Adult Neurogenesis

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Abstract

Adult neurogenesis is the generation of new neurons in the adult brain. Adult neurogenesis is an exception, as the brain of mammals is non-neurogenic. Rodents and primates have two neurogenic zones, the hippocampus and the olfactory bulb. Lower vertebrates often have many more sites of adult neurogenesis. In the peripheral nervous system, there is high neurogenesis in the olfactory epithelium. Neurogenesis in the olfactory bulb originates from precursor cells in the wall of the lateral ventricle, the subventricular zone. New interneurons in the bulb are produced. In the dentate gyrus of the hippocampus, new excitatory granule cells are generated that add to the mossy fiber tract into

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hippocampal area CA3. Numerous factors are known to regulate neurogenesis: it seems that adult neurogenesis responds very sensitively to external stimuli. The current hypothesis is that adult hippocampal neurogenesis critically contributes to hippocampal function, allowing life-long adaptation processes. If adult neurogenesis fails, this might contribute to several important diseases, including dementias, major depression and schizophrenia, as well as temporal lobe epilepsy.

Keywords

Adult neurogenesis • Astrocyte-like stem cells • B cells • Brain-derived neurotrophic factor (BDNF) • C cells • Catastrophic interference • Chain migration • Concrete learning stimuli • Cortical neurogenesis • Dendritic fine-tuning • Doublecortin (DCX) • Homotypic migration • Immediate neuronal progenitor cells • Mossy fibers • Neural stem cells • Neuroblast • NG2 cells • Pattern separation • Pax6 • Phylogenetic reduction • Postmitotic astrocytes • Radial glia-like stem cells • Regenerative neurogenesis • Rostral migratory stream (RMS) • Spatial learning • Subgranular zone • Temporal lobe epilepsy

Abbreviations

Ascl1	Official symbol of gene Achaete-scute complex homolog 1 (Drosophila), also known as Mash1
BDNF	Brain-derived neurotrophic factor
BLBP	Brain lipid-binding protein, an antigen characteristic of radial glia
CA1	Cornu ammonis, area 1, the third relay station in the trisynaptic backbone of the hippocampus, projecting to the subiculum and back to the cerebral cortex
CA3	Cornu ammonis, area 3, the second relay station in the trisynaptic backbone of the hippocampus, projecting to CA1
DCX	Doublecortin, protein related to the cytoskeleton and associated with plasticity, expressed during intermediate stages of adult neurogenesis
Dlx1	Official symbol of gene Distal-less homeobox 1
EGF	Epidermal growth factor
FGF2	Fibroblast growth factor 2
GABA	Gammy amino butyric acid, inhibitory neurotransmitter
GFAP	Glial fibrillary acidic protein, intermediate filament that is the classical astrocytic marker, also expressed by the radial glia-like "stem cells"
GLAST	Glutamate transporter, an antigen expressed by radial glia
Hes	Official symbol of genes in the Hairy and enhancer of split family of genes
IGF1	Insulin-like growth factor 1
LTP	Long-term potentiation
miRNA	Micro-ribo nucleic acid, short regulatory pieces of RNA
NeuroD	Alias of gene Neurod1, neurogenic differentiation 1

NG2	Acronym standing for "neuron glia 2," the proteoglycan that allows identification of the class of cells named after this antigen, a particular group of cells, partially with precursor cell properties that share some neuronal and glial features
Ngn1	Official symbol of gene Neurogenin 1
Pax6	Official symbol of gene Paired box gene 6
Prox1	Official symbol of gene Prospero-related homeobox 1
PSA-NCAM	Polysialylated form of the neural cell adhesion molecule, surface molecule expressed during intermediate stages of adult neurogenesis
RMS	Rostral migratory stream, the route of migration for neuroblasts between the SVZ and the olfactory bulb
SGZ	Subgranular zone, the germinative matrix in the adult hippocampus, situated between the band of granule cells in the dentate gyrus and the hilus
SVZ	Subventricular zone, in adult neurogenesis the germinative matrix immediately below the ependyma
Tbr1	Alias of gene Eomes
Tbr1	Official symbol of gene T-box brain gene 1
VEGF	Vascular endothelial growth factor

Brief History

Adult neurogenesis was not recognized before the mid-1960s, when Joseph Altman first described neurogenesis in the hippocampus of adult rats. He, and in 1977 Michael Kaplan, also described that cell proliferation in the walls of the ventricles produced new neurons in the olfactory bulb. The reports were more or less treated as curiosities, but Stephen Goldman's and Fernando Nottebohm's discovery of neurogenesis in adult songbirds, where the production of new cells fluctuates with seasonal song learning, somewhat changed the perspective. It was not until the 1990s, however, that when neural stem cells were discovered in the adult brain and methodological advances facilitated the detection of newborn neurons, the field really gained momentum. Heather Cameron and Elisabeth Gould published the detailed re-discovery of adult hippocampal neurogenesis. Arturo Alvarez-Buylla and colleagues described adult olfactory neurogenesis of rats and mice in great anatomical detail. From Fred H. Gage's group came a wide spectrum of publications on various aspects of adult neurogenesis, including the first report linking adult hippocampal neurogenesis to behavior and, in 1998, the first report on new neurons in the adult human hippocampus. Since then the field experienced exponential growth. Especially the function of newborn neurons in the adult hippocampus was intensively explored and links to important diseases such as dementias, major depression, schizophrenia, and temporal lobe epilepsy have been made. The discovery of adult neurogenesis substantially changes our view on how the brain works.

