Stem Cell Biology and Regenerative Medicine

H. Georg Kuhn
Amelia J. Eisch *Editors*

Neural Stem Cells in Development, Adulthood and Disease

Humana Press

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This book is dedicated to our teachers, our students, and our families. We thank them for the support, patience, and inspiration they have given us throughout the years.

We specifically thank our parents, who provided the genetic material that made this book possible. Thank you for everything.

Preface

The previous assumption that the adult mammalian brain cannot generate new neurons has been definitively refuted with the discovery of continually ongoing neurogenesis in discrete regions of the central nervous system (CNS) in all mammalian species studied so far. The characterization of neural stem cells, their progeny and lineages, and the signaling pathways that guide these processes has fundamentally changed our view on development and repair of the CNS and even the peripheral nervous system as well.

It is in this context that we recruited prominent researchers in the field of neural stem cells and neurogenesis to provide their perspective on this rapidly changing field. The ten chapters in this book—briefly introduced below—cover a range of topics that will be of interest to both the established neurogenesis researcher as well as to readers with general interests in nervous system science. The chapters are designed to stand alone, yet even greater understanding of the field can be gained by reading the book in its entirety, following the chapters in the order they are presented.

Adult neurogenesis exists in numerous mammalian and nonmammalian species. Chapter "Adult Neurogenesis and Regeneration: Focus on Non-mammalian Vertebrates" by Ferretti and Prasongchean highlights that the contribution of adult neurogenesis to repair processes varies greatly between vertebrates. These species-specific traits provide an important opportunity to study differences in cellular and molecular mechanisms of adult neurogenesis and its contribution to neuronal cell turnover and structural repair in the CNS.

In mammals including humans, neural stem cell activity is most commonly observed in the juvenile and adult brain within the subventricular zone of the lateral ventricles and within the subgranular zone of the dentate gyrus. In chapter "Differential Intrinsic and Extrinsic Regulations of the Two Adult Neurogenic Regions," Guo and Zhao focus on comparing these regions and highlighting differential regulation of neurogenesis between these sites in order to evaluate their respective regenerative potentials.

A strong link between adult hippocampal neurogenesis and affective disorders such as depression and anxiety has been proposed because behavioral changes and neurogenesis are coregulated by alterations in serotonin neurotransmission. In chapter "The Role of Adult-Born Dentate Granule Neurons in the Regulation of Mood," Guo, Gatchel, and Sahay address whether the role of adult-born neurons in modulation of anxiety and depression-like behaviors is dependent on their proposed role in hippocampal function such as in pattern separation.

Although new neurons are continually produced in the adult human brain and may in the future serve as a replacement for dying neurons, adult neurogenesis itself could be affected by diseases. Chapter "Stem Cells and Neurogenesis in Relation to Dementia and Alzheimer's Disease Mouse Models" by Lucassen and colleagues and chapter "Hippocampal Neurogenesis in Neurodegenerative Movement Disorders" by Kohl and colleagues examine how the production of neurons is regulated by chronic neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's disease. It appears that immature neurons are very vulnerable to changes in cellular milieu within the neurogenic regions in these diseases and their animal models. The resulting deterioration in neurogenesis may further reduce the ability of the adult brain to cope with pathological changes and may enhance specific cognitive and affective symptoms in these disorders.

In epilepsy, in contrast to neurodegenerative diseases, it has been speculated that new neurons could aggravate the occurrence of subsequent spontaneous seizures. In chapter "Linking Adult Neurogenesis to Epilepsy Through Epigenetics," Cho and Hsieh review the evidence for an involvement of seizure-induced neurogenesis in the onset and progression of the disease with a particularly focus on ectopic dentate gyrus granule cell production. Furthermore, epigenetic alterations, which may control behavior of hippocampal neural stem cells and their progeny after seizures, are discussed as possible contributor to epilepsy.

Increased production of neurons in the dentate gyrus is assumed to elevate hippocampal function. Chapter "Activity-Based Maintenance of Adult Hippocampal Neurogenesis: Maintaining a Potential for Lifelong Plasticity" by Kempermann explores the conditions under which it could be possible to inspire and maintain a high level of neuronal production. Maintaining a pool of recruitable progenitor cells through means of environmental stimulation and physical exercise may provide an important and natural approach to prevent the aging-related decline in hippocampal neurogenesis.

