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Milestones of neuronal development in the adult hippocampus

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Adult hippocampal neurogenesis originates from precursor cells in the adult dentate gyrus and results in new granule cell neurons. We propose a model of the development that takes place between these two fixed points and identify several developmental milestones. From a presumably bipotent radial-glia-like stem cell (type-1 cell) with astrocytic properties, development progresses over at least two stages of amplifying lineage-determined progenitor cells (type-2 and type-3 cells) to early postmitotic and to mature neurons. The selection process, during which new neurons are recruited into function, and other regulatory influences differentially affect the different stages of development.

Throughout adult mammalian life, hippocampal neurogenesis generates new granule cell neurons that become integrated into the dentate gyrus. This process is tightly regulated and can be influenced by many factors. Regarding the recent flood of papers on this regulation, some critical minds have longed to see the one stimulus that does not affect adult neurogenesis.

In most studies, only few parameters have been assessed to characterize the regulation of adult neurogenesis. The most widely used parameters are cell proliferation in the subgranular zone (SGZ), cell survival, and neuronal differentiation. Consequently, the patterns of regulation appeared relatively uniform. But should it really be true that non-specific stimuli (e.g. hormonal changes [1] or physical activity [2]), functionally specific stimuli (e.g. learning [3] and the experience of complexity [4]) and pathological stimuli (e.g. ischemia [5], trauma [6] or seizures [7,8]) all have 'identical' effects on adult neurogenesis, in that they plainly just induce proliferation of a precursor cell or increase their survival?

If adult hippocampal neurogenesis is functionally relevant and an evolutionary benefit is linked to it, it does not make sense that adult neurogenesis would react with highest sensitivity to every kind of stimulus in the same way, even if the net result of more new neurons might look very similar. We therefore suspect that the impression of a standardized neurogenic response is due to a still relatively simplistic view of what actually constitutes adult neurogenesis. Rather, adult neurogenesis and its regulation can be understood only if quantitative considerations such as cell counts are combined with a qualitative assessment of neuronal development. How a stimulus influences the quality of neuronal development might be as meaningful as the numbers of newly generated neurons. This will be of particular importance for contexts in which normal adult neurogenesis apparently fails, such as in temporal lobe epilepsy [9], or where its failure has been discussed as a possible factor, such as in major depression [10–12]. However, the prerequisite for understanding the altered function is characterization of normal neuronal development in the adult dentate gyrus.

In general, the mere existence of adult hippocampal neurogenesis is undisputed today. But many individual results are controversial because of the methodological limitations of birth-marking dividing cells with bromodeoxyuridine (BrdU) and the problem of deciding on sufficient criteria to identify a cell as a neuron. These technical issues have been extensively discussed elsewhere [13,14]. The field is now at the point where we can begin to describe adult neurogenesis in more detail and thereby add precision to our conclusions.

Limitations to describing neuronal development in the adult

Adult neurogenesis and embryonic neurogenesis differ in at least two fundamental aspects. Adult neurogenesis proceeds in an environment that is not generally programmed to promote neurogenesis because its own development has long ceased. Neuronal development in the adult thus needs protection from the anti-neurogenic influences of the surrounding brain tissue and requires the maintenance of a permissive microenvironment.

The second fundamental difference is that adult neurogenesis shows nothing of the orchestrated and massively parallel progression of developmental stages. In the adult hippocampus, neurons of all developmental stages can be found at any given time point. Thus, adult neurogenesis is an individualized process, not a population event. We use 'neurogenesis' as a term encompassing this entire multistep process, not the division of a neuronal progenitor cell alone.

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On a superficial level, describing adult hippocampal neurogenesis is very straightforward. A dividing precursor cell gives rise to daughter cells, which migrate away from the site of division [15] and start to differentiate into neurons [16]. They first extend dendrites towards the molecular layer and later an axon that projects to area CA3 [17]. Over four to seven weeks they become integrated into the local neuronal circuits [18]. Besides the challenging fact that all these steps in different cells are found in parallel and in immediate neighborhood, many open questions surround every particular step.

The biggest problems with this superficial description are that: (i) it does not reflect that the population of dividing cells is heterogeneous [19,20]; (ii) neurogenesis is intricately interspersed with gliogenesis [21]; (iii) most of the newly generated cells die [16,22,23]; (iv) the temporal resolution of the model is too low to address where exactly regulation occurs; and (v) it is not clear when in development the fate choice decision towards actual neuronal differentiation is made and, thus, where the crucial restriction point 'of no return' is.