Adult Neurogenesis Is Neuronal Development Under the Conditions of the Adult Brain

For most part, brain development ceases around birth or shortly thereafter, and the lack of persistent neurogenesis is considered one of the reasons why our brain regenerates so poorly and many neuropsychiatric disorders are chronic and irreversible. Lower vertebrates, in contrast, show widespread adult neurogenesis and often astonishing levels of regeneration. Whether this truly amounts to a so-called "phylogenetic reduction" is controversial because actually only few species have been studied. In all "higher" animals, however, adult neurogenesis is undisputedly an exception, not the rule. For adult neurogenesis two key requirements have to be met: stem cells must exist, from which the new neurons might develop, and an environment that allows neuronal development to occur. This environment is also relevant for maintaining and presumably protecting the stem cell pool and is usually referred to as "the niche." Most of the brain is not permissive for adult neurogenesis at least under physiological conditions. These are the "non-neurogenic" areas.

Neuronal development is an immensely complicated process that involves several discernable and many unnoticeable steps. The processual nature of adult neurogenesis is important: adult neurogenesis is no single event but an orchestrated sequence of many, beginning with the proliferation of a stem cell and ending with the integration of a new, fully functional neuron.

Of Neurogenic and Non-neurogenic Regions

In rodents and primates, adult mammal neurogenesis occurs in two "canonical" neurogenic regions. The subventricular zone (SVZ) of the walls of the ventricles and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus (Fig. 1).

The adult SVZ is not strictly identical to the SVZ of the developing cortex (as defined by the famous Boulder Committee) because in the adult no ventricular zone exists any more. In rodents, the precursor cells sit directly underneath the ependyma or, as in humans, rabbits, and cows, are separated from the ependyma only by a hypocellular gap. The adult SVZ produces various kinds of interneurons (the current count is seven) in the olfactory bulb. The neurogenic zone hence stretches out from the walls of the ventricle into the olfactory bulb. In the hippocampus, adult neurogenesis produces new granule cell neurons.

There are diverse reports on adult neurogenesis in other brain regions than the SVZ and the SGZ but they have not yet led to abandoning the distinction of SVZ and SGZ as neurogenic zones. In some species, especially among birds and fish, adult neurogenesis is far more widespread; zebrafish, for example, have 16 neurogenic regions in the adult. In mammals, the reports on adult neurogenesis in the neocortex, hypothalamus, amygdala, piriform cortex, etc., are to date supported by highly variable levels of evidence, in some cases only single studies still raising methodological concern. But while most bats do not seem to have adult hippocampal neurogenesis (but show neurogenesis in the olfactory bulb), rabbits appear to have

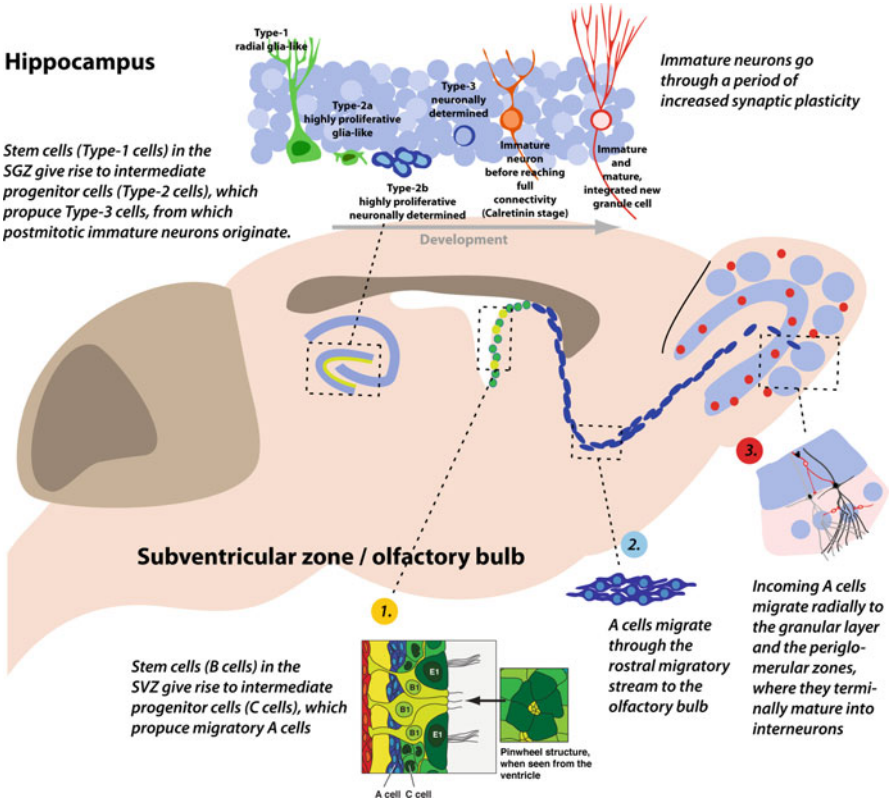


Fig. 1 *The neurogenic regions of the adult rodent brain*. Rodents and primates, as well as most mammals studied so far, have two "canonical" neurogenic regions, the dentate gyrus in the hippocampus and the subventricular zone (*SVZ*)/olfactory bulb. Both regions share certain cardinal features of neurogenesis, including a characteristic sequence of stages and events, but other than that have little in common

more (e.g., in the caudate nucleus). It cannot be maintained, though, that "simple" animals have generalized adult neurogenesis, while more complex brains would have less. There is, for example, no clear sign of adult neurogenesis in Drosophila. One hypothesis is that adult neurogenesis correlates with the overall level of plasticity and adaptability rather than inversely with brain size or complexity.

Neurogenic regions in the stricter sense, that is, SVZ and SGZ, are characterized by the presence of neurogenic precursor cells and a microenvironment that is permissive for neuronal development to occur. The relevance of the microenvironment has been established in transplantation studies, which showed that precursor cells from non-neurogenic areas, such as the spinal cord, can produce neurons if transplanted, for example, into the dentate gyrus. The problem is, however, that there are only very few such studies and that some results are ambiguous. The consequence is that the exact contribution of cell-autonomous versus microenvironmental factors is still not fully resolved.