Neural stem cells in the adult brain are multipotent and the generation of oligodendrocytes opens the exciting possibility to respond to myelin loss by generating new myelin sheaths. Chapter "Neural Stem Cells and Demyelinating Disease" by Crawford and Franklin explores possibilities for local as well as transplanted stem cells and oligodendrocyte precursor cells to induce remyelination, reinstate rapid axonal conduction, and resolve functional deficits of demyelinating diseases.

Spinal cord injury requires reestablishment of sensory and motor connections across complex damaged neural tissue. Chapter "Stem Cell-Based Therapies for Spinal Cord Regeneration" by Sandner and colleagues provides an overview on the current status of neural stem cell transplantation in spinal cord injury and discusses

future perspectives for the generation of astroglia, oligodendroglia, and neurons in spinal cord repair.

The final chapter, "Direct Reprogramming of Somatic Cells into Induced Neuronal Cells: Where We Are and Where We Want to Go," by Masserdotti and Berninger on direct reprogramming of somatic cells into neurons merges the knowledge that has accumulated on the induction of a stem cell state in somatic cells, the so-called induced pluripotency, with the more specific requirements for directed neuronal development within a neurodegenerative environment. It gives thus an outlook towards exciting future strategies of creating neurons in regions of the CNS that have no or only limited capacity for neurogenesis.

Taken together, these reviews by leading researchers emphasize the multifaceted progress made in understanding the functional role of neural stem cells in the adult nervous system. This is an extremely exciting time to study neural stem cells, and there is significant hope that the findings discussed in this book will translate into clinical advances in the near future.

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List of Abbreviations

5-hmC	5-hydroxymethylcytosine
5-mC	5-methylcytosine
α-syn	α-synuclein
Aβ	β-amyloid peptide
AD	Alzheimer's disease
APP	Amyloid precursor protein
BAC	Bacterial artificial chromosome
BAF	Brahma-associated factors
BAM	*B*rn2 (Pou3f2), *A*scl1, and *M*yt1l
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
bHLH	Basic-helix-loop-helix
BMP	Bone morphogenetic protein
BrdU	Bromodeoxyuridine
CA	Cornu ammonis
CaMK	Calcium-/calmodulin-dependent protein kinase
C/EBP	CCAAT/enhancer binding protein
ChRC	Chromatin remodeling complex
CNP	2', 3'-cyclic nucleotide 3'-phosphodiesterase
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CSPG	Chondroitin sulfate proteoglycans
DCX	Doublecortin
DG	Dentate gyrus
DNMT	DNA-methyltransferase
EAE	Experimental autoimmune encephalomyelitis
EEG	Electroencephalography
EGF	Epidermal growth factor

ES cells	Embryonic stem cells
fMRI	Functional magnetic resonance imaging
FXR2	Fragile X relative protein 2
GABA	γ-aminobutyric acid
GCL	Granule cell layer
GFP	Green fluorescent protein
GRP cells	Glial-restricted precursor cells
HAT	Histone acetylase
HBD	Hilar basal dendrites
HD	Huntington's disease
HDAC	Histone deacetylase
HdMT	Histone demethylase
HMT	Histone methylases
IEG	Immediate early gene
IPC	Intermediate progenitor cell
iPSC	Induced pluripotent stem cells
lncRNA	Long non-coding RNAs
MAG	Myelin-associated glycoprotein
MAP	Microtubule-associated protein
MCT1	Monocarboxylate transporter 1
MEF	Murine embryonic fibroblasts
MGE	Medial ganglionic eminences
miRNA	microRNA
Mol	Molecular layer
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSC	Mesenchymal stem cell
NEP	Neuroepithelial cell
NeuN	Neuronal nuclear antigen
NFTs	Neurofibrillary tangles
NRP cells	Neuronal-restricted precursor cells
NT-3	Neurotrophin-3
NSC	Neural stem cell
OB	Olfactory bulb
Omgp	Oligodendrocyte myelin glycoprotein
OPC	Oligodendrocyte progenitor cell
PCNA	Proliferating cell nuclear antigen
PD	Parkinson's disease
PDGF	Platelet-derived growth factor-β
PEDF	Pigment epithelium-derived factor
piRNA	Piwi-interacting RNAs
pMN	Motor neuron progenitor
PNS	Peripheral nervous system
PS	Presenilin
PSA-NCAM	Polysialylated neural cell adhesion molecule