The fifth of these problems is particularly worth considering, because adult neurogenesis is such a rare event in comparison with the immense production of neurons during embryonic brain development. Also, there must be a point in adult neurogenesis at which the number of new immature cells, whose amplification can be induced by a wide range of rather non-specific stimuli, is matched against the actual functional needs of the hippocampus. At this point, only a subset of immature new cells is selected and recruited into function [3,4].

A model of six developmental milestones

We here propose a model that characterizes several easily identifiable developmental milestones in adult hippocampal neurogenesis. Criteria are morphology and expression of some key markers.

The six milestones of neuronal development (Figure 1) are:

- (stage 1) the division of a stem cell (type-1 cell), giving rise to
- (stages 2–4) three consecutive stages of putative transiently amplifying progenitor cells, which differ by their proliferative potential and their increasing neuronal differentiation. These stages lead finally to the exit from the cell cycle and to
- (stage 5) a transient postmitotic stage, during which network connections are established and the selection for long-term survival occurs, and finally to
- (stage 6) the stage of the terminally differentiated new granule cells.

Breaking down adult neurogenesis into these few accessible steps opens up a new view on how neuronal development occurs under the condition of the adult hippocampus. One might have perceived adult neurogenesis as an automated program to neuronal differentiation, originating

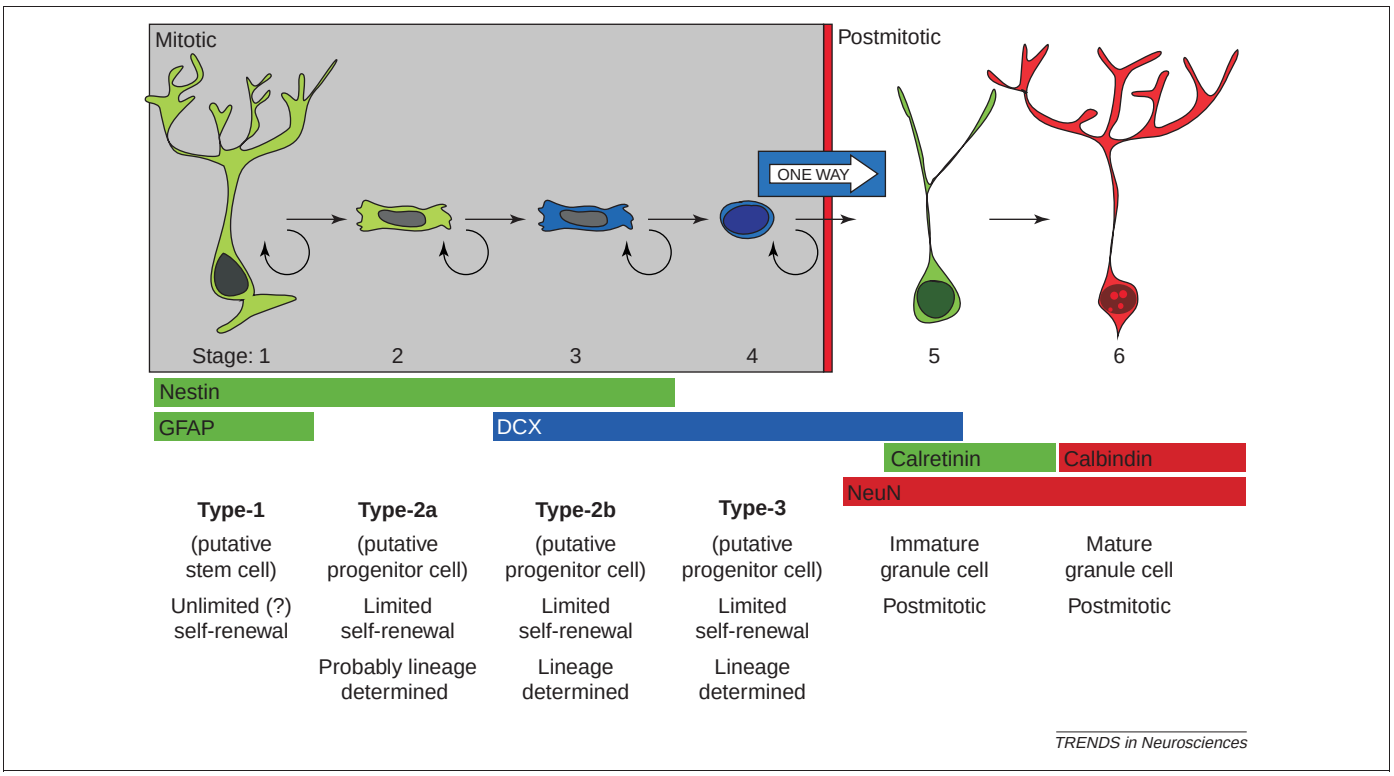

Figure 1. Proposed sequence of cell types in adult hippocampal neurogenesis. Six stages of neuronal development in the adult hippocampus can be readily identified on the basis of morphology, proliferative ability, and expression of markers such as nestin, glial fibrillary acidic protein (GFAP), doublecortin (DCX), calretinin, calbindin and NeuN. Development originates from the putative stem cell (type-1 cell; stage 1) that has radial glia and astrocytic properties and is probably identical to the astrocyte-like B-cell, first identified as the stem cell of this region by Seri *et al.* [19]. Neuronal development then progresses over three stages of putative transiently amplifying progenitor cells (type-2a, type-2b and type-3 cells; stages 2–4), which appear to be increasingly determined to the neuronal lineage because *in vivo* no overlap with any glial markers has been found in these cells, to an early postmitotic stage (indicated by the 'one-way' sign). This transient early postmitotic period is characterized by calretinin expression (stage 5). Confocal images of these stages are found in Figure 2, and quantification is in Figure 3. Distinction of cells as stem cells, transiently amplifying progenitor cells and lineage-determined progenitor cells is hypothetical and remains to be proven *in vivo*. Modified from Ref. [42].

from proliferating stem cells and corrected only by cell death mechanisms. By contrast, adult neurogenesis turns out to be a complex process with many developmental steps sensitive to different regulatory influences.