Non-neurogenic regions might contain precursor cells that show a neurogenic potential at least ex vivo but no appreciable neuronal differentiation under undisturbed in vivo conditions and appropriately no anatomically discernible "niche." The term of the stem cell niche has been borrowed from hematology and by now been extended to essentially all stem cell systems of the body. The niche is a defined microanatomical structure that surrounds the stem cells and their immediate progeny and consists of supporting and guiding cells, as well as vasculature, and possibly immune cells. Niche cells and stem cells form a functional unit. The question how niche-free stem cells, if they exist, might exert their (largely unknown) function is not clear. The best candidates for precursor cells in the non-neurogenic zones are the so-called NG2 cells, which share some features with neurons (the name of their lead antigen NG2, for Neuron Glia 2, hints at their position between the two worlds) but are not neurons and do not seem to be able to develop into neurons under normal conditions. A subset of NG2 cells rather represents precursor cells in the oligodendrocytic lineage.

All Adult Neurogenesis Originates from Neural Stem Cells

One of the reasons why the response to Altman's and Kaplan's discoveries of adult neurogenesis was only lukewarm was the fact that they could not provide a clear answer to the question, where the new neurons should come from. Altman speculated correctly that "some precursor cell" should be the origin of adult neurogenesis but only with the description of neural stem cells in the adult brain, these precursor cells became tangible. Strictly speaking, the term stem cell should be reserved for cells, whose "stemness" properties, that is, the ability to self-renew indefinitely and to differentiate into more than one lineage, has been clearly demonstrated. In practical terms, however, the term is used much more loosely and the precursor cells in the adult SGZ and SVZ are routinely referred to as stem cells. Their stem cell properties have indeed been demonstrated in cell culture experiments but the exact analogy between the cells in vitro and their in vivo counterpart has not been fully established. Within the systematics of stem cell biology, the precursor cells of the adult brain are "multipotent" in that they can produce (at least ex vivo) all three cardinal lineages of the adult brain: neurons, astrocytes, and oligodendrocytes. In vivo this trilineage potential has not been convincingly shown. For most purposes, in the context of adult neurogenesis, the first precursor cell in the lineage is called the "stem cell," their progeny "progenitor cells," whose migratory daughter cells are sometimes referred to as "neuroblast." At the latest, during the stage of the migratory neuroblast, the cells might exit from the cell cycle and enter a postmitotic maturation stage.

In vivo, the stem cells of the adult brain that lifelong produce new neurons share many similarities with astrocytes and are considered direct relatives of radial glia, a particular type of glia during development. In cortical development, radial glia serves both as precursor cell and as guidance structure. The stem cells of the adult brain do not show the full radial morphology (in that they do not stretch out between

the ventricular wall and the pial surface) but show other key features of radial glia, including the expression of characteristic markers such as BLBP, GLAST, and GFAP. The stem cells are thus often referred to as radial glia-like or astrocyte-like. Nevertheless, there are many semantic issues and factual controversies around the nomenclature and the exact nature of the precursor cells of the adult brain, which need to be taken into account when statements from different reports are compared.

The precursor cells of the SVZ and SGZ are not identical but are highly regionalized. Almost all precursor cells of the SVZ are of ultimately ventral origin (and appropriately produce interneurons and oligodendrocytes). Precursor cells of the dentate gyrus are dorsal (and thus generate excitatory neurons and astrocytes). But whereas there is no evidence to date that within the SGZ additional heterogeneity exists (with the exception of certain subtle functional differences between the ventral and the dorsal dentate gyrus), the SVZ is divided into several subregions that each appear to produce particular types of olfactory bulb interneurons and oligodendrocyte progenitor cells migrating into the neocortex.

Adult Hippocampal Neurogenesis

In the hippocampus, adult neurogenesis is limited to one single subregion, the dentate gyrus, and only one type of neuron is produced (Fig. 2). Adult neurogenesis lifelong generates new excitatory granule cells, the principal cells of the dentate gyrus that receive input from the entorhinal cortex and project to area CA3. The dentate gyrus is the first relay station in the classical trisynaptic backbone of the hippocampal circuitry (Fig. 3). From the second synapse in CA3, the pathway continues via the Shaffer collaterals to CA1 and from there via subiculum and entorhinal cortex back to cortical regions. The axons of the granule cells are called mossy fibers, and the mossy fiber tract represents a bottleneck in the circuit in that a large number of sparsely firing granule cells converge on few pyramidal neurons in CA3. This fact is presumably of relevance for the functional contribution of the newborn neurons. The newly generated granule cells allow an increase in the strength of the mossy fiber connection.

The precursor cells of the dentate gyrus reside in the so-called subgranular zone, usually abbreviated as SGZ, a narrow band of cells between the densely packed layer of granule cells and the hilus. The radial glia-like stem cells of the SGZ are referred to as type-1 cells. This nomenclature is meant to be neutral with respect to the specific properties of the different types of cells found in the course of adult neurogenesis. These exact properties are only slowly emerging, but they seem to correspond quite well to the traditional scheme of "stem cell–progenitor cell–neuroblast." Type-1 cells have many astrocytic properties, including the expression of GFAP and have a radial morphology in that they send a tree-like process into the molecular layer. In the SGZ they have vascular endfeet. Type-1 cells divide rarely. Their progeny are highly proliferative intermediate progenitor cells as well as, presumably, postmitotic astrocytes (also referred to as horizontal astrocytes, because they lack the radial morphology). The intermediate progenitor cells are highly