PTB	Polypyrimidine tract-binding
PTSD	Posttraumatic stress disorder
PvL	Periventricular leukomalacia
RGL	Radial glia-like cell
RMS	Rostral migratory stream
RXR	Retinoid X receptor
SCI	Spinal cord injury
SE	Status epilepticus
SEZ	Subependymal zone
Shh	Sonic Hedgehog
siRNA	Short interfering RNAs
SGZ	Subgranular zone
Sox2	Sex determining region Y-box 2
SVZ	Subventricular zone
TET	Ten-eleven translocation
TF	Transcription factor
TR	Transcription repressor
TTF	Tail-tip fibroblasts
TUNEL	Tdt-mediated dUTP-biotin nick end labeling
VPA	Valproic acid
VZ	Ventricular zone
xCT	Na^+-independent cystine-glutamate exchange antiporter

Adult Neurogenesis and Regeneration: Focus on Nonmammalian Vertebrates

Patrizia Ferretti and Weerapong Prasongchean

The Neurogenic Niches

The study of mammalian neurogenesis has primarily focused on rodents, although information about postnatal neurogenesis in other species, including primates and humans, is slowly becoming available. In brief, adult neurogenesis in the rodent brain has been clearly identified in at least two brain regions (Figs. 1 and 2a, b): the subgranular zone (SGZ) of the dentate gyrus in the hippocampus (Altman and Bayer 1990; Eriksson et al. 1998; Palmer et al. 1995) and the subventricular zone (SVZ; also known as the subependymal zone, SEZ) of the forebrain lateral ventricle (Alvarez-Buylla and Garcia-Verdugo 2002; Doetsch et al. 1997). One striking aspect of adult neurogenesis, particularly in neuronal progenitors originating in the SVZ, is the ability of newly produced neurons to migrate far from the site where they were born and integrate within already established neural networks (Lie et al. 2004). In this respect, adult neurogenesis differs greatly from embryonic development, where maturation of different cell types occurs stepwise as a highly concerted and integrated process.

NSCs of the adult SVZ (Fig. 2b) are slowly proliferating, radial, glia-like astrocytes known as quiescent stem cells or type B cells, which express GFAP, vimentin, nestin, and prominin (Coskun et al. 2008; Doetsch et al. 1997). These cells are polarized with a basal process terminating onto blood vessels and an apical process,

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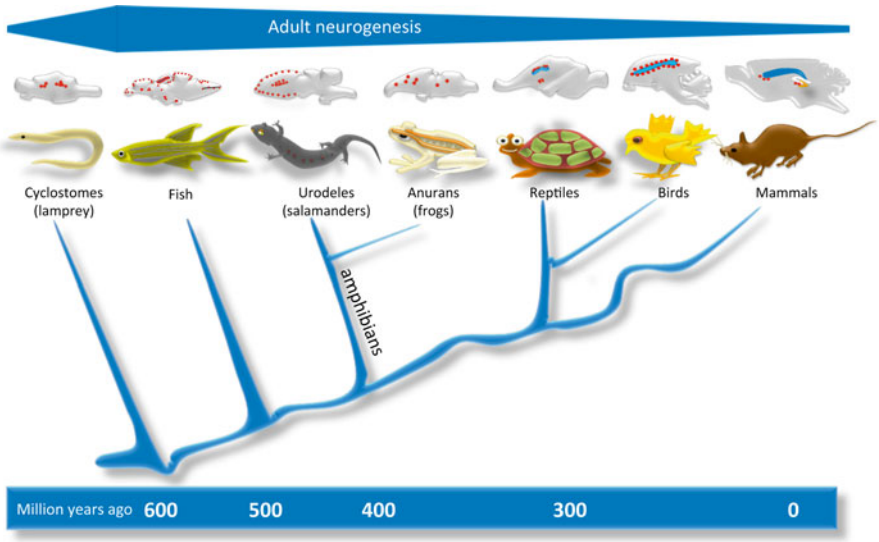

Fig. 1 Schematic representation of the phylogenetic tree and differences in neurogenic regions in vertebrates. The *red dots* indicate regions where proliferation/neurogenesis has been reported. Drawings are not to scale