After dividing cells have been labeled with the proliferation marker BrdU, which persists in daughter cells and can be detected immunohistochemically, only certain combinations of markers and morphologies of labeled cells are observed at different intervals after BrdU injection (Figure 1). We conclude that there is a progression from expression of immature to mature markers combined with suggestive changes in morphology.

Stage 1: type-1 cells are the radial-glia-like stem cells of the adult dentate gyrus

The putative stem cell of the adult SGZ is a radial-glia-like cell with a characteristic morphology [19,20] (Figure 2). We have named these cells type-1 cells [20]. The soma of these cells is roughly triangular, and a long and strong apical process reaches into the granule cell layer branching sparsely until it reaches the inner molecular layer, where it disperses in many small processes. Type-1 cells express the astrocytic marker glial fibrillary acidic protein (GFAP) and share several other astrocytic features such as vascular end-feet and the electrophysiological properties of astrocytes [20,24]. Type-1 cells express nestin and make up approximately two-thirds of the nestin expressing cells of the SGZ in the adult mouse. However, they account for only 5% of cell divisions among the nestin-expressing cells of the SGZ [20,25]. It seems surprising that type-1 cells divide at all. The likeliest explanation is that type-1 cells divide asymmetrically. Although final proof (and real-time observation) is lacking, their morphology during M-phase of the cell cycle is suggestive of a characteristic type of asymmetric division (Figure 2e). The daughter cell, which would bud off at the base of the type-1 cell, is also nestin-expressing and has been named the type-2 cell [20,24].

Type-1 cells have the appearance of radial glia. Radial glia in the embryonic ventricular zone have stem cell properties and region-dependent neurogenic potential [26–29]. In the hippocampus, radial-glia-like cells provide scaffolding, which is necessary for normal development of the dentate gyrus [30]. The finding that radial-glia-like cells in the adult brain have stem cell properties [19,31,32] links them to new concepts of radial glia function during embryonic brain development.

Type-1 cells in the adult dentate gyrus overlap with the astrocyte-like B cells, first identified as the putative stem cells of this brain region by Seri and Alvarez-Buylla [19]. However, as one can see in Figure 2(a), not all GFAP-expressing cells with the morphology of radial glia are positive for the precursor cell marker nestin: type-1 cells are probably a subset of B cells. The nomenclature with letters had been originally devised to describe the subventricular zone (SVZ), the second neurogenic region of the adult brain [31–33]. Adult hippocampal neurogenesis differs considerably from neurogenesis in the adult olfactory system and inducers of hippocampal neurogenesis do not necessarily act on SVZ neurogenesis [34,35]. Precursor cells of the SVZ do not appear to be identical to the cells

