18 Basic Aspect: Neurorepair After Stroke

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Abstract

This chapter discusses the scientific premise of stem cell-based therapies aimed at repairing damage produced by cerebrovascular insults in the adult human brain. Understanding the principles that govern stem cell biology will be of crucial importance to help designing treatment strategies for regenerative medicine. For this reason, the chapter is divided in two sections. The first part will touch upon pivotal basic research that has paved the way to a fuller comprehension of neurogenesis in the developing and adult brain. Interestingly, many molecular mechanisms that play roles in neurogenesis are shared between brain development and adulthood. Therefore, studies that have focused on brain formation have also guided investigations around homeostatic neurogenesis, as well as regenerative repair of the adult brain. The second section of this chapter will introduce recent biomedical investigations around the possibilities of initiating ectopic neuroregenerative programmes, or boosting physiological cell turnover rates for regenerative repair. In this context, we will discuss opportunities for promoting endogenous neurogenesis that may be generated from actual or potential stem cells residing throughout the brain. Finally, we will discuss experimental approaches aiming to replace lost neurons using endogenous sources.

18.1 Stem Cell Biology and Endogenous Neurogenesis in Brain

18.1.1 Stem Cell Biology: General Principles

Stem cells are cells that can proliferate to maintain their own population, often referred to as self-renewal, and give rise to different cell types through a process of differentiation. In the embryo, they allow formation of all organs in the body. They are still present in adulthood, although decimated in numbers and limited in their potency to organ-specific cell types. In the adult organism, stem cells are continuously active for homeostatic turnover of cell populations in dynamic organs, such as skin and blood, or they contribute to regenerative repair after injury. Maintenance of adult stem cell pools is ensured through tissue-specific stem cell niches, in which dividing cells may give rise to other stem cells, as well as progeny destined for differentiation into postmitotic cells. Stem cells can exist in dormant or activated states, which depend on their

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metabolism, transcriptional and epigenetic profile, as well as spatio-temporal orientation. Thanks to their capacity for self-renewal and multipotency, stem cells have been proposed for therapeutic strategies. For instance, transplantation of haematopoietic stem cells obtained from the bone marrow has been successful in remitting diseases of the blood, such as leukaemia and plasma cell myeloma.

18.1.1.1 Tracking Cell Generation

A challenge in regenerative medicine is the ability to trace self-renewing cells, quantifying their turnover rates, and characterizing their maturation in different postmitotic cell types. Researchers have taken advantage of a wide variety of methods that allow them to follow the life of stem cells. For instance, Ki67 is a protein that is transiently expressed during the cell cycle, and is commonly adopted to measure numbers of dividing cells in immunohistochemical staining or in flow cytometry [1]. Labeled thymidine analogues, such as BrdU and EdU, are another means of tracking dividing cells [2]. The advantage of using these over Ki67 expression is that cells stably integrate the analogues into their genome. Thus, one could quantify newly created cells even past their mitotic phase. Using these analogues, however, carries a risk of overestimating cell division, because BrdU and EdU can be detected even in the progeny of cells that have previously been labeled. Finally, experiments employing thymidine analogues are only suitable for short-term, preclinical studies due to dangerous side effects associated with their administration.

A novel approach has been devised to measure cell renewal rates in human individuals. This method takes advantage of a dramatic surge in environmental Carbon^{14} (^{14}C) levels, which increased due to nuclear bomb testing over the past century [3]. The concentration of ^{14}C rapidly increased up until the early 1960s, and has been decreasing through uptake by the biotope through photosynthesis since 1963 when an international test ban treaty was agreed to and over-ground nuclear bomb tests were discontinued. Atoms of carbon (incl. isotopes) present in the environment are incorporated into cells during DNA synthesis and the amount of integrated ^{14}C reflects the isotope concentrations at the time of cell division [4]. Thus, measuring the ^{14}C concentration in genomic DNA of a cell population enables the determination of that population's turnover dynamics. By means of ^{14}C dating, researchers in our lab have been able to infer turnover rates of various cellular populations, including those of the heart and central nervous system [5–7].

18.1.2 Endogenous Neurogenesis

18.1.2.1 Stem Cells in Brain Development and Adulthood

During neurodevelopment, neurons and macroglia (i.e. astrocytes and oligodendrocytes), arise from various regions around the ventricles, hollow structures filled with cerebrospinal fluid (CSF) [8]. Lineage-tracing technologies have allowed stable expression of fluorescent protein reporters into progenitors of neural stem cells (NSCs) and have enabled appreciation of the neurogenic process. Thanks to experimental designs that track differentiation of NSCs, researchers have, for instance, established that pyramidal neurons of the neocortex and astrocytes both originate from radial glia that span their processes between the ventricular zone, on the basal side, and the pial surface, on the apical side [8, 9]. In contrast, interneurons are formed in subpallidal regions and tangentially migrate to populate cortex [8].