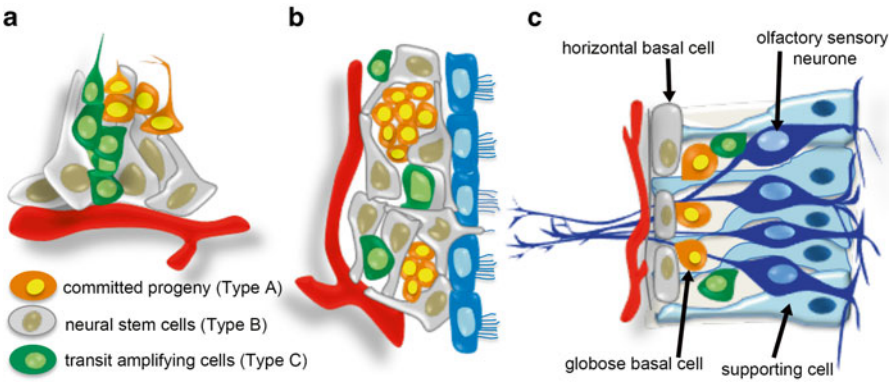

Fig. 2 Schematic representation of neurogenic niches in different regions of the rodent nervous system showing shared architecture. (**a**) Dentate gyrus of the hippocampus. The same cell types described in the SVZ niche (*panel B*) are present in the dentate gyrus, but unlike the SVZ there is not a direct connection to the ventricular surface. (**b**) Subventricular zone (SVZ). Type B cells are slowly proliferating astrocytic/radial glial neural stem cells bridging the ventricular cavity lined by ependymal cells (*pale blue*) and blood vessels (*red*); type C cells are transit-amplifying progenitors, and type A cells are the migratory lineage-committed cells. Multipotent globose basal cells originate from horizontal basal cells; they are akin to the transit-amplifying cells (C cells) and generate new olfactory receptor neurons and possibly new support cells (A cells). Blood vessels are present in close proximity to the olfactory epithelium, but their interaction with horizontal basal cells remains currently unclear. (**c**) Olfactory epithelium. Horizontal basal cells are believed to be homologous to B cells

Table 1 Summary of neurogenesis and regenerative capability in the central nervous system across species

Species	Adult CNS	Telencephalon	Mesencephalon	Cerebellum	Spinal cord	Retina	Olfactory system
Fish	Neurogenesis	+	+	+	+	+	+
	Regeneration	+	?	+	+	+	+
Urodeles	Neurogenesis	+	+	?	+	+	+
	Regeneration	+	+	?	+	+	+
Anurans	Neurogenesis	+	+/–	?	–	+	+
	Regeneration	–	–	?	–	–	–
Reptiles	Neurogenesis	+	?	+	+/–[a]	–	+
	Regeneration	+	?	?	–[a]	–	+
Birds	Neurogenesis	+	?	–	–	–	+
	Regeneration	+	?	–	–	–	?

[a]No neurogenesis is seen in lizards; however, turtles can regenerate nerves in transected spinal cords, but do not seem to undergo neurogenesis to replace lost neurons

a short primary cilium, extruding into the ventricle (Mirzadeh et al. 2008; Tavazoie et al. 2008). NSC cells give rise to transit-amplifying cells, also called type C cells, which thereafter generate migrating neuronal precursors, neuroblasts (type A cells), and oligodendrocytes. Neuroblasts expressing PSA-NCAM (polysialylated neural cell adhesion molecule) and DCX (doublecortin) migrate along the rostral migratory stream to the olfactory bulb where they differentiate into olfactory interneurons (Alvarez-Buylla and Garcia-Verdugo 2002; Lledo et al. 2008). Therefore, neurogenesis in the rostral SVZ is crucial for the renewal of interneurons in the adult olfactory bulb. The architecture of the human adult SVZ differs from that of rodents. It displays a hypocellular region that separates the putative astrocyte-like neural progenitors from the ependyma (Quinones-Hinojosa et al. 2006). The fate and migratory ability of these cells in adult brains have been much debated (Ferretti 2011 and references herein). It appears that robust neuroblast migration occurs only early in life and is negligible by 2 years of age (Sanai et al. 2011). This shorter-lived, postnatal, neurogenic activity may be due to a reduced importance of olfaction in humans compared to rodents.