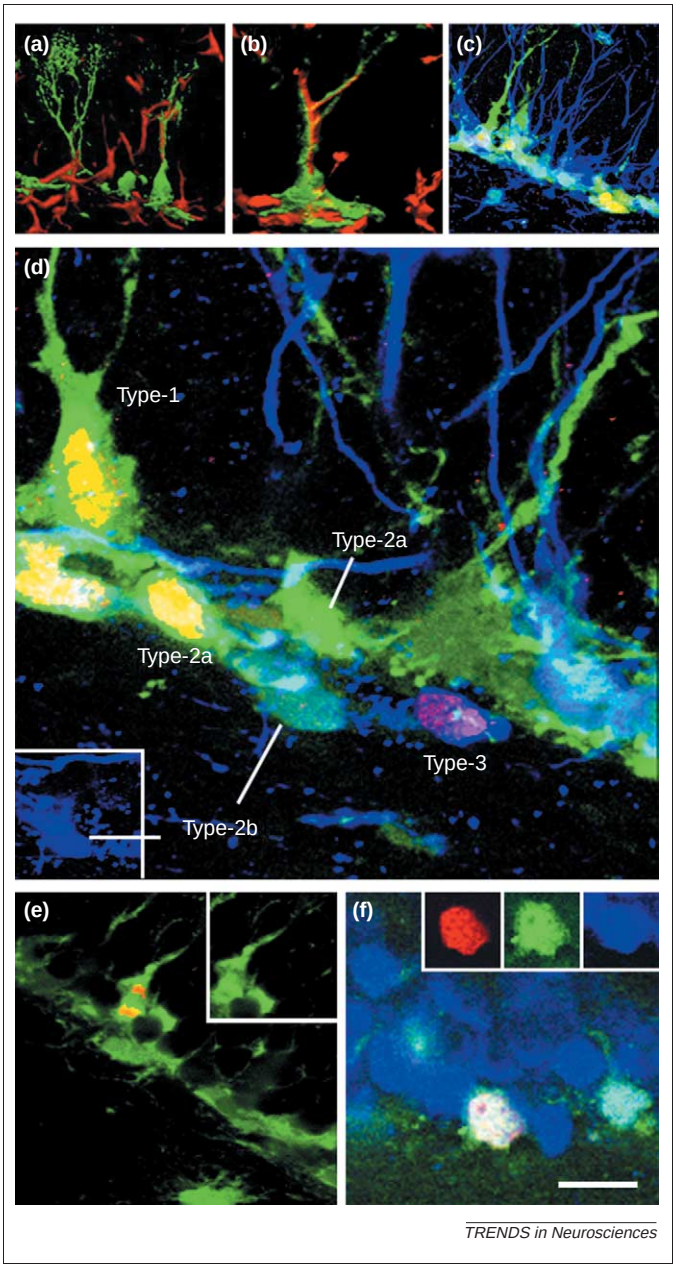

Figure 2. Cell types in neuronal development of the adult hippocampus. Confocal microscopic images from mouse brain depict the different cell types in adult hippocampal neurogenesis (methods are as described in [21,25,42]). **(a)** Type-1 cells have a radial glia-like appearance with the cell body in the subgranular zone of the dentate gyrus and a strong process reaching into the granule cell layer. They supposedly are the bipotent stem cell of this region. They express nestin [nestin green fluorescent protein (nestin-GFP; green)] and glial fibrillary acidic protein (GFAP; red). Note that not all radial-glia-like processes are nestin-positive. **(b)** The colocalization of GFAP and nestin within the process can be appreciated in this image. **(c)** Immature neurons express doublecortin (DCX; blue); this is also present in their dendrites. The young dendrites do not show a clear relationship to the similarly oriented nestin-GFP-positive processes of the type-1 cells (green). The insert shows DCX-expression (blue) in the weakly nestin-GFP-positive type-2b cell indicated. **(d)** The four proliferative types of precursor cells are distinguished by their morphology and the absence and presence of nestin (nestin-GFP; green) and DCX (blue). For details, see Figure 1. Red identifies cells that were labeled with proliferation marker Ki67. In general, all four cell types can divide. **(e)** A mitotic figure in a type-1 cell suggestive of an asymmetric division, detected by immunohistochemistry for phospho-histone H3, an M-phase marker (red). The insert shows the nestin-GFP expression only. **(f)** The early postmitotic stage of neuronal development is characterized by expression of Ca^{2+}-binding protein calretinin (green) in cells that were birth-marked with the thymidine analog bromodeoxyuridine (BrdU) one week earlier (red). Blue indicates expression of the granule cell marker NeuN. Inserts display the three channels for the triple-labeled cell individually. Scale bar in (f): 100 μm (a–c), 10 μm (d), 60 μm (e) and 15 μm (f).

found in the SGZ [36]. Our use of a preliminary but distinct nomenclature for the hippocampus is intended to avoid a premature conceptual equalization with the situation in the SVZ. It does not question the data obtained by Seri *et al.* and it is not meant to introduce just another nomenclature. At a later time and based on functional criteria, a definite and intuitive nomenclature should be devised. The numbering is a relatively neutral means of describing the different categories of cells.