Historically, the brain has been considered to be an organ where no neuronal replacement takes place. The earliest reports of adult neurogenesis date back to Altman's "Are new neurons formed in the brains of adult mammals?" in 1962 [10]. The author used thymidine-based methods to record addition of new neurons to the dentate gyrus and olfactory bulb of rodents. Sadly, his work was either ignored or harshly criticized by contemporary scientists. A decade later, Kaplan also tried to persuade, though without success, the scientific community of the existence of neurogenesis in the adult rodent and cat hippocampus, olfactory bulb and cortex

[11]. Rakic strongly opposed this idea, with his publication "Limits of Neurogenesis in Primates" [12]. It was not until the 1990s that research in neurogenesis finally started thriving, largely because of advances in available methodology. Several groups now reported that NSCs could be isolated from the adult rodent brain [13, 14] and also that neurogenesis takes place in vivo in both experimental animals [15] and humans [16]. In addition, it was during this time found that adult neurogenesis is positively regulated by factors such as exercise and enriched environment [17, 18].

It is now widely accepted that the mammalian brain retains neurogenic capacity in a few small regions throughout the lifespan of an individual. New neurons originate in neurogenic niches that contain NSCs, which display proliferative and multipotent properties, even during physiological conditions. In the adult mammalian brain, the germinal niches are found in the subventricular zone (SVZ), which is a narrow band flanking the lateral walls of the lateral ventricles, as well as in the subgranular zone of the hippocampal dentate gyrus (Fig. 18.1). More than a thousand new neurons are generated