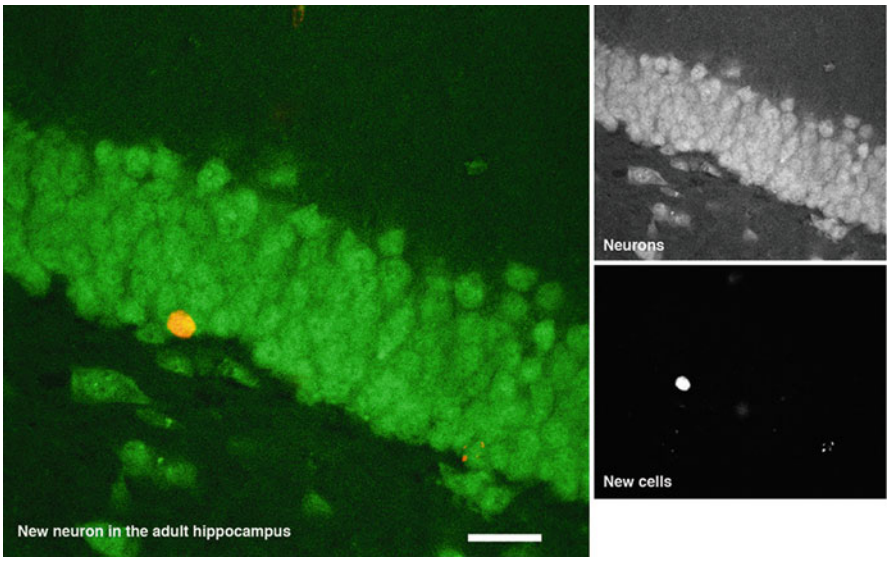
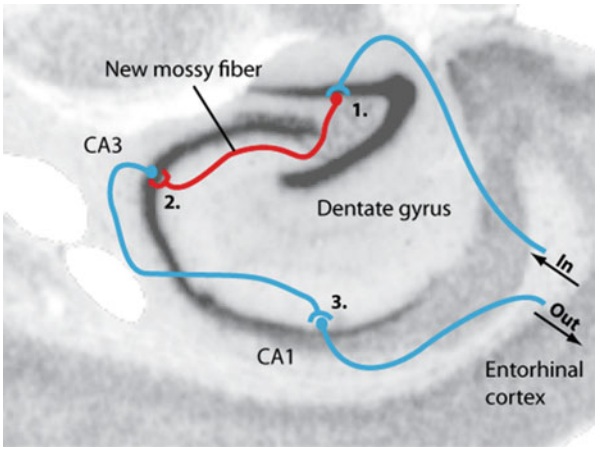


Fig. 2 *A newborn neuron in the adult hippocampus*. In the dentate gyrus of an adult mouse a newborn granule cells (*orange*) is highlighted in the band of granule cell neurons (*green*). The BrdU method (BrdU stands for the thymidine analog Bromodeoxyuridine) allows birthdating and permanently labeling newborn cells, because BrdU is incorporated into the DNA of dividing (precursor) cells and remains detectable in the progeny, here a newborn neuron. As BrdU must be exogenously applied and has a short bioavailability the time of the cell division is known. BrdU is depicted in the upper right inset, neuronal marker NeuN in the lower right inset (Image courtesy of Klaus Fabel, Dresden. Scale bar, 20 μm)

Fig. 3 *The basic circuitry of the hippocampus*. In a very simplifying but quite useful view the hippocampus has a trisynaptic backbone. Adult hippocampal neurogenesis produces neurons and their fibers that connect the input structure, the dentate gyrus, with area CA3, which serves as an autoassociative memory system. From CA1 the encoded memories are transferred ("consolidated") to cortical regions



proliferative and are referred to as type-2 cells. One can distinguish type-2a from -2b cells because at this stage the transition occurs from glia-like cells to cells expressing neuronal lineage markers. A key marker is transcription factor Prox1, which in the adult brain is specific to granule cells. Type-1 and type-2a as well as few type-2b cells express stem cell gene Sox2; many type-1 cells are Pax6-positive. In the course of development, the cells go through the characteristic sequence Pax6-Ngn2-Tbr2-Tbr1 known from the embryonic development of cortical glutamatergic neurons.

From stage 2b onward the cells also express cytoskeletal protein doublecortin (DCX) and the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), but molecules linked to "plasticity." Both stay on postmitotically, presumably until full connectivity has been established. DCX is a useful surrogate marker for adult neurogenesis but it is important to realize that DCX alone does not prove neurogenesis. Many NG2 cells throughout the brain express DCX but do not generate new neurons. First synaptic input from interneurons reaches the cells at the type-2 stage and the cells are also responsive to ambient GABA. The local interneurons presumably play a central role in steering neuronal development.

The next developmental stage is referred to as type-3 cells. At this stage the newborn cells show a more rounded morphology and begin to extend neurites, even though they can still proliferate. Migration into the granule cell layer might occur at this stage but is limited. Most cells remain in the SGZ and the inner third of the granule cell layer.

Cell cycle exit occurs at the level of type-2 or -3 cells. The postmitotic maturation stage lasts approximately 4–7 weeks. Initially, the cells extend their axons to area CA3 and their dendrites toward the molecular layer. During the first 3–4 weeks the cells express calcium-binding protein calretinin, which is later exchanged for calbindin that remains expressed in all granule cells. The cells are responsive to GABA, but GABA acts as excitatory neurotransmitter and promotes further maturation. In contrast, mature granule cells are massively inhibited by the network of interneurons, so that overall activity in the dentate gyrus is very sparse. In fact, it is the newborn neurons that provide synaptic plasticity (long-term potentiation, LTP) to the dentate gyrus (see also below). The newborn neurons thus go through a phase of increased excitability, which has large impact on the function of the dentate gyrus. Presumably, the increased synaptic plasticity is also the key trigger for the ultimate recruitment of the cells for long-term integration and survival. At a certain point in time, roughly 7 weeks after they are born, GABA becomes inhibitory and the phase of enhanced plasticity is over.