Precursor cells from the hippocampus are multipotent *in vitro* [37]. *In vivo*, there is evidence of both neurogenesis and gliogenesis in the dentate gyrus [21,38], but at present it is not clear whether both originate from the type-1 cell. In contrast to type-1 cells, the newly generated astrocytes of the adult SGZ express not only GFAP but also S100β [21,39], which is another established marker for mature astrocytes [40]. In the SGZ we have never found S100β-positive cells to be in cell cycle. New S100β-positive astrocytes are probably derived from an as-yet elusive progenitor cell within the glial lineage [21]. From data by Seri *et al.*, it seems likely that this intermediate cell also originates from the radial-glia-like precursor [19] but final proof of their multipotency *in vivo* is still lacking.

Stages 2–4: type-2 and type-3 cells are the transiently amplifying progenitor cells in the neuronal lineage

Type-2 cells have a distinct morphology, with plump short processes that are oriented tangentially. They are GFAP-negative and highly proliferative [20,25] (Figure 2d). Type-2 cells have an irregularly shaped dense nucleus. Their morphology suggests that they are capable of tangential migration [15].

Type-2 cells come in two subtypes, both nestin-positive, one negative and one positive for immature neuronal marker doublecortin (DCX) [20,25,41,42], which are therefore named type-2a and type-2b, respectively [25]. Type-3 cells are DCX-positive but nestin-negative. They are also proliferative. It appears that this stage is one of great morphological changes [42]. The nucleus of type-3 cells is rounded. These cells all express the polysialated form of neural cell adhesion molecule (PSA-NCAM), but so do some type-2 cells [20]. PSA-NCAM has early been noted to mark a stage of precursor cell activity in adult hippocampal neurogenesis [43,44]. Type-1 cells, by contrast, are always negative for PSA-NCAM.

Type-2 and type-3 cells share many features and the three identified stages might actually reflect a continuum that could either have many more potentially identifiable sub-stages or be subsumed under one single stage of development. Consequently, it is not clear how type-2 and 3 cells relate to the D cell described by Seri *et al.* [19]. One could argue that our specification according to morphology plus antigen expression divides the group of D cells into three subtypes. However, this subdivision makes sense because, as will be discussed, type-2 and type-3 cells do not behave uniformly in response to stimuli.

Many type-2 cells have a complex pattern of electrophysiological responses, clearly different from those of astrocytes and similar to the membrane properties described for oligodendrocyte precursor cells [20]. A small number of type-2 cells show Na^+ currents and thus early signs of neuronal differentiation. Electrophysiology thus confirmed the heterogeneity of progenitor cells in the adult dentate gyrus and suggested that functional neuronal differentiation can begin on the level of type-2 cells [20,24]. As DCX and PSA-NCAM expression and morphology indicate, type-3 cells are even further advanced in the expression of neuronal features.

Taken together, in the adult SGZ, four distinct cell types with certain precursor cell properties can be found. One might want to apply a classical concept of stem cell biology [45,46] to these cells and consider type-1 cells as stem cells, type-2a and type-2b cells as transiently amplifying, lineage-determined progenitor cells, and type-3 cells as neuroblasts. But this categorization is hypothetical and remains to be verified. All of these cells divide, and over time the distribution of BrdU-labeling shifts through these four cell types [25] (Figure 3). The unidirectional redistribution of BrdU-labeled cells is suggestive of a marker progression and thus development.

Stage 5: calretinin expression marks a transient postmitotic stage in early neuronal development

DCX-expression persists onto the next stage, at which the maturing granule cells become postmitotic. The majority of cells reach this stage only 3 d after the initial division. During this time the population of new cells is expanded fourfold or fivefold. On day 3, two-thirds of these cells already express postmitotic markers. Within four more days the number of new cells drops dramatically and at 2.5 weeks after the initial division reaches a level that remains stable over months and years [16]. This curve implies that expansion occurs on the level of precursor cells, whereas recruitment for terminal differentiation, and thereby the final decision for long-lasting survival, takes place during the early postmitotic period.

This early postmitotic stage of adult granule cell development is characterized by the transient expression of Ca^{2+}-binding protein calretinin (Figure 2f), which is later exchanged for calbindin in mature granule cells [42]. At this stage, the cells retain the vertical morphology of (late) type-3 cells, with a rounded or slightly triangular nucleus and a clearly visible apical dendrite (Figure 2c). We hypothesize that this is also when the new cells send out their axon. Axonal contact in the target region CA3 has been found as early as 3–5 d after division [47]. Calretinin expression is also found in new neurons of the olfactory system, but it appears there earlier, when the cells are still migrating to their target zone [48,49].