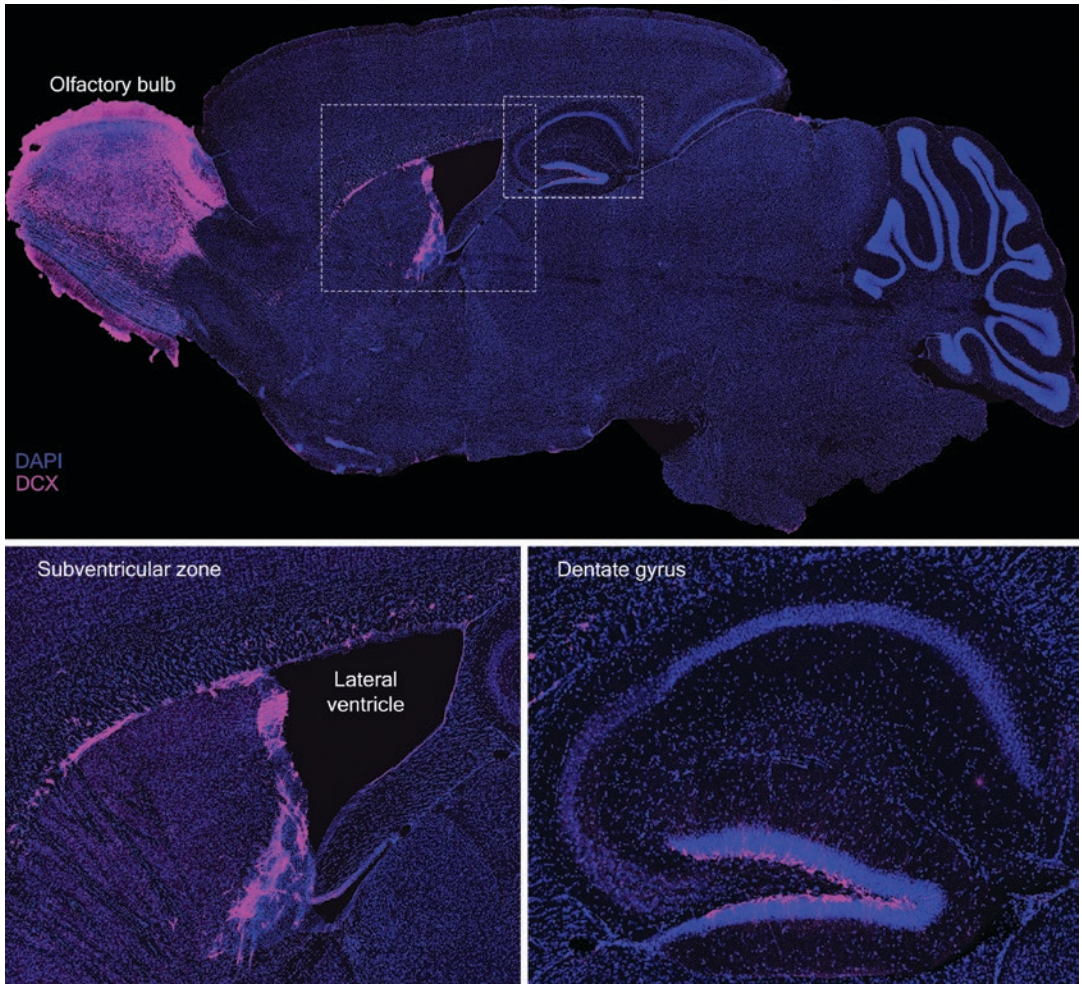

Fig. 18.1 Neurogenic regions in the adult mouse brain. An immunohistochemical staining against the neuroblast-specific protein Dcx was performed on a sagittal tissue section through an adult mouse brain. This shows that new neurons are generated in two regions: the subventricular zone, from which newly generated neuroblasts migrate to the olfactory bulb, and the hippocampal dentate gyrus

every day throughout adulthood [5, 19]. Newborn neurons display a transient, hyper-excitable electrophysiological profile [20], which may give them a special role in information processing. For example, they may be important for storing similar experiences as distinct memories [21]. The functional relevance of adult-born neurons is demonstrated in instances of impaired neurogenic activity, whether in the context of experimental manipulation, or due to neurological conditions. Animal models of impaired hippocampal neurogenesis display difficulties in learning and memory abilities, such as contextual learning and retention of spatial information [22]. Additionally, several human diseases have been suggested to be associated with altered neurogenic behavior in the hippocampus. These include both psychiatric diseases, such as schizophrenia and depression, as well as neurodegenerative conditions, such as Alzheimer's disease [22]. In the SVZ of most mammals, new immature neurons migrate to the olfactory bulb where they are important for certain aspects of odor discrimination [23]. Thus, neurogenesis appears to be important for neural circuit function in the brain regions where it occurs. On the whole, however, adult neurogenesis is extremely limited: it only occurs in such small, specialized niches; the vast majority of neurons in the brain cannot be replaced if they are lost.

There are species-specific differences in neurogenesis. Interestingly, humans do not have any olfactory bulb neurogenesis [6]. This may be because humans, unlike rodents, rely very little on their sense of smell for survival. Hippocampal neurogenesis, on the other hand, is very active in adult humans [5]. In addition, retrospective birth dating and IdU labeling of neural cells using ^{14}C have allowed scientists to identify an additional and unique site of adult neurogenesis in the human brain. It appears that interneurons are continuously added in the human striatum, a structure that lies adjacent to the SVZ [24] (Fig. 18.2). To this date, the origin of these cells remains unclear, although it is tempting to specu-

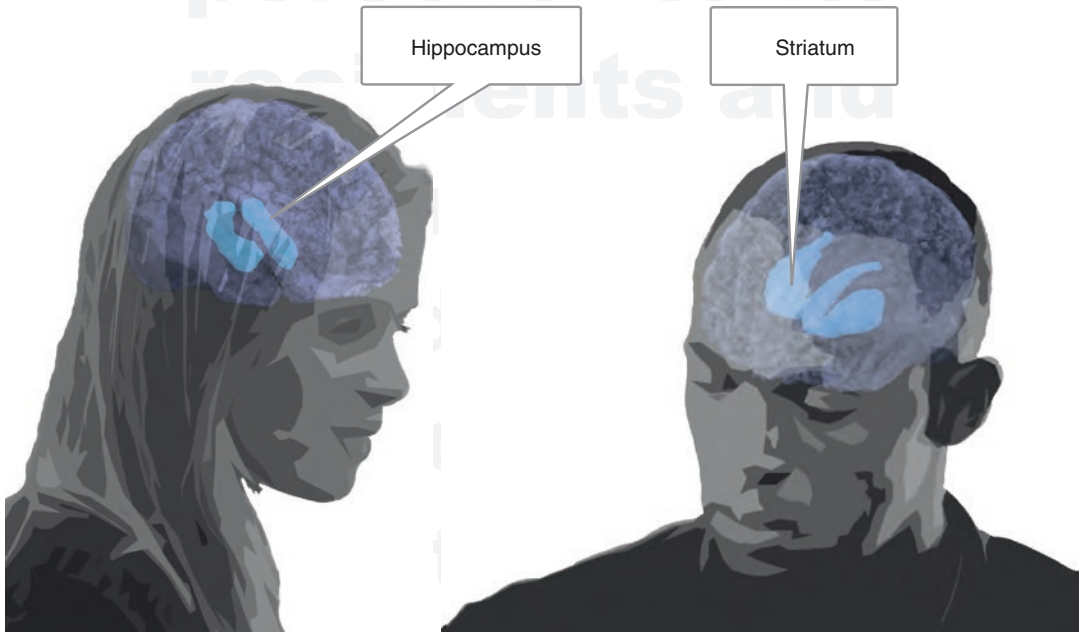

Fig. 18.2 Regions in the healthy adult human brain where new neurons are added throughout adulthood. As in most mammals, adult neurogenesis takes place in the dentate gyrus of adult humans. However, in contrast to most other mammals, adult neurogenesis does not occur in the human olfactory bulb. Instead, new neurons are continuously being added to the striatum

