmented neurogenesis.

5. Approaches for studying neural stem cells have inherent limitations

Why are there discrepancies and even contradictions between proposed stem cell lifecycle models? In part, differences may arise because the approaches used to probe stem cell division and differentiation are inherently limited. For instance, consider pulse labeling of dividing cells with nucleotide analogs: while this method precisely detects cells that have been engaged in division at the time of label injection, pulse labeling may not account for cells that have a particularly protracted S phase or prolonged periods of quiescence, or that have undergone multiple rounds of division, thus diluting the pulse label beyond the limits of detection. Further, pulse labeling presents a static picture of a cell population and may not provide enough resolution to analyze the dynamic stem cell lifecycle. Or consider clonal analysis and cell-fate mapping (e.g., after recombination in stem or progenitor cells): while these methods are effective in detecting stem cells' accumulated progeny within a particular brain region, they do not report on stem cells' early division and loss events, or prove that cells have actually duplicated their DNA; they may be distorted by cell migration in or out of the designated clone boundaries, and may be prone to complex artifacts underlaid by a pre-existing bias of the stem cell distribution [62,63].

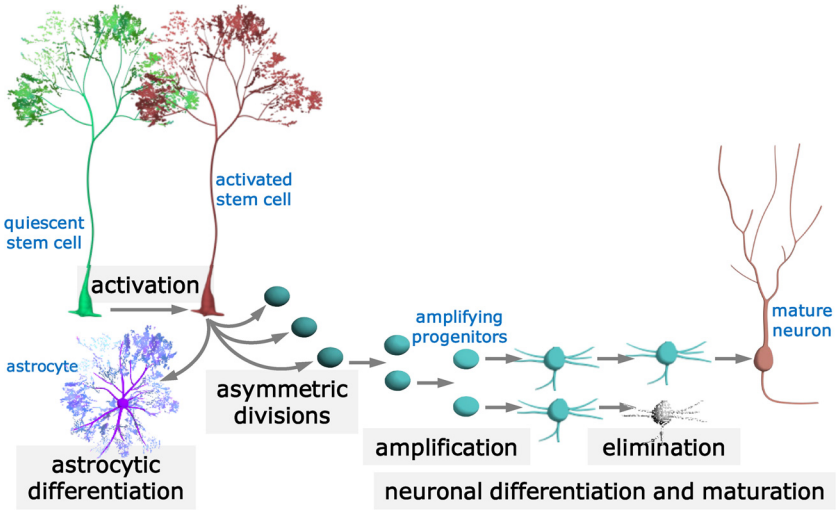

Fig. 2. Limited lifecycle ("disposable stem cell") model of stem cell division and differentiation.

Finally, consider direct observation through surgical intervention, with endoscopes or glass windows: while this method allows direct access to the process of neurogenesis in the live brain, it inevitably alters cell division's parameters due to imposed trauma and may induce compensatory changes in stem and progenitor cell division (for instance, brain trauma may induce stem cell divisions that do not lead to an increase in neuronal production [64–67]. Thus, the field awaits new or hybrid approaches that would bypass or minimize each method's inherent limitations, allowing us to better delineate the stem cell lifecycle in the adult brain.

6. Open questions

For all described models of how neural stem cells are maintained and divide, there are still several critical questions waiting to be resolved or reconciled, even if simplistic responses are often taken for granted. These outstanding questions relate, among other things: to the criteria and identification of stem cells and the existence of cells that may escape regular means of detection; the possibility of stem cell symmetric division and self-renewal under normal conditions, and whether the stem cell pool can be prematurely exhausted; a common or separate astrocytic/neuronal precursor(s); the prolonged lifecycle of the amplifying progeny of stem cells and the potential of their reversal to the stem cell state. Here, we present some of these questions (Fig. 3), and discuss the challenges that confound their resolution. While we focus on adult hippocampal neural stem cells, the same questions may be relevant for other neuronal and non-neuronal stem cell types, and their turnover during normal or malignant tissue growth:

***(a) Neural stem and progenitor cells must be better defined and identified*:** The very term of art of this field – "neural stem cell" – is not fully defined or settled for the adult brain. Traditionally, tissue-specific stem cells are defined by the ability to self-renew and, in some settings (e.g., for hematopoietic stem cells), to fully reconstitute a damaged tissue [68–71]. Stem cells are also often, but not always, expected to: produce various differentiated cell types; support or replenish their pool by symmetric divisions; and refrain from frequent divisions that could introduce increased risk for the integrity of their genome. Still, a range of stem cell types do not fit these criteria, including exclusively or predominantly unipotent stem cells (e.g., germ stem cells or skin stem cells), cells that engage in rapid continuous divisions (e.g., stem cells for enterocytes), and stem cells that enter the division cycle to preserve their genome integrity (e.g., hematopoietic stem cells entering the cell cycle to switch from non-homologous end-joining to more efficient modes of DNA repair) [68,72,73]. In the same vein, hippocampal neural stem cells apparently satisfy only some generic stem cell criteria: for instance, there is not yet unequivocal proof that they can continuously self-renew, or divide symmetrically under normal conditions, or furnish endogenous repair of the damaged brain tissue.

Nevertheless, a population of adult hippocampal cells carrying most of the characteristics of *bona fide* stem cells has been identified and

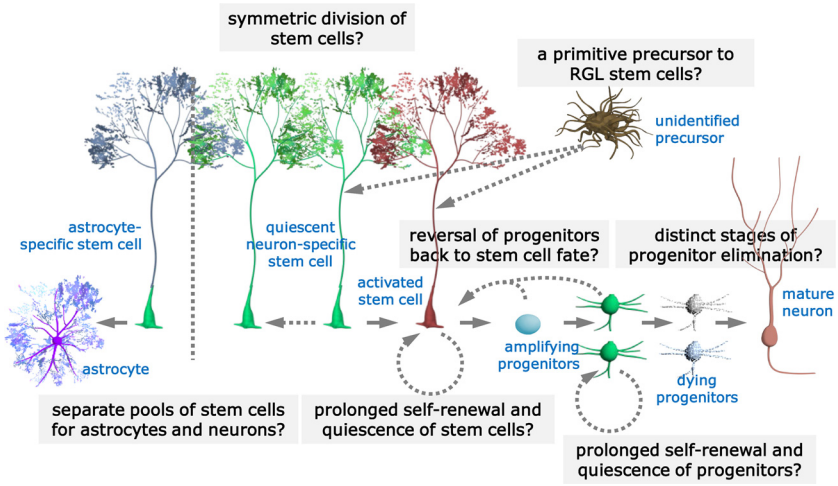

Fig. 3. Outstanding questions on the lifecycle of adult neural stem cells.

investigated in much detail (remarkably, these cells have been morphologically described long before their stem nature has been proposed or proven [74,75]). Several lines of evidence provide strong proof that cells with radial morphology, with the triangular soma located in the subgranular zone of the dentate gyrus and a single or branched process traversing the entire granule cells layer, act as the key type of stem cells, producing new neurons and new astrocytes [46,56,76–79] (see [80] for a detailed morphological description of these cells and their niche). Such cells have been variously described as Type-1 cells, quiescent neural progenitors (QNP), radial astrocytes, and adult radial glia cells; here these cells are referred to as RGL cells.

RGL cells can be recognized in the dentate gyrus by their conspicuous morphology under conventional or electron microscopy. In practice, they are mainly identified immunocytochemically with relevant markers such as nestin, glial fibrillary acidic protein (GFAP), or vimentin, or, predominantly, through the use of reporter transgenic mouse lines in which RGLs are marked by expression of fluorescent proteins such as GFP, CFP, and mCherry [57]. In these lines, expression of the fluorescent markers is driven by regulatory elements of genes that are preferentially expressed in RGLs: nestin, GFAP, Gli1, Sox2, Hes5, or Lfng [53,55–57,81–85]. To a large degree, these reporters mark the same cell types; however, the extent of overlap, the completeness of cell-scoring of a particular class, and the brightness and half-life of the fluorescent markers may differ. Therefore, even if a variety of reporters label the bulk of a particular cell type (e.g., various lines of Nestin-GFP reporters, or the Nestin-based reporters vs. the Gli1, Sox2-or Hes5-based), one should be aware that smaller fractions of that cell type may not be captured by a particular reporter and, conversely, that a particular reporter may mark additional cell types even when compared to its close analog.

A point to consider is that relying on a fluorescent protein as a proxy for a reporter gene may both reveal cells that express low levels of that gene product (and thus increase the sensitivity of detection) and highlight cells that ceased to express that gene (and thus lead to false positives). At the same time, the latter feature allows us to follow the progeny of stem cells that no longer express the reporter gene, but still carry the reporter fluorescent protein. In other words, this feature allows prolonged tracing of stem cells' progeny until the fluorescent signal is diluted below detection limits. Along the same lines, consider that Cre-ER protein may linger in progeny cells past the period of activity of the promoter elements driving its expression, thus skewing the range of cells presumed to be the traced stem cell's progeny.