Adult hippocampal neurogenesis is high during very early periods of life, actually well before one can reasonably speak of "adulthood" but clearly differs from postnatal neurogenesis. Between 3 and 4 weeks postnatally (in mice and rats), the SGZ has formed and "adult" neurogenesis is found. The rate of neurogenesis massively decreases during the first weeks and months of life but remains constant at a very low level. Even in the oldest rodents studied to date, adult hippocampal neurogenesis has been found and remains regulatable.

Adult hippocampal neurogenesis is a mixture of turnover and lasting addition to the dentate gyrus network. In young rats, a net growth of the dentate gyrus was found and genetic tracing revealed that a substantial proportion of granule cells is adult-generated. Although turnover and replacement of old granule cells cannot be categorically excluded, it seems that the turnover primarily affects immature new cells.

Neurogenesis in the Adult SVZ/Olfactory Bulb

In contrast to the SGZ, neurogenesis in the olfactory system is spread out over a large distance. Precursor cell proliferation occurs in the SVZ and the newborn neuroblasts (referred to as A cells) migrate the long distance into the olfactory bulb, where they terminally differentiate, mostly into granule cells in the granule cell layer, to a small percentage into periglomerular neurons. With the exception of a very small group of excitatory glutamatergic interneurons that forms a separate lineage, all newborn neurons in the olfactory bulb are inhibitory interneurons. A subgroup of periglomerular neurons uses dopamine as neurotransmitter besides GABA.

The radial glia-like stem cells of the SVZ are called B cells and extend a cilia-bearing process to the ventricular surface. Bundles of processes from several B cells are surrounded by a rosette of ependymal cells in a "pinwheel fashion." B cells also have a basal process that touches blood vessels. The cilium plays an important role in regulating the precursor cell activity. Dividing B cells give rise to intermediate progenitor cells, here referred to as C cells, which in turn produce the migratory A cells. The ependymal cells (E cells) do not seem to serve as precursor cells under physiological conditions but might act as back-up in pathological situations.

The A cells migrate via the "rostral migratory stream" (RMS) to the olfactory bulb. The RMS begins near the roof of the ventricle in the anterior SVZ and travels ventrally until, at its "knee," it turns caudally toward the olfactory bulb. In rodents, the RMS is surrounded by a tube of astrocytes but this structure is not required for migration to occur and is absent in many species. The route of migration seems preformed by the primordial olfactory ventricle, which is obliterated in most cases in adulthood but might occasionally persist as fluid-filled cavities in humans. The mode of migration along the RMS is called "chain migration" or homotypic migration. In this distinct form of migration, the migrating cells take turns in using each other as guidance structure. It is not clear at present how migration occurs, when (e.g., in the human brain) very few cells are on the route and no chains are discernible.

Once in the olfactory bulb, the cells start to migrate radially to their final destination in the granule cell layer or the periglomerular zones. At this stage, they begin to express further signs of neuronal differentiation. Electrophysiological maturation is also held back until this point.

There is no indication of glial migration into the olfactory bulb. SVZ-derived NG2-positive oligodendrocyte precursor cells, many of them expressing DCX as well, leave the SVZ and RMS toward cortical regions.

Neurogenesis in the Adult Olfactory Epithelium and Other Parts of the Peripheral Nervous System

Adult neurogenesis is also found in the peripheral nervous system. In the olfactory epithelium, we find a massive turnover of receptor neurons; this is actually the largest site of adult neurogenesis in the body. The assumption is that due to their exposure to noxae the receptors are particularly vulnerable and need to be constantly replaced. The identity of the stem cell in the olfactory epithelium has not been fully revealed. The "horizontal basal cell," the "globose basal cell," and the "sustentacular cell" (the latter resembling a radial cell) are the candidates. Irrespective of this, at the next stage, we find transient amplifying intermediate progenitor cells and so-called immediate neuronal progenitor cells that give rise to immature and finally mature olfactory receptor neurons. Because olfactory receptors are odor-specific, the axons of the newborn neurons need to find their way to the glomerulum in the olfactory bulb that processes that particular smell.

Scientific interest in neurogenesis in the olfactory epithelium is to a considerable degree fueled by the idea that the readily assessable olfactory epithelium might provide something like a window into neuronal and brain development. Researchers have even tried to gain insight into complex disorders such as schizophrenia from biopsies of the olfactory epithelium.

Adult neurogenesis has also been described for dorsal root ganglia, but the evidence is still weak. A stronger case can be made for the enteric nervous system and carotid bodies.

Reactive Neurogenesis

In a number of cases, adult neurogenesis has been reported under pathological conditions, where physiologically no neurogenesis occurs. The best documented case is the striatum after ischemia, where a small number of interneurons might form. These cells are of SVZ origin and must hence use a direct path of migration. In rats, this process has been shown to be very long-lasting and it seems that there is an involvement of microglia in mediating the response. The functional relevance is unknown and to date there is no information that something similar can be found in other species. Reactive neurogenesis was also convincingly found after excitotoxic lesion in the adult retina. Here, Müller glia were the origin of new amacrine cells. However, no regenerating photoreceptors were detected.

Cortical neurogenesis has also been induced after highly targeted ablation of individual neurons, here presumably without any overt immune response. Cell death seemed to be sufficient to trigger neurogenesis. The findings were very intriguing but we lack follow-up reports and a more detailed analysis of the underlying mechanisms.