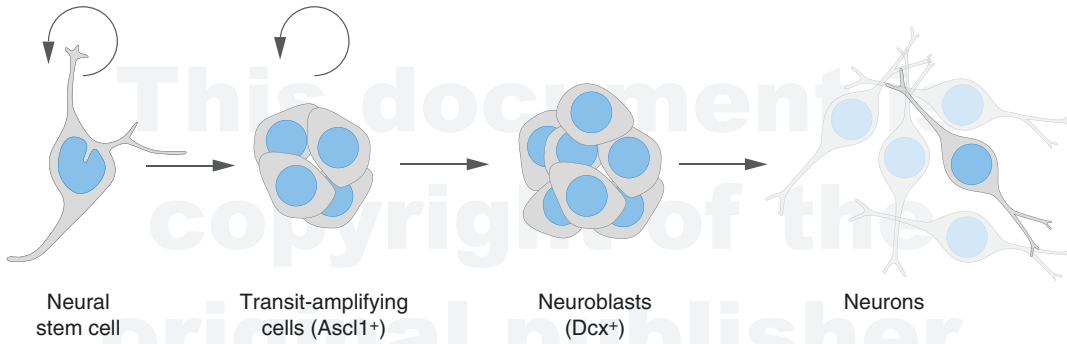

Fig. 18.3 Mechanism by which neural stem cells generate neurons in the adult neurogenic niches. Neural stem cells divide to give rise to transit-amplifying cells. These, in turn, divide a few times in rapid succession to generate neuroblasts, which mature into neurons

late that neuroblasts may be migrating from the neurogenic niche at the SVZ.

Cell types that differ along stages of differentiation can be found in the neurogenic niches. Specifically, astrocyte-like cells are adult NSCs [25]. They share several molecular and functional properties with parenchymal astrocytes, such as expression of many astrocyte genes. Recent studies have suggested that the NSC population may be heterogeneous in the progeny it gives rise to, as well as its cell turnover rates. Indeed, NSCs residing in different locations around the lateral ventricles seem to be predetermined in the type of olfactory bulb interneurons they will give rise to [26]. Additionally, Llorens-Bobadilla and colleagues took advantage of state of the art single-cell RNA sequencing technologies to segregate several subpopulations, based on their self-renewal rate, along the quiescent-to-activated axis [27]. They have shown that recruitment of dormant NSCs into the cell cycle is accompanied by diminished glycolytic and lipid metabolism and a simultaneous surge in transcriptional activity of lineage-primed transcription factors (i.e. pro-neuronal or glial) and genes implicated in protein synthesis. NSCs develop into transit amplifying cells that can be identified through immunohistochemical staining of Ascl1 (Achaete-scute homolog 1) (Fig. 18.3). These cells are short-lived and go through brief rounds of division before generating neural progenitors. Finally, neuroblasts expressing doublecortin (DCX) and PSA-NCAM are formed in the neurogenic niche. These give rise to proliferative clusters and become committed to the neuronal lineage, being identified as immature neurons. Following another cell division phase, neuroblasts depart for a migratory journey that will take them to their final destination in the brain [28].

The germinal niche is also characterized by a specific microenvironment and ultrastructure that is crucial for stem cell behavior [29, 30]. In particular, studies have shown that NSCs extend a process into the lateral ventricle, thus gaining a privileged access to growth factors contained in the CSF [29, 31]. Subsequently, Tavazoie and colleagues have proposed that the proximity of NSCs and transit amplifying cells to the cerebral vasculature supports their proliferation [32].

18.1.2.2 Molecular Basis for Self-Renewal and Fate Determination of NSCs

Cell turnover rates and lineage commitment are dictated by molecular mechanisms that are highly regulated in a spatio-temporal manner. Numerous studies have identified many of the molecular cues that instruct NSCs towards expansion, or differentiation into neurons and glia. Additionally, researchers have been able to interfere with the neurogenic process using transgenic animal models, viral vectors, and small molecules that enhance or inhibit molecular signaling activity. As a result, they have determined ways for boosting proliferation or favoring differentiation.