The radial glia-like NSCs in the hippocampal dentate gyrus are largely organized in a similar fashion to those in the SVZ (Fig. 2a, b) and share expression of several NSC markers, such as GFAP, Sox2, and nestin (Ming and Song 2011). In the hippocampus, DCX-expressing neuroblasts migrate only a relatively short distance to reach the inner granule cell layer where they differentiate into dentate granule neurons. Hippocampal neurogenesis is believed to play a role in some types of hippocampus-dependent learning and memory (Gage et al. 2008; Kempermann 2011).

The true potential and identity of NSCs in the adult mammalian spinal cord have been a matter of debate. Cells that are capable of generating neurons and glia in vitro are also present in the central canal of the spinal cord, but postnatal adult neurogenesis is not known to occur. However, some DCX-positive cells have been reported in young animals where progenitor-like cells segregated within a specific domain (Sabourin et al. 2009). Interestingly, a recent study in 8-week-old mice suggested that sensory stimulation induced progenitor cell proliferation and differentiation into GABAergic neurons in the spinal cord (Shechter et al. 2011).

In adult mammalian spinal cords, the majority of proliferative activity is observed within the ependyma, particularly in the dorsal aspect. The existence of a very small cell population of proliferating cells adjacent to the ependyma, and immunogenically distinct from the ependymal cells, has recently been reported, but the lineage relationship with progenitor cells in the central canal, if any, is not known (Hamilton et al. 2009). In addition to the restricted regions of the central canal, radial glia-like stem/progenitor cells have been proposed to be present beneath the pial surface (Petit et al. 2011).

Similar to the brain, the mammalian-injured spinal cord cannot regenerate. Nonetheless, proliferative activity significantly increases following injury, although these newly born cells appear to primarily contribute to the replacement of a small number of oligodendrocytes and the glial scar (Barnabe-Heider et al. 2010). Some proliferating cells observed in the parenchyma following injury are thought to originate from a population of nestin- and DCX-expressing cells in the meninges, although there is no evidence that these cells mature into neurons (Decimo et al. 2011).

Adult neurogenesis occurs also within the olfactory neuroepithelium, where locally generated sensory neurons, which have a relatively rapid turnover, and neurons derived from the olfactory bulb are continuously rewired (Mackay-Sim 2010). Recent studies have provided clues to the molecular cues governing stem cell renewal and differentiation in the adult olfactory neuroepithelium. It has been proposed that p63 plays a crucial role in maintaining an undifferentiated state of horizontal basal cells, which is the putative stem cell of the olfactory neuroepithelium, and that fine modulation of ACTβB and GDF11 interplay controls the decision towards a neurogenic or gliogenic fate (Fletcher et al. 2011; Gokoffski et al. 2011; Packard et al. 2011).

NSCs of the SVZ and hippocampus closely interact with vascular cells and more mature neural cells in the so-called neurovascular niche (Fig. 2a-b) through extracellular matrix components, cell–cell interactions, and soluble signals (Andreu-Agullo et al. 2009; Kazanis and ffrench-Constant 2011; Kazanis et al. 2010; Shen et al. 2008; Tavazoie et al. 2008). Strikingly, a similar organization to that observed in stem cell niches of the brain is found in the olfactory epithelium, but at present there is no information on the cross talk between the putative stem cells of this neuroepithelium, horizontal basal cells, and blood vessels (Fig. 2c) (Iwai et al. 2008; Leung et al. 2007). In the adult spinal cord, the relationship between the neural stem niche and blood vessels remains unclear, although proliferating putative NSC/progenitor cells, observed mainly in the dorsal aspect of the central canal, appear to be closely associated with blood vessels (Hamilton et al. 2009).

Composition differences of neural niches in the brain have been described in various vertebrates, and it is clear that the NSC niche in nonmammalian vertebrates is more widely distributed along the brain rostrocaudal axis, extending to the spinal cord in certain lower vertebrates, such as fish and salamanders (Holder et al. 1991; Kaslin et al. 2009; Waxman and Anderson 1985). Notwithstanding recent progress in the ability to track cells from several species to study NSCs and their lineages, the lack of highly specific markers for neural stem cells and their early progeny, combined with interspecies differences in expression of available markers, is hampering rapid progression and unequivocal interpretation of comparative studies. Nestin expression, for example, which was once considered to be the landmark of NSCs, has been shown in a wide range of stem/progenitor cells, as well as prominin expression. Furthermore, we do not yet fully understand the functional role and implication of adult neurogenesis in different species, which will be crucial for elucidating the selective forces that are involved in the maintenance of this trait.