The cells now also express postmitotic neuronal marker NeuN, the most widely used indicator for 'mature neurons'. Even 1 d after a single injection of BrdU, one can find a considerable number of cells labeled for both BrdU and NeuN; after only 3 d their number reaches a maximum [21,42]. The majority of these immature neurons are subject to a selection process, during which they are either recruited into function or eliminated [22,23].

Stage 6: mature new granule cells express calbindin

Approximately 2–3 weeks after becoming postmitotic and expressing calretinin, the new cells switch the expression of calretinin to calbindin. Calbindin is found in all mature

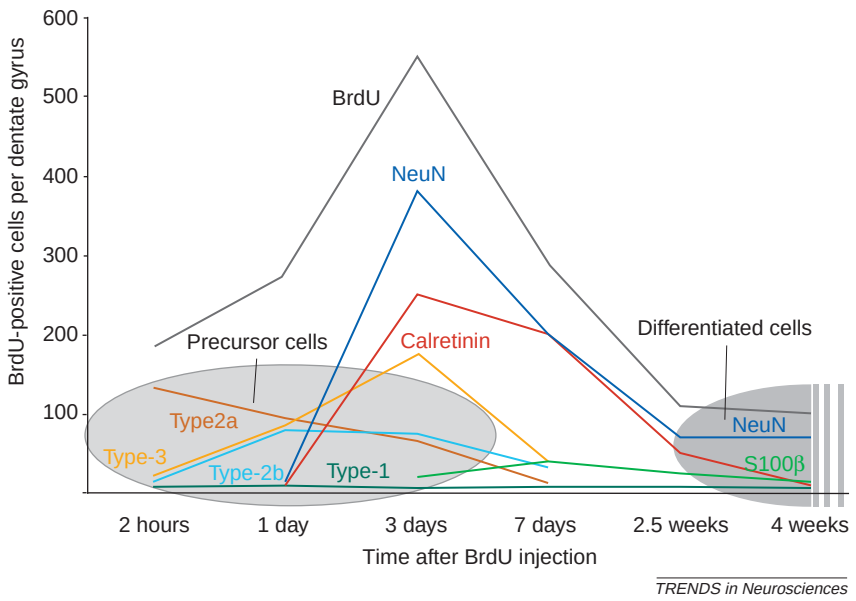

Figure 3. Numbers of new cells during different stages of neuronal development. The curves show numbers of cells of different phenotypes labeled with the thymidine analog bromodeoxyuridine (BrdU), over time after the injection of BrdU. The gray line on top gives the total number of BrdU-labeled cells; the colored curves below give the number of BrdU-marked cells expressing calretinin, NeuN and S100β (a marker for mature astrocytes), and the number of BrdU-marked cells of the different precursor cell types. The progression through expression of different markers is also depicted in Figure 1. The largest expansion of new cells occurs within the first three days after the injection of BrdU. Selection, or otherwise elimination, of new cells occurs on the level of calretinin-positive, early postmitotic neurons. The schematic drawing compiles data from Refs [21,25,42]; please refer to the original articles for exact numbers of cells.

granule cells. Calbindin expression alone, however, does not yet mark full maturation. It takes 4–7 weeks for the new cells to become functionally indistinguishable from older granule cells [18,50]. Details about this final stage of maturation are not yet known. At this time the new neurons must find their place in the hippocampal circuitry, including their connections to the local network of interneurons. The number of new cells, however, does not change further once this stage has been reached.

Selective responses of different progenitor cell types to neurogenic stimulation

In the sequence of four progenitor cell stages, the cells respond differently to different neurogenic stimuli. Numbers of type-1 cells do not seem to change in response to the neurogenic stimuli investigated so far [25]. Neither environmental enrichment nor voluntary wheel running, two robust physiologic neurogenic stimuli, increased the low proliferative activity of type-1 cells [25].

The relative constancy of type-1 cells is in accordance with the hypotheses that these cells are the stem cells of the hippocampus [33]. As a stable foundation of this system they would be controlled largely by stem-cell-specific genetic programs and not directly participate in the acute fluctuations of adult neurogenesis. Nevertheless, type-1 cells have a very suggestive communicative morphology, with processes into the molecular layer and on blood vessels in the SGZ. Therefore, type-1 cells might mediate relevant information without this resulting in their increased proliferation.