Interestingly, the signaling pathways implicated in neurogenesis are conserved through development and into adulthood. Examples of these include Sonic Hedgehog (Shh), Notch, and BMP (bone morphogenetic protein). Knocking out Shh during corticogenesis severely impairs patterning of the cortical lamina and fate determination of neural progenitors [33]. Notably, Shh is also required to regulate adult neurogenesis, and acts as a mitogen in concert with epidermal growth factor (EGF) to control proliferation of astrocyte-like NSCs and their progeny in the SVZ [34]. Likewise, Notch has been implicated in both developmental and adult neurogenesis, where it plays roles in regulating stem cell quiescence and lineage fate determination, favoring astroglia differentiation over neuronal maturation [35]. In addition, developmentally active BMP signalling contributes to morphogenesis of the nervous system and participates in the switch between neuro- and astrogenesis. It is, furthermore, implicated in adult neurogenesis, where its activity halts proliferation and maturation of neuroblasts, while imposing quiescence of stem cells or glial differentiation [36]. Interestingly, ectopically infused Noggin, a natural inhibitor of BMP signaling, alters fate determination preferences at the germinal niche, favoring production of neurons [36].

18.1.2.3 Neurogenesis in the Injured Brain

Investigations on the regenerative potential of mammalian brains principally comes from preclinical studies employing animal models that aim at mimicking mechanisms and symptoms of human pathologies. Importantly, species may diverge significantly in their cell turnover capacity. For instance, great differences in regenerative capabilities are seen between mammals and certain amphibian species, which show superior reparative potential after brain insults. Considering in what ways brains differ in their reaction to injury may help shaping more effective therapies that mimic dynamics of regeneration as seen in more permissive species, such as the salamander. The amphibian brain is quickly and efficiently repopulated with cells after neuronal loss, at least if the neuronal loss is limited. This is apparent in the midbrain of salamanders subjected to chemical ablation of dopaminergic neurons. In this context, the neuronal population is completely regenerated through a process of proliferation and differentiation of ependymoglia stem cells that is finely regulated through dopaminergic signaling [37]. Neurons can be replaced even if as much as a third of one brain hemisphere is removed [38], and all neuronal subtypes are apparently replaced in their correct proportions [39]. However, even in salamanders, these neurons fail to reestablish long-range neuronal connections (millimeters) [39].

In contrast to the relatively effective brain repair of salamanders, injury-induced production of neurons in quiescent regions is very restricted in the mammalian brain. This poses great limitations to the possibility of achieving satisfactory functional recovery. Mammals and amphibians also diverge in the context of scar formation. Shortly after damage to the mammalian, but not the amphibian, central nervous system, astrocytes and pericytes are recruited to build a scar that seals the site of damage, and separates it from the remaining, viable tissue. Astrocytes undergo a process of reactive gliosis, during which they become hypertrophic and extend their processes to form a physical and chemical barrier between lesioned and intact tissue [40]. Pericytes are responsible for extracellular matrix deposition and formation of connective tissue, which further encapsulates the lesion [41]. In the acute phase after a brain insult, the scarring process seems to prevent spreading of damage. However, it may, in the long run, hinder formation of new connections, and eventually restoration of functional neural networks. Interestingly, when salamander organs are subjected to insults, they do not form scars that segregate damaged regions from the intact tissue [42]. Thus, absence of scarring processes may partly explain the greater regenerative potential seen in amphibian brains.

One additional limitation to successful neuronal replacement is that axons regrow very poorly in the central nervous system. This is in part because many neuronal subtypes in the central nervous system form poor growth cones at the tip

of their regrowing axons, which hampers their successful outgrowth [43]. But in addition to such neuron-intrinsic limitations, poor axonal regrowth is also a product of the environment of the central nervous system. The scar that forms around an injury constitutes a chemical barrier that inhibits axonal regrowth [44]. But even in the healthy central nervous system, axonal regrowth is poor, as demonstrated by observations of peripheral neurons attempting to grow their axons into the spinal cord [45]. In the peripheral nervous system, severed axons regrow much more effectively. One explanation for poor axonal growth in the central nervous system is that axons here are not myelinated by Schwann cells, as they are in the periphery. Schwann cells have an important role in guiding regrowing axons [46]. In the central nervous system, axons are myelinated by oligodendrocytes, which do not have this guiding role. In fact, central nervous system neurons whose axons project into the periphery, such as lower motor neurons, can regrow their Schwann cell-ensheathed axons if they are severed in the periphery [47].