Our current understanding of the molecular basis of adult neurogenesis in mammals mainly comes from studies in rodents. As recently reviewed by Faigle and Song (Faigle and Song 2013), many of the key pathways utilized during neural development (e.g., Wnt, Notch, and Shh signaling) also underpin adult neurogenesis (Table 2). Furthermore, adult NSC proliferation and neurogenesis appear to be regulated by neurotransmitters (e.g., dopamine and serotonin) linking brain activity and homeostasis of neurovascular niches (Young et al. 2011). Although certain aspects of the relationships between neurogenesis and function most likely differ across species (Barker et al. 2011), the molecular mechanisms governing neurogenesis are

Table 2 List of some of the signalling molecules/pathways that have been implicated in the modulation of adult neurogenesis

Morphogens	Cytokines	Neurotransmitters	Transcription factors	Adhesion/Guidance molecules
Wnt	FGF	Dopamine	Sox2	DCX
Notch	EGF	GABA	Tlx	PSA-NCAM
Shh	BDNF	Glutamate	NeuroD	UNC/Netrin
BMP	VEGF	Serotonin	Pax6	Slit/Robo
	IGF		CREBA	Ephrins
	erithropoietin		Tbr2	Nogo/NgR1
				Plexin-B2

likely to be very conserved. Although only fragmentary information is currently available for such mechanisms in nonmammalian species, our overall knowledge of adult neurogenesis across species is increasing and will be discussed in the following section.

Adult Neurogenesis in Nonmammalian Amniotes

Neurogenesis in the adult avian and reptile brain is significantly more extensive than in mammals. The SVZ of both birds and reptiles displays some important differences compared to rodents. For example, transit-amplifying cells (type C) do not seem to be present (Fig. 3), as assessed by [3H]-thymidine tracking and electron microscopy studies (Alvarez-Buylla et al. 1998; Garcia-Verdugo et al. 2002). There is also evidence that radial glia-like cells are not homogeneously distributed in the periventricular regions, but present at a higher density in discrete areas. These results would suggest that the SVZ of birds is in an "intermediate" state between the germinal zone of the developing brain and the SVZ of adult rodents and has more extensively maintained embryonic-like pockets of cells (Alvarez-Buylla and Lim 2004). The DCX-positive neuroblasts generated in the SVZ migrate along radial glia fibers to the subjacent forebrain, where neurogenesis takes place (Goldman 1998). Both in vivo and in vitro tracking of single SVZ cells infected with a lacZ-expressing lentivirus suggests that neurons and radial glia generated in the adult avian brain share a common progenitor (Maden et al. 2013).

Telencephalic maturation occurs relatively late in songbirds, whose brain at birth is smaller than that of non-song-learning birds. The telencephalic neurons born in the embryo are generally long-lived and are not replaced by newly born neurons generated after hatching; these cells are associated with postnatal brain growth during the first 4 months of life. [3H]-Thymidine injections pre- and post-hatching have demonstrated neuronal birth in the telencephalon in juvenile and adult brains, and the differences in their contribution to different brain regions depend on the age of