In a number of studies, regenerative neurogenesis was boosted by growth factor infusions (e.g., in hippocampal region CA1 and the hypothalamus). Again, independent follow-up studies are missing and the available information remains

inconclusive. In summary, thus, there is some indication that reactive neurogenesis is generally possible in the adult rodent brain but the phenomenon is neither widespread nor particularly strong. One cannot truly speak of regenerative neurogenesis in these case because as yet we do not have evidence that the lesion-induced neurogenesis really provides "regeneration" and contributes to functional restoration.

In other species, for example, fish and amphibians, widespread regenerative neurogenesis takes place. A particularly prominent example is the Axolotl (*Ambystoma mexicanum*) that after amputation can regrow its entire tail including the spinal cord. An intriguing question is why "higher" animals lost this ability for regeneration. While this might have been the price to pay for other benefits accumulated in the course of evolution, we might also learn from these species how regeneration is controlled or might become inducible.

Regulation

Because neurogenesis is a multistage process, involving a sequence of cells, regulation of neurogenesis is complex even though the end result, the generation of more or fewer newborn neurons, might suggest otherwise. The majority of publications on adult neurogenesis deals with "regulation" but the term can stand for very different events and specific results. Regulators encompass behaviors as well as genes, humoral factors, and cell–cell interactions. They stand in a hierarchical order: behavior does not affect neurogenesis directly but through a cascade of factors, all of which are "regulators" themselves. Publications on adult hippocampal neurogenesis greatly outnumber those on neurogenesis in the adult SVZ and olfactory bulb. This is also reflected in our knowledge about the regulation of neurogenesis in both areas. As it is rare that both regions are investigated in the same study, no explicit and concrete comparison is possible. But there are clear differences, some of which relate to the different regionalization of the precursor cells, the different developmental lineages involved, and the different types of neurons that are produced.

Adult neurogenesis seems to be very sensitive to a very broad range of stimuli and factors that alone or together affect the net result. Jokingly, one could say that it would be much more interesting to find factors that do not influence adult neurogenesis than just adding more molecules to the list. But in the course of neuronal development, decisions at preceding stages limit the range of regulatory options at later stages. Dendritic fine-tuning, for example, can obviously be only the target of regulatory events, if the cells have been selected for survival. In terms of cell numbers, proliferation of precursor cells determines the size of the population of the potential new neurons but it is at the level of cell survival that the actual decision whether or not a cell is lastingly integrated is made.

A key point is that adult neurogenesis is a highly polygenic trait. This fact is easily overlooked in straightforward studies exploring the roles of individual genes.

Single genes explain very little of the variance in adult neurogenesis, and trait variability is very large. This baseline variability, however, is only the basis on which any regulation might act, making adult neurogenesis an immensely variable quantitative trait.

Age is a prominent negative regulator of neurogenesis in that "adult" neurogenesis is high in young animals, actually well before they can be rightfully called "adult," and very low in old animals. But the decline is hyperbolical and neurogenesis actually is thus very stable over most part of life. This implies that the impact of aging on changes in adult neurogenesis is different at different stages of life and decreases after adolescence. Age effects on olfactory bulb neurogenesis have not been studied in much detail and the overall decrease seems to be lower than in the hippocampus. This might have to do with the different functions that the new cells have in the two systems, possibly indicating that in the hippocampus we see such strong decrease very early because after an initial buildup phase only very few cells are needed.

The most notorious negative regulator of adult neurogenesis, again only extensively studied in the hippocampus, is stress. The rediscovery of adult neurogenesis in 1992/1993 through Elisabeth Gould and Heather Cameron, both working with stress researcher Bruce McEwen, took place in this context. This perspective shaped the first few years of adult neurogenesis research in the 1990s. It was found that acute stress massively downregulated adult neurogenesis, directly mediated by stress hormone corticosterone and possibly other indirect mechanisms. Under chronic stress, however, the picture is far more complicated and no unambiguous stress effect can be identified. There is a tendency to relate all negative regulation of adult neurogenesis of unknown cause to stress, but this scheme appears too simple. The question of how stress regulates adult neurogenesis is of great relevance for the hypothesis linking adult neurogenesis to the pathogenesis and course of major depression.

On the positive side, both physical and cognitive activity stimulate adult hippocampal neurogenesis. Because both situations provide some level of stress, the boundaries are somewhat blurred. Intriguingly, the two paradigms affect different aspects in the regulation of adult neurogenesis: while physical activity promotes the proliferation of the precursor cells, the more cognitive stimuli, for example, the experience of so-called enriched environments or concrete learning stimuli, enhance the recruitment and survival of newborn neurons. These two mechanisms are additive in their effect, suggesting that a nonspecific stimulus, such as physical activity, can increase the potential for neurogenesis, and a presumably specific cognitive stimulus can make use of this increased potential. This synergy concretely links regulation of adult neurogenesis to brain function and is relevant for evolutionary considerations and the question how adult neurogenesis might contribute to brain function across the life span. The idea is that locomotion and physical activity provide an intrinsic mechanism that signals to the brain that situations of greater cognitive challenge might arise (see below). In the olfactory bulb, in contrast, no effect of physical exercise has been found but richness of olfactory experience promotes neurogenesis.

Individual molecules with regulatory effects on adult neurogenesis are numerous, ranging from transcription factors over paracrine signaling molecules, extracellular matrix, and cell–cell contacts, to neurotransmitters, growth factors, hormones, and cytokines. It seems that there is hardly any factor that does not have an effect on adult neurogenesis. This might again reflect the sensitivity of this system of plasticity to a wide range of signals. The big and as yet unanswered question is how the neurogenic niche and the precursor cells integrate over this flood of regulatory cues and bring specificity into the regulation.