Type-2 cells, by contrast, divide under resting conditions and respond to physiologic stimulation. Wheel running as a physiological but very general stimulus on adult neurogenesis induced the proliferation of type-2 cells but not of type-3 cells [25]. Environmental enrichment, by contrast, affected the division of neither type-2 nor type-3 cells but still increased net neurogenesis [25]. This finding underscores that the distinction of type-2 and type-3 cells is not merely academic. In both stimulatory paradigms, the number of calretinin-expressing cells in the SGZ increased [42]. This suggests that, independent of their different pro-proliferative action, both physiological neurogenic stimuli induced the fate choice decision toward actual neuronal differentiation.

Perspective

The proposed model not only adds details to the description of neuronal development in the adult hippocampus but also allows concepts of different stages and degrees of 'stemness' to be applied to adult neurogenesis. The population of neuronal precursor cells in the adult dentate gyrus is heterogeneous and the regulation of neuronal development involves effects on several of these levels, even if the details of this response are not yet clear. The most important goal for future studies is to clarify the lineage relationships within the adult dentate gyrus. How are neurogenesis and gliogenesis linked? Are type-1 cells bipotent or even multipotent *in vivo*? How lineage-determined or even lineage-restricted are type-2 and type-3 cells? How is the natural course of neuronal development in the adult hippocampus altered by pathology and how might disturbed neuronal development in the adult brain contribute to neuropsychiatric disease?

Research on adult hippocampal neurogenesis is currently making the crucial step from phenomenology to an understanding of mechanisms. Considering adult neurogenesis as development rather than an event prepares the

ground for appreciating the complexity of the underlying biology.