Injury-induced neurogenesis has been recorded, though limited, in a few regions of the adult mammalian brain, including the striatum and the hypothalamus [48, 49]. Reports have suggested that neurogenesis may also occur in the rodent neocortex, though findings remain, to this date, controversial [50]. The regenerative process may involve actual stem cells resident in the neurogenic niche, which migrate towards sites of damage. It may also call into play cells that are not normally identified as self-renewing, multipotent cells. These are defined as potential stem cells, which play other, unrelated roles in the healthy brain, but start proliferating and generating neurons following injury. Parenchymal astrocytes have been identified as a source of quiescent stem cells, which support neuronal functioning during tissue homeostasis, but can engage in neurogenic programs following damage [48].

18.1.2.4 Stimulating Endogenous Neurogenesis After Stroke

Reactive neurogenic activity has been recorded by several research groups following ischemic insults (Fig. 18.4). Studies have, for instance, implicated cells from the germinal niche, which engage in proliferative programs and migrate towards sites of damage. Astrocyte-like NSCs from the SVZ have been shown to undertake a regenerative response after an ischemic injury in rodents [51]. This mainly occurs in the striatum, a region that in humans is commonly afflicted by stroke. Additionally, ependymal cells, which are co-inhabiting the SVZ but do

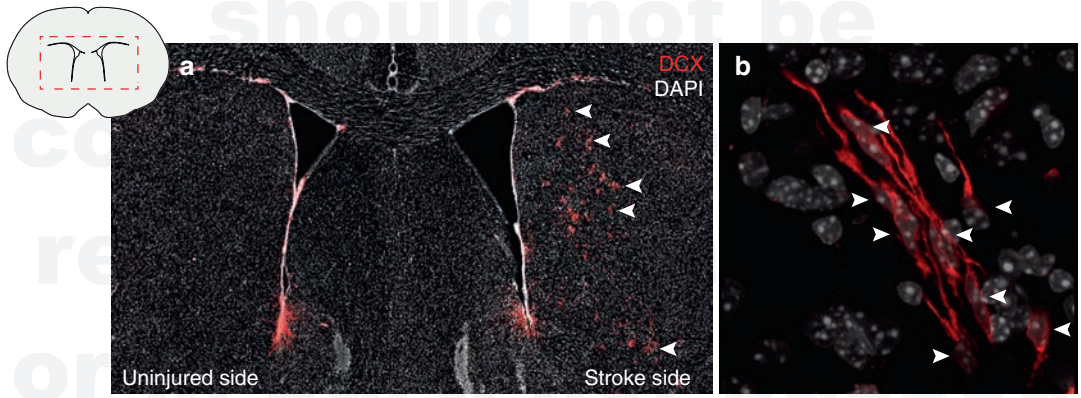

Fig. 18.4 Stroke-induced neurogenesis in the mouse striatum. A coronal section from a stroke-injured mouse brain was imaged 7 weeks after the experimental stroke, which was inflicted using transient middle cerebral artery occlusion. Neuroblasts have appeared in the ischemic striatum (**a**; arrowheads highlight examples of neuroblasts). The panel in (**b**) shows a magnification of migrating striatal neuroblasts (arrowheads highlight individual neuroblasts). Such stroke-induced striatal neuroblasts derive from both the nearby subventricular zone and from local striatal astrocytes

not normally display neurogenic activity, seem to give rise to astrocytes and neuroblasts after stroke in mice [52].

Interestingly, neurogenic responses may become evident outside of the canonical germinal niches. There, parenchymal astrocytes that are conventionally busy coordinating neuronal communication and other homeostatic processes, may reveal an intrinsic, otherwise quiescent potential for neurogenesis [48]. Similar to NSCs of the neurogenic niches, striatal astrocytes undergo a proliferative phase, during which they upregulate the proneural transcription factor Ascl1. Transit amplifying cells expressing Ascl1, subsequently, turn into clusters of DCX^+ neuroblasts. With the end of cell division, astrocyte-originated neuroblasts assume the form of migrating cells that translocate to sites in the tissue where they complete maturation into neurons. Further research will elucidate whether addition of astrocyte-derived neurons may be sufficient for neural networks to repair themselves and achieve detectable functional recovery. Additionally, future investigations will help understanding whether human astrocytes can also be forced towards neuronal lineages and, thus be considered for development of stem cell-based therapies. In support of this view, both Arsenijevic and colleagues [53] and Palmer and Gage [54], have identified a population of progenitors in the human cortex, which displayed potential for multilineage differentiation in cultures. The study, however, did not detect the identity of these progenitor cells, nor were the findings confirmed by other reports [55, 56].