***(b) Multiple classes of RGL neural stem cells in the adult and aging dentate gyrus*?** Most of the RGL cells in the adult brain express an overlapping set of markers and have similar basic structure. However, they can also vary substantially in their morphology, raising a question of whether neural stem cells, even if they express the same marker, are inherently diverse or whether this perceived diversity primarily reflects dynamic changes of the same cell type. Among possibilities:

- The difference in morphology reflects a true heterogeneity of neural stem cells, perhaps with a different productivity and lifecycle (e.g., horizontal cells [81] vs. regular RGLs), or even specialization (neurons vs. astrocytes) [51].
- The observed variants represent various stages of the lifecycle of the same stem-cell type; for instance, their activation and entry into the cell cycle vs. progressive divisions vs. quiescence, or these variants correspond to different stages of stem cells' conversion into astrocytes [52,57].
- The difference in RGL cells' morphology, particularly in the aging brain, reflects age-dependent changes [86], different kinetics of morphological changes upon differentiation, selection of rare types upon aging, or greater tolerance for atypical RGL morphology in the aging brain.

While much of this uncertainty may be resolved using targeted transcriptional profiling of cells with different morphology or ingenious tracing techniques, the question would benefit from new or combined techniques for stem cell identification and long-term tracing in the fixed or live brain.

(c) Unidentified precursors for RGL stem cells? The issue of stem cell variability overlaps with another formal possibility – the existence of an as-of-yet unidentified cell type that gives rise to RGLs. Although the bulk of published data strongly suggests that the RGL cells present the key and most primitive class of hippocampal stem cells, unequivocal proof is still lacking. These presumptive cells might be very rare, may lack the conventionally used markers, divide very slowly (and thus escape identification when labeling with nucleotide analogs), or be activated only in certain conditions or upon certain type of trauma. Again, we may require alternative methods of stem cell detection to find such cells, if they indeed exist.

(d) Separate pools for new neurons and new astrocytes? Another question that arises from the potential variability of stem cells is whether there exist dedicated stem cells that produce either neurons or astrocytes. Indeed, while lineage tracing data suggest a common origin for neurons and astrocytes [44,52], direct observation [47] indicates that events when an activated RGL cell (Ascl1-Cre-marked) produce astrocytes are rare. Conceivable scenarios include

- entirely separate pools of RGLs for neurons and for astrocytes, which differ only slightly and overlap in their expression of the majority of markers;
- a stochastic decision of otherwise identical RGL to engage into a program that leads to the production of either a neuron or an astrocyte, but not both;
- the production of neuronal precursor(s) switching to production of astrocytes, or a direct conversion of an RGL into a regular parenchymal astrocyte, akin to what happens in the perinatal period.

New markers and new techniques for stem cell tracing may help resolve this still open question.

***(e) Preservation and exhaustibility of the neural stem cell pool*:** The pool of tissue-specific stem cells does not necessarily have to diminish, even if their productivity decreases. For instance, the number of hematopoietic stem cells, per conventional assays, does not decrease significantly with age, although their ability to reconstitute hematopoiesis upon serial transplantation is compromised [71,87,88]. For neural stem cells, it is often assumed that their pool is preserved, even if their propensity to produce new neurons is diminished. However, other reports indicate that the number of RGLs is drastically reduced with age, even though the rate of their disappearance continuously decreases [52,89]. Effectively, such a rate decrease means that an ever-diminishing pool of RGLs is always preserved in the old brain, albeit at low levels. Moreover, somewhat counterintuitively, the output of each stem cell increases with age [52], even as, with a drastically reduced stem cell pool, this is not enough to restore neurogenesis to "young" levels. One has to bear in mind, however, that while the morphology and immunocytochemical prolife of RGLs is often assumed to be similar in the young and old brain, this may not be the case [51,86]. Indeed, even a cursory analysis suggests that RGLs have a broader gamut of morphologies in the old brain. Even if a continuum of morphological variants of RGLs expressing the same marker exists in the dentate gyrus, there is still a challenge of deciding on the threshold for scoring a particular cell as a *bona fide* RGL.