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References

1 Gould, E. *et al.* (1992) Adrenal hormones suppress cell division in the adult rat dentate gyrus. *J. Neurosci.* 12, 3642–3650
2 Van Praag, H. *et al.* (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* 2, 266–270
3 Gould, E. *et al.* (1999) Learning enhances adult neurogenesis in the hippocampal formation. *Nat. Neurosci.* 2, 260–265
4 Kempermann, G. *et al.* (1997) More hippocampal neurons in adult mice living in an enriched environment. *Nature* 386, 493–495
5 Liu, J. *et al.* (1998) Increased neurogenesis in the dentate gyrus after transient ischemia in gerbils. *J. Neurosci.* 18, 7768–7778
6 Gould, E. and Tanapat, P. (1997) Lesion-induced proliferation of neuronal progenitor cells in the dentate gyrus of the adult rat. *Neuroscience* 80, 427–436
7 Bengzon, J. *et al.* (1997) Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *Proc. Natl. Acad. Sci. U. S. A.* 94, 10432–10437
8 Parent, J.M. *et al.* (1997) Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J. Neurosci.* 17, 3727–3738
9 Parent, J.M. and Lowenstein, D.H. (2002) Seizure-induced neurogenesis: are more new neurons good for an adult brain? *Prog. Brain Res.* 135, 121–131
10 Jacobs, B.L. *et al.* (2000) Adult brain neurogenesis and psychiatry: a novel theory of depression. *Mol. Psychiatry* 5, 262–269
11 D'Sa, C. and Duman, R.S. (2002) Antidepressants and neuroplasticity. *Bipolar Disord.* 4, 183–194
12 Kempermann, G. and Kronenberg, G. (2003) Depressed new neurons? – Adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. *Biol. Psychiatry* 54, 499–503
13 Cooper-Kuhn, C.M. and Kuhn, H.G. (2002) Is it all DNA repair? Methodological considerations for detecting neurogenesis in the adult brain. *Brain Res. Dev. Brain Res.* 134, 13–21
14 Hayes, N.L. and Nowakowski, R.S. (2002) Dynamics of cell proliferation in the adult dentate gyrus of two inbred strains of mice. *Brain Res. Dev. Brain Res.* 134, 77–85
15 Kuhn, H.G. *et al.* (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J. Neurosci.* 16, 2027–2033
16 Kempermann, G. *et al.* (2003) Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* 130, 391–399
17 Stanfield, B.B. and Trice, J.E. (1988) Evidence that granule cells generated in the dentate gyrus of adult rats extend axonal projections. *Exp. Brain Res.* 72, 399–406
18 Jessberger, S. and Kempermann, G. (2003) Adult-born hippocampal neurons mature into activity-dependent responsiveness. *Eur. J. Neurosci.* 18, 2707–2712
19 Seri, B. *et al.* (2001) Astrocytes give rise to new neurons in the adult mammalian hippocampus. *J. Neurosci.* 21, 7153–7160
20 Filippov, V. *et al.* (2003) Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. *Mol. Cell. Neurosci.* 23, 373–382
21 Steiner, B. *et al.* (2004) Differential regulation of gliogenesis in the context of adult hippocampal neurogenesis in mice. *Glia* 46, 41–52
22 Biebl, M. *et al.* (2000) Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. *Neurosci. Lett.* 291, 17–20
23 Young, D. *et al.* (1999) Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nat. Med.* 5, 448–453
24 Fukuda, S. *et al.* (2003) Two distinct subpopulations of nestin-positive cells in adult mouse dentate gyrus. *J. Neurosci.* 23, 9357–9366
25 Kronenberg, G. *et al.* (2003) Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *J. Comp. Neurol.* 467, 455–463
26 Malatesta, P. *et al.* (2000) Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. *Development* 127, 5253–5263
27 Noctor, S.C. *et al.* (2001) Neurons derived from radial glial cells establish radial units in neocortex. *Nature* 409, 714–720
28 Noctor, S.C. *et al.* (2002) Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. *J. Neurosci.* 22, 3161–3173
29 Kriegstein, A.R. and Gotz, M. (2003) Radial glia diversity: a matter of cell fate. *Glia* 43, 37–43
30 Forster, E. *et al.* (2002) Reelin, Disabled 1, and β1 integrins are required for the formation of the radial glial scaffold in the hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* 99, 13178–13183
31 Doetsch, F. *et al.* (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J. Neurosci.* 17, 5046–5061
32 Doetsch, F. *et al.* (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 97, 703–716
33 Alvarez-Buylla, A. *et al.* (2001) A unified hypothesis on the lineage of neural stem cells. *Nat. Rev. Neurosci.* 2, 287–293
34 Rochefort, C. *et al.* (2002) Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. *J. Neurosci.* 22, 2679–2689
35 Brown, J. *et al.* (2003) Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *Eur. J. Neurosci.* 17, 2042–2046
36 Seaberg, R.M. and van der Kooy, D. (2002) Adult rodent neurogenic regions: the ventricular subependyma contains neural stem cells, but the dentate gyrus contains restricted progenitors. *J. Neurosci.* 22, 1784–1793
37 Palmer, T.D. *et al.* (1997) The adult rat hippocampus contains primordial neural stem cells. *Mol. Cell. Neurosci.* 8, 389–404
38 Kempermann, G. *et al.* (1997) Genetic influence on neurogenesis in the dentate gyrus of adult mice. *Proc. Natl. Acad. Sci. U. S. A.* 94, 10409–10414
39 Kempermann, G. *et al.* (1998) Experience-induced neurogenesis in the senescent dentate gyrus. *J. Neurosci.* 18, 3206–3212
40 Savchenko, V.L. *et al.* (2000) Microglia and astrocytes in the adult rat brain: comparative immunocytochemical analysis demonstrates the efficacy of lipocortin 1 immunoreactivity. *Neuroscience* 96, 195–203
41 Brown, J.P. *et al.* (2003) Transient expression of doublecortin during adult neurogenesis. *J. Comp. Neurol.* 467, 1–10
42 Brandt, M.D. *et al.* (2003) Transient calretinin-expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. *Mol. Cell. Neurosci.* 24, 603–613
43 Seki, T. and Arai, Y. (1993) Highly polysialylated neural cell adhesion molecule (NCAM-H) is expressed by newly generated granule cells in the dentate gyrus of the adult rat. *J. Neurosci.* 13, 2351–2358
44 Seki, T. (2002) Expression patterns of immature neuronal markers PSA-NCAM, CRMP-4 and NeuroD in the hippocampus of young adult and aged rodents. *J. Neurosci. Res.* 70, 327–334
45 Weissman, I.L. *et al.* (2001) Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. *Annu. Rev. Cell Dev. Biol.* 17, 387–403
46 Seaberg, R.M. and van der Kooy, D. (2003) Stem and progenitor cells: the premature desertion of rigorous definitions. *Trends Neurosci.* 26, 125–131
47 Hastings, N.B. and Gould, E. (1999) Rapid extension of axons into the CA3 region by adult-generated granule cells. *J. Comp. Neurol.* 413, 146–154
48 Li, Z. *et al.* (2002) Cell dynamics of calretinin-immunoreactive neurons in the rostral migratory stream after ibotenate-induced lesions in the forebrain. *Neurosci. Res.* 42, 123–132
49 Jankovski, A. *et al.* (1998) Proliferation, migration and differentiation of neuronal progenitor cells in the adult mouse subventricular zone surgically separated from its olfactory bulb. *Eur. J. Neurosci.* 10, 3853–3868
50 van Praag, H. *et al.* (2002) Functional neurogenesis in the adult hippocampus. *Nature* 415, 1030–1034