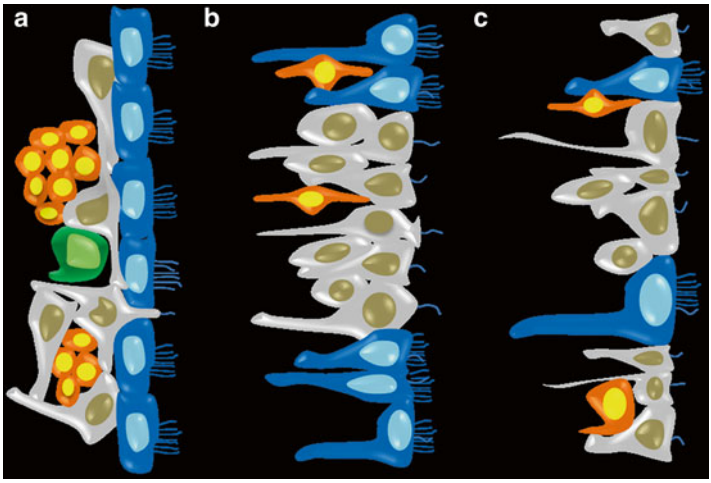

Fig. 3 Schematic representation of the ventricular zone in different amniotes. (**a**) Mammals, (**b**) birds, and (**c**) reptiles. The same type of cells described in Fig. 2 are present in the three species, but in different ratios. Type A cells (orange) are arranged radially in reptiles, radially or tangentially in birds, and tangentially in mammals. Types B cells (pale gray) and ependymal cells (*pale blue*) display radial expansions in reptiles and birds, while in mammals type B cells have lateral expansions and ependymal cells are cuboidal. The cell in green is a transit amplifying progenitor cell. Drawings modified from García-Verdugo et al., Brain Research Bulletin, Vol. 57, No. 6, pp. 765–775, 2002

the animal (Alvarez-Buylla et al. 1994). Although brain growth stops at around 4 months of age in the canary, neurogenesis continues to replace neurons that die in certain brain regions (Alvarez-Buylla et al. 1992).

Neurogenesis in nonmammalian amniotes can be clearly associated with function, particularly in songbirds, where seasonal variations in neuronal turnover are observed in vocal control brain nuclei. These nuclei include the large forebrain nucleus, hyper-striatum ventralis, or high vocal center (HVC), which contains a population of interneurons that turns over in the adult brain and the hippocampus (Nottebohm 1985). Neuronal turnover in the HVC is photoperiodic—greater at times during the breeding season, when songs are modified in the spring, than in the autumn. Neurons born in the latter period, however, appear to have a longer life span and may therefore play a role in long-term storage of song-related information (Alvarez-Buylla and Kirn 1997).

The mechanisms of seasonal control of neurogenesis remain poorly understood, but hormonal control appears to be important for its regulation. Manipulation of sex steroids affects song learning, but other hormones are also believed to play a role in song acquisition (Barnea and Pravosudov 2011). Reduction in testosterone levels, which is photoperiodically regulated in starlings, results in a marked reduction in the size of song control nuclei (Hall and Macdougall-Shackleton 2012). The effect of testosterone can be directly mediated via the androgen receptor or by some of its

androgenic (e.g., 5alpha-dihydrotestosterone) or estrogenic (e.g., 17beta-estradiol) metabolites (Yamamura et al. 2011). However sex steroids do not seem to play a role in NSC proliferation and neuronal production, but rather in the recruitment and migration of newborn neurons. DCX, which is a well-known player in cell migration in developing and adult mammalian brains, may be one of the targets of testosterone. Significantly, DCX expressional changes are concomitant with seasonal changes in HVC size and are synergistically modulated by androgen and estrogen (Balthazart et al. 2008; Yamamura et al. 2011). Recruitment and survival of neurons by testosterone have also been associated with upregulation of both VEGF and its endothelial VEGFR2 tyrosine kinase receptor. Inhibition of the latter reduces endothelial cell division in the HVC, as well as the number of newly generated neurons, linking neurogenesis and angiogenesis (Louissaint et al. 2002).

Although studies have shown intense neurogenesis and an enlarged adult avian hippocampus, particularly in food-storing species where spatial learning is crucial (Barnea and Pravosudov 2011), there are no studies to date on neurogenesis in the adult avian spinal cord. Nonetheless, because neurogenesis can be detected at embryonic day 11 (E11), when the fairly mature spinal cord continues to regenerate, but not at E15, a regeneration-incompetent stage, occurrence of post-hatching neurogenesis in the spinal cord seems unlikely (Ferretti and Whalley 2008; Whalley et al. 2009).

Similar to what observed at late stages of development, the post-hatched avian spinal cord does not regenerate, nor do brain neuronal populations that do not normally turn over in the adult. This is in contrast to the regenerative ability displayed by neurons that do turn over in the adult brain and whose ablation results in some recovery in song production (Scharff et al. 2000). While stab injury to the hippocampus induces a proliferative response, it is not yet known whether neurogenesis ensues (Law et al. 2010). The use of transgenic songbirds may facilitate tracking the fate of the proliferating cells present in the injured hippocampus and resolve this issue (Agate 2009). If the hypothesis that neuronal populations in avian