Regulatory influence of the niche consists of direct cell–cell communication, for example, through gap-junctional contacts between the precursor cells, and short-range signaling molecules secreted from the various cell-types involved (precursor cells, endothelia, astrocytes, microglia, immature neurons, etc.) as well as extracellular matrix.

The first key regulatory event in adult neurogenesis is the maintenance of precursor cells. Paracrine signals, such as Shh, Bmp, Notch, and Wnt, play the presumably expected roles in controlling this step, which is essential for the long-term persistence of adult neurogenesis. On the cell-autonomous side, we know a number of relevant transcription factors, most notably Sox2 and, in the glutamatergic lineage, Pax6 are necessary at this stage. In how far radial glial genes and structural proteins like nestin, which show a certain specificity for precursor cells, are required for this key function is not clear.

The most frequently assessed biological function is “proliferation” of the precursor cells. Proliferation stands not only for maintenance but also for the expansion of the precursor cell pool. Here, we find classical mitogenic factors, especially epidermal growth factor (EGF), but the effect can also be highly indirect as in the case of physical exercise. Because it stands at the beginning of development, expansion of the precursor cells is a rather nonspecific regulation but very central in that consecutive regulatory events can only act upon cells that have been produced in the first place. Intriguingly, genetic manipulation of the cell cycle of the precursor cells is not only sufficient to induce expansion but also increases neurogenesis, suggesting that neuronal development is somehow linked to this initial step.

Regulation occurs also at the level of neuronal differentiation, which implies that the molecular program for neuronal development and specification is turned on in the progenitor cells. The basis of this regulation are the proneuronal transcription factors such as NeuroD or Ascl1 in the hippocampus, or, for example, Dlx1/2 in the SVZ. A particular case in the hippocampus is transcription factor Prox1, which in the brain is specific for granule cells of the dentate gyrus. In how far neuronal differentiation in adult neurogenesis is subject to actual extrinsic regulatory influences is not clear.

In the SVZ, neurogenesis is also regulated at the level of migration but details are sparse. In the hippocampus, migration is so minute that the phenotype can hardly be explored. But mutations of reelin result in overshooting migration here.

At least in the hippocampus, adult neurogenesis is to a large degree quantitatively regulated through the control of survival. The long-term recruitment is activity dependent, and a complex machinery exists to translate the pro-survival stimulus

into actual survival. Cells that were not recruited are eliminated by apoptosis, so that part of the regulation of survival consists of the negative regulation of apoptosis. A key survival factor, as far as the available information goes, is brain-derived neurotrophic factor (BDNF) which is released by neurons in response to activity. BDNF, on the other hand, is a "usual suspect" and unlikely to be the only key player. Generally, several growth and neurotrophic factors are positive regulators of neurogenesis. Besides EGF and BDNF, known key factors in this class are fibroblast growth factor 2 (FGF2), insulin-like growth factor 1 (IGF1), and vascular endothelial growth factor (VEGF).

Inflammatory and immunological signals are increasingly recognized as being relevant for the regulation of neurogenesis not only under pathological but also physiological conditions. The role of the immune system, however, is two-edged. On one side, baseline neurogenesis appears to be supported by the immune system, most notably microglia and T lymphocytes, on the other side, many cytokines and stronger inflammation damages precursor cells and neurogenesis. How this balance is controlled remains to be found out.

At the heart of all regulation of neurogenesis we find the transcriptional control that is responsible for steering the developmental program at the level of genes. From stem cells to mature neurons, transcription factor profiles are the signature of "neurogenesis." One of these characteristic sequences, found in the hippocampus and one particular lineage in olfactory bulb neurogenesis, is the sequence Pax6-Ngn1-Tbr2-Tbr1. At the precursor cell stage, we find the expression of Hes genes. Neuronal differentiation is linked to Neurod1 expression. As mentioned, Prox1 expression, which comes on at the type-2b stage in the hippocampus is specific for granule cell development.

Gene expression is also controlled by epigenetic mechanisms and the inhibition of, for example, histone deacetylation increased neurogenesis. Activity-dependent regulation has many long-lasting effects on adult neurogenesis. Epigenetic changes at the precursor cell level are a plausible explanation for these phenomena. The first reports, however, are not much more than a first glimpse into this presumably extensive aspect of regulation. The same applies more or less to the role of microRNA that is only beginning to be explored.

Function

In the olfactory bulb, input from the olfactory epithelium constantly changes because of the turnover of the receptor neurons. The idea is that the new interneurons facilitate the resulting permanent rewiring. In this sense, neurogenesis in the olfactory bulb would be dependent on neurogenesis in the olfactory epithelium. And in fact, tampering with the olfactory epithelium influences neurogenesis in the olfactory bulb. The new interneurons improve olfactory bulb function, including olfactory discrimination and olfactory learning. Not very many details are known, partly due to the fact that olfaction is very difficult to study in practical terms and the electrophysiology of the bulb has not explored to the same level of detail as in the

hippocampus. Pierre-Marie Lledo from Paris set the stage with most of the studies that define our present knowledge about the function of neurogenesis in the olfactory bulb.

Throughout the years in which adult hippocampal neurogenesis was considered a curiosity, a key argument to justify the negligence was that the function of the new cells was unknown, and hence one must assume this function, if it existed at all, could not be of much relevance. It now turns out that adult neurogenesis actually provides fundamental functionality to the hippocampus. There is increasing evidence that the function of the dentate gyrus is based on the contribution of the new neurons.

The long-term potentiation (the electrophysiological correlate of learning at the synaptic level) that can be measured in the dentate gyrus under physiological conditions is entirely a contribution of the highly plastic new neurons. The existing granule cells, in contrast, are strongly inhibited by interneurons and their firing rate is very sparse.