While stroke-induced striatal neurogenesis has been demonstrated in a series of independent studies, it remains to be clarified whether neuronal replacement also occurs in other regions of the injured mammalian brain. Recent investigations have proposed that a cortical lesion may redirect migration of NSCs from the SVZ towards sites of damage [57]. Once in the cortex, however, the neural progenitors show propensity for an astroglial lineage fate. Similar to striatal glia, parenchymal astrocytes residing in the cortex may also harbor neurogenic potential, which may be revealed following injury. In line with this perspective, Sirko and colleagues [42] showed that astrocytes isolated from cortical regions subjected to stab wound injury, are able to form neurospheres, which are indicative of their multilineage potential in vitro. The authors, however, did not see the same response in vivo, perhaps demonstrating a hostile cortical microenvironment that prevails over intrinsic neurogenic capabilities of astrocytes. Other investigations have proposed the occurrence of neuronal turnover in the cortex, following an ischemic insult [58], although findings remain, to this day, controversial. Most importantly, while histological analysis and ^{14}C dating could demonstrate occurrence of striatal neurogenesis in healthy human brains [24], neuronal turnover in cortical regions could not be detected, neither in healthy subjects [7] nor in patients afflicted by stroke [59].

18.1.2.5 Therapeutic Implications of Endogenous Neurogenesis

Stroke is one of the major causes of death and long-lasting adult disability in the world. Cognitive and motor impairments derive from extensive neuronal death, associated with loss of cerebral blood flow. This underscores the importance of developing therapeutic strategies that are aimed at replacing neurons lost to injury to allow recovery of function. In fact, an advantage of treatments that focus on promoting endogenous neurogenesis concerns their extended therapeutic window. Indeed, thrombolytic treatments, which can be used in a subset of patients afflicted by stroke, will only be effective if administered within hours after an ischemic episode has occurred. In reality, a small portion of victims of a cerebrovascular insult qualifies for this therapeutic option. By contrast, stimulation of endogenous regenerative processes may still be effective throughout the chronic phase after stroke, which is mostly associated with remodeling and reorganization of surviving cells. Indeed, while proliferation of SVZ cells occurs in the first 2 weeks after stroke in rodents, migration of neuroblasts into the ischemic region has been recorded for as long as 16 weeks later [60]. Notably, approaches that rely on regeneration of

affected regions of the brain may also involve transplantation of neuronal progenitors, obtained for instance from embryonic sources or reprogramming of the patient's own cells. These therapeutic strategies are discussed in the next chapter.

Several preclinical investigations have, so far, supported the idea that the adult mammalian brain remains capable of stroke-induced remodeling and regeneration, at least to some extent. Clinical evidence, however, suggests that processes of reactive neurogenesis possibly occurring in the brains of patients afflicted by an ischemic insult are still not sufficient to achieve satisfactory recovery. The limited impact that endogenous neurogenesis has on functional recovery may derive from several issues. On the one hand, it is possible that contribution of actual (and potential) stem cells is too restricted, at least in terms of numbers of newly generated neurons. On the other hand, it remains to be clarified whether young neurons can effectively integrate in the pre-existing circuits and, in fact, recover function of cells lost to injury. Therapeutic strategies that focus on promoting repair based on endogenous neurogenic processes may thus try to tackle and improve these aspects of injury-induced regeneration.

A significant portion of neural progenitors generated by NSCs after stroke dies before achieving full neuronal maturation. Arvidsson and colleagues estimated that new neurons had replaced only 0.2% of dead neurons 6 weeks after stroke in rats [51]. However, in a follow-up study, the authors found that the neurogenic response still continued 1 year after stroke [61], making it possible that neuronal replacement had become about an order of magnitude higher at this time point. Although it remains unclear to what extent lost neurons should be replaced in order to achieve significant neural circuit restoration, new therapies may focus on boosting proliferation, differentiation, and survival of newly created progenitors. For this purpose, one may target molecular cues that have been implicated in the regulation of endogenous neurogenesis, such as Notch and BMP signaling [62]. Alternatively, ectopic manipulation of mitogens and neurotrophic factors, such as EGF and BDNF, have shown to enhance proliferation of progenitors, as well as survival of neuronal cells in the striatum [63]. In order to achieve recovery of function, proliferating neural progenitors may also need to give rise to region-appropriate neuronal cell types that are depleted after stroke. Interestingly, Arvidsson and colleagues [51] have shown in rats that neuroblasts originating from the SVZ migrate to striatal injury sites, where they mature into medium spiny neurons, the most commonly affected neuronal population. Studies in mice, on the other hand, have shown that stroke-induced neurogenesis primarily replenishes calretinin-expressing interneurons, rather than the neuronal subtypes that were lost [64].

For effective functional integration to occur, one also needs to consider the complex network of connections established among neurons in a circuit. Communication between neurons wired together occurs through spreading of electrical stimulation that travels from one cell to the next. This electrical activity makes up behavior. Newly generated neurons need, therefore, to find their place in the circuitry and properly connect to neighboring cells, in order to compensate for the loss of activity that follows stroke-induced neuronal death. The first experimental evidence of functional integration of newly generated striatal neurons comes from studies carried out by Hou and colleagues [65]. The authors could demonstrate that new neurons form synaptic structures reminiscent of pre-existing connections. They furthermore showed that neurons generated after stroke are integrated in the surrounding circuitry, being able to collect input from presynaptic cells, and fire action potentials to the neighboring neuron. Notably, the process of scar formation may be relevant to further enhancing this aspect of regeneration: Certain aspects of the scarring processes, such as the formation of a physical barrier and deposition of extracellular matrix, may indeed prevent axons of new cells from sprouting and reorganizing into the lesioned tissue [66]. Taken together, preclinical investigations are promising and have repeatedly demonstrated that there are ways of boosting endogenous neurogenic processes to

improve cell replacement after ischemic injuries, which may become feasible to employ as therapeutic strategies in the future.

It may sound self-evident that new neurons could mediate improved brain repair after injury. However, the mechanism by which such improvement would occur is not obvious. There are several ways in which new neurons could have an impact on spared neuronal circuits. Perhaps the most straightforward way is that each lost neuron would be replaced with a new one of exactly the same subtype—that the neuronal replacement strategy is so perfect that all lost neuronal subtypes are regenerated in their correct proportions. It is indeed possible to imagine future scenarios where stem cell differentiation protocols are refined to the extent that it is possible to generate all types of neurons required. However, this on its own will not be enough to restore the brain to its pre-injury state, because of two reasons. The first is that neuronal function is dependent on neuronal connections. Perfect replacement of neurons therefore requires perfect replacement of all their connections. This will likely be very difficult to achieve because the patterning signals that guided neural connectivity during brain development are largely absent in the adult brain. For this reason, neuronal connections—particularly long-range connections—are likely difficult for new neurons to reestablish. The second reason why neuronal replacement will not be enough to perfectly restore brain function is that brain injury leads to extensive scarring and tissue remodelling. To restore a chronic stroke lesion to its former state would therefore require biological tissue engineering on an altogether different scale than what seems realistic today. For these reasons, neuronal replacement is more likely to have beneficial effects through other mechanisms than direct replacement:

One possibility is that new neurons improve circuit function through the neurotransmitters they secrete. For example, in transplantation strategies to treat Parkinson's disease, dopaminergic neurons are grafted to the striatum, where dopamine is needed—even though dopaminergic neurons are normally located in the substantia nigra, far from the striatum [67]. The important thing with this neural transplantation strategy is that dopamine levels are restored in the striatum, even though pre-existing neuronal connections are not recreated. The same strategy may be applicable for other damaged circuits.

Another possibility is that new neurons act like relays that indirectly reconnect two spared neurons that used to be directly connected to each other. This has been shown to occur in a rat model of spinal cord injury, where transplanted NSCs developed into neurons that received synaptic connections from cortical motor neurons, and themselves developed projections that targeted neurons distal to the lesion [68].

A third possibility is that regeneration strategies focus only on generating interneurons. Although interneurons constitute only 10–15% of all neurons in the rodent brain [69], they are capable of modulating the output of surrounding neurons [70], such that their impact on neural circuits is large compared to their small numbers. This suggests that it might only be necessary to generate a small number of interneurons to achieve a significant functional impact on brain function. As described above, the few neurons generated in the rodent striatum in response to lesions are primarily interneurons [48, 64], which suggests that this is the strategy actually employed by the healing striatum. One study estimated that a few hundred interneurons had been generated 7 weeks after stroke in mice [48]. In another study, a similar number of transplanted interneurons were shown to have a beneficial effect on functional recovery in a mouse model of Parkinson's disease [71]. Therefore, even a small number of interneurons may be enough to promote some level of functional recovery after brain injury.

18.2 Conclusion

Today, research on therapeutic neuronal replacement is still in its infancy. New studies need to promote neuronal replacement in regions other than the striatum, either through stimulation of local neurogenic programmes or through redirection of migrating neural progenitors. Neocortical regions are relevant targets for new (pre-)clinical

investigations, due to their common involvement in stroke-related pathology and their implication in long-lasting cognitive deficits and motor impairments. Additionally, the field of regenerative neuroscience needs to develop safe and selective therapies that precisely tackle the process and/or cell population of interest, without unwanted, off-target effects. As our understanding of the molecular mechanisms that regulate NSC behavior improve and strategies for clinical delivery are refined, technologies to restore cognitive and motor function will continue to develop.

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