Critically, most of the above considerations relate to the basal, unperturbed process of neurogenesis in the healthy adult brain. As noted above, seizures, Alzheimer's disease, or ablation of long-range GABAergic projections can lead to overstimulation of stem cell division followed by an accelerated diminishment of the stem cells pool [59–61].

***(f) Recurrent quiescence*:** Long presumed to be an obvious neural stem cells prerequisite, whether they go through cycles of recurrent

quiescence is still under debate. In the "classical" definition of stem cells, RGLs would be expected to alternate duplication of their genome (whether symmetrically or asymmetrically) with periods of prolonged quiescence. With age, their pool might diminish due to attrition, but may even increase if symmetric divisions counteract the loss of RGLs.

One of the limitations in investigating multiple cycles of cell division and quiescence is the scarcity of labels that would differentially mark populations and cohorts of dividing cells. A new technique for quadruple labeling dividing cells that we have recently developed [90,91] may help bypass these limitations by allowing to use three nucleotide analogs (triple S-phase labeling), with the fourth label used for yet another marking of dividing cells (e.g., using a cell cycle marker Ki67), or for phenotypic identification of the dividing cells (e.g., using a GFP-expressing reporter allele). This approach allows for the multiple birth-dating of stem cell cohorts, the identification of well-defined (and otherwise elusive) functional subpopulations of stem cells, and the combination of various labeling paradigms within one experimental design. We have validated this method for stem cells of the hippocampus and the subventricular zone (SVZ), testis and intestinal epithelium, and used it to determine the parameters of cell division in the adult brain [90]; it was also recently used to dissect the recruitment, division, and depletion of chondroprogenitors during the longitudinal bone growth [92].

***(g) Symmetric divisions*:** The question of how cell division works in the adult hippocampal stem cell pool under normal conditions is still contentious. Symmetric division of stem cells is presumed to be an essential mechanism for preventing exhaustion of the stem cell pool [68,69]. For neural stem cells, switching to a symmetric-division mode has been proposed as a link to cognitive and emotional state or disease [93,94], and as a means for brain repair and rejuvenation. Evidence for a symmetric mode of division is usually based on finding pairs of closely positioned nucleotide-labeled cells, or by tracing genetically labeled cells and determining the occurrence of pairs of stem cells within the same clone. It is usually assumed in such analyses that individual neural stem cells, whether dividing or not, are distributed randomly, at least within small subdomains of the dentate gyrus. Further, observation of a bias towards very closely located labeled cells is usually interpreted as a strong indication of a symmetric division (rather than of preexisting bias in stem cell distribution). But although the assumption of randomness is critical for this interpretation of experimental data, it has never been rigorously tested, and the potential biases in stem cell distribution and division have never been compared (in part because it is not obvious how to evaluate such bias directly).

We have recently addressed this assumption by comparing the biases in the distribution of all stem cells and their dividing subset in 3D, i.e., focusing on the similarity of the potential biases of these distributions. After examining the spatial geometry of neural stem cell distribution and division, we concluded that even when bias in the distribution of dividing stem cells is observed, it can be explained solely as the preexisting bias in the distribution of all (dividing and non-dividing) hippocampal stem cells [62,63]. Moreover, we found that age-dependent disappearance of stem cells tends to randomize the distribution of the remaining cells [63].

The pre-existing bias and non-randomness in the distribution of stem cells may have various explanations: non-random positioning during embryonic development, selective elimination during development or adulthood, the effect of a particular niche (e.g., a blood vessel) on location or division of adjacent stem cells, or non-randomness of induced recombination. In any case, assumptions that stem cells and their dividing subset are distributed randomly, or that the potential biases in their distribution are similar, may potentially compromise interpretation of the experimental data. Note that these findings do not disprove the possibility of symmetric division of neural stem cells, but rather bring attention to the problem of detecting true symmetric divisions and the need to reevaluate some of the existing claims. It is also conceivable that disease or drastic insult may alter the incidence of symmetric divisions of RGLs: for instance, kainate-induced seizures induce symmetric divisions of some RGLs [59]. It is challenging to determine whether RGLs can engage in symmetric divisions under less extreme conditions.

***(h) Asymmetry, productivity, prolonged quiescence, and transdifferentiation of advanced progenitors*:** Transiently amplifying progeny of RGLs (defined as type-2 cells, amplifying neuronal progenitors/ANPs, transit amplifying progenitors/TAPs) are presumed to be a short-lived cell type that are in the process of symmetric division, or differentiating into neurons, or being eliminated. However, direct observation through a skull glass window is compatible with the possibility that these cells can persist for long time, divide asymmetrically, and perhaps withdraw from cycling, thus endowing them with features characteristic of true stem cells [47,49]. Taken together with the data suggesting that Type-2-like cells may act as stem cells [48], these results raise the possibility of trans-or de-differentiation of a particular cell type into an RGL cells (e.g., progeny of an RGL cell changing its morphology and profile and converting back into an RGL cell) or simply taking the function of stem cells upon themselves; note that this scenario is formally similar or equivalent to the possibility of unidentified precursors for RGL cells (paragraph *[c]* above) residing in the hippocampus or migrating from elsewhere.

***(i) Elimination of neuronal progenitors*:** Most of newborn neuronal progenitors and young neurons are eliminated. The kinetics of such elimination indicates different rates at different steps of the division/differentiation cascade [52,95]. While the earliest stages of programmed elimination are executed by microglial cells in a highly defined manner [95], the details of elimination at the later stages are scarce. These later stages of elimination may have different mechanistic underpinnings and different functional role (e.g., modulation of neuronal differentiation and connections).

***(j) Activation of stem cells' productivity in the aged brain*:** Aging is characterized by a dramatic decrease in cell proliferation, production of new neurons, and stem cell pool, in the dentate gyrus. It is also accompanied by a broadening of the phenotypical and functional heterogeneity of stem cells [86] and randomization of remaining stem cells' distribution [62].

Remarkably and somewhat counterintuitively, the rate of stem cells' attrition decreases with age almost tenfold and their output increases almost fivefold [52]. Effectively, this rate changes imply that even though the number of stem cells decreases with age, their efficiency and normalized output increases. This finding may explain the remarkable activation of neurogenesis in the aging brain upon certain types of stimulation [96–98] and hint at the prospects of rejuvenating stem cell in the aged brain to increase their neuronal output. At the same time, the overall consistency of the stem cell maintenance blueprint lifecycle across the lifespan remains unclear. It is conceivable that aging of stem cells and/or of their niche may diversify or even switch the scenarios (Fig. 1) characteristic of the young adult and adult brain.

It is worth emphasizing that while these processes may be common to other neurogenic zones (SVZ, RMS, or hypothalamus during first weeks of age [99], these regions may be different even in their key features: note, for instance, presence of early precursors that do not express nestin in the SVZ [100] or symmetric division of stem cells in the SVZ [101].

7. Implications for human neurogenesis

Much of the current interest in adult hippocampal neurogenesis stems from its possible role in human brain plasticity and the assumption that mouse models reproduce key features of human neurogenesis. Without covering this critical assumption and the ensuing debate in detail, we will point to a few important considerations:

- The morphology of stem cells and their progeny may be different in humans (particularly in the aging brain) from rodents, and the

search for proper RGL cells with the expected markers in the human brain may be misleading or incomplete.

- The dynamics of cycling and the differentiation of stem cells and their progeny in the human brain may be different from the rodent brain, skewing the presumed presentation; for instance, neuronal differentiation takes a remarkably longer time in the human brain than in the rodent brain, and this alone could distort the conventional assumptions on the differentiation cascade in the human brain.
- The dynamics of marker expression (or even the exposure of the key epitopes used to identify the expected progenitor class) may be different between the human and rodent brain. Even the most widely used markers of neural progenitors (Nestin, Dcx, PSA-NCAM,) may overlap (even if temporarily) with what would be considered a reliable marker of a differentiated neuronal cell (e.g., Prox1), leading to a false attribution of a particular cell type. For instance, it is not clear for how long the Dcx protein can be detected in functional young human neurons; ironically, technical advances that increase the efficiency of detecting a particular marker may lead, instead of resolving controversies, to a misattribution of young differentiated neurons as neuronal precursors.
- Prolonged stress and disease may affect the marker expression (cf. rapid decay of Dcx protein in stressed animals [102]), decreasing the estimate for the rate of neurogenesis in the human hippocampus.

To conclude, some of the basic features of the blueprint of how human and animal neural stem cells maintain their quiescence, become activated, differentiate, and disappear are not yet resolved. The potential relevance of hippocampal neurogenesis to the prospects of slowing down or reversing cognitive decline in the aging or diseased human brain adds to the urgency of resolving these outstanding questions and to developing new approaches to study brain stem cells.

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