The two key behavioral tests, on which most of our knowledge about the function of newborn neurons rests, are the Morris water maze and contextual fear conditioning. The first is a test of spatial learning, in which rodents learn to navigate to a hidden escape platform in a circular pool of water by using cues in the room. In the second test, rodents learn to associate a spatial context with an unpleasant experience so that presentation of the context alone elicits the learned fear behavior. The experiments were based on the suppression of adult neurogenesis by various means, ranging from pharmacological approaches with cytostatic drugs to irradiation and the genetically engineered ablation of newborn neurons. The resulting literature is quite confusing in its details and no true overarching theory has emerged yet, but the direction has become clear. Computational models have greatly helped to shape the hypotheses to be tested.

One key function of the dentate gyrus is “pattern separation,” an important prerequisite for learning, especially, for example, in spatial or episodic learning. Pattern separation allows the hippocampus to distinguish information that belongs together from those that does not. This in turn is necessary for relating information to its context. Pattern separation especially relates to the temporal domain. The new neurons help to provide information that is to be learned with a “time stamp.” The new neurons alter the network properties of the dentate gyrus. They prevent that new information interferes with previously learned information, a problem called “catastrophic interference.” The new neurons allow the hippocampus to flexibly integrate new information into existing contexts. This latter effect appears to be more lasting and in contrast to the improved information processing on time, which is probably related to the highly plastic immature neurons, is related to the permanent integration of the new neurons. The contribution of the newborn neurons is, hence, an investment for the future that allows gradual improvement and an optimization of the mossy fiber connection. This connection apparently needs to be as narrow as possible but obviously as strong as necessary to cope with the cognitive challenges experienced by the individual.

Medical Relevance of Adult Neurogenesis

Adult neurogenesis does not seem to be about regeneration, although we can probably learn a lot for regenerative medicine from physiological neuronal development under the conditions of the adult brain. Adult neurogenesis contributes to normal brain function: in the olfactory bulb new neurons seem to stabilize and maintain the network integrity in the face of constantly changing input; in the hippocampus they provide a unique means to optimize the network structure. This function can obviously fail and hence cause problems in the context of disease. For the olfactory bulb, it has been suggested that the peculiar early loss of smell in Parkinson's disease might be due to the lack of dopaminergic support to neurogenesis. Otherwise, there are few direct links to human disease because humans do not rely much on their smell and even anosmia can go unnoticed for long time.

The case is different in the hippocampus, where adult neurogenesis might be central to the function of the dentate gyrus so that disturbance of adult neurogenesis could lead to more severe dysfunction. There is no evidence that adult neurogenesis is necessary for hippocampal function. Even the animals with suppressed adult neurogenesis showed signs of hippocampal learning in the water maze: it was their efficiency in using hippocampus-dependent strategies that was reduced. This implies that in the context of disease, only particular aspects of hippocampal function are impaired. Failing adult hippocampal neurogenesis has thus been brought into connection with the pathogenesis and course of dementias, age-related cognitive decline, major depression, and schizophrenia. In all cases, the problem with adult neurogenesis would not explain the disease but those aspects of it that are related to hippocampal function. Conversely, supporting or even enhancing adult neurogenesis might become a useful target to address these diseases. In the case of dementias, stimulating adult neurogenesis by physical and cognitive training or pharmacological intervention might help to build a neurogenic reserve that allows functional compensation in the case of increasing functional losses. In the case of depression and schizophrenia, targeting adult neurogenesis might reinstall lost flexibility, and thereby improve coping with the challenges of the world. The theories circling around these issues are still rather vague and speculative but they have the unusual appeal of linking the therapeutic approach to an endogenous potential for plasticity.

Temporal lobe epilepsy is a particular case, in that here the new neurons might actually be part of the problem. Acute seizures stimulate adult hippocampal neurogenesis, while prolonged epilepsy results in impairment of the neurogenic niche. The increased neurogenesis due to acute seizures results in a number of abnormal features, including the appearance of basal dendrites that form aberrant connections, increased migration and dispersion of the newborn cells, a proportionally greater rate of proliferation at the later stages of development, and altered electrophysiological contributions to the networks. These changes do not prove that temporal lobe epilepsy actually originates from impaired adult neurogenesis but new neurons at least seem to contribute to the persistence of the problem.

In many pathological conditions, adult neurogenesis responds to the damaging stimulus, presumably in a rather nonspecific way. Hypoxia, ischemia, trauma, infection, etc., all initially stimulate neurogenesis. In the hippocampus, no migration toward the lesion occurs. In the SVZ, such migration may be found and very low numbers of newborn neurons have been reported, most convincingly in the striatum after ischemia. As stated above, this reactive neurogenesis is not regenerative or restorative and its functional relevance is presumably low. Nevertheless, the process is interesting and important for more than academic reasons. The brain is far less passive in response to insult than previously thought and we are only at the beginning of understanding the impact of maintained plasticity on brain health.

Outlook

Key to the future of adult neurogenesis research in the twenty-first century will be the clear identification of its functional role and relevance in human cognition. While progress is clearly made on the side of our insight into the general functional relevance of adult hippocampal neurogenesis in rodents, our information about adult neurogenesis in humans is still very limited. Quite generally, the position of adult neurogenesis in the evolution of the brain is largely uncharted terrain. We need to answer the question, if and why adult neurogenesis provides an evolutionary advantage.

With respect to the functions themselves, many questions are open and a unifying theory is still missing. Regulation of adult neurogenesis is amazingly complex and very few aspects have as yet been covered. It has become clear that piling up information about individual genes and factors alone will not lead to an integrative picture.

Despite and because of these difficult open questions, adult neurogenesis research is a booming field with large translational impact for medicine. “New neurons” have a particular appeal, and this fascination extends well beyond the circles of researchers in different disciplines but into the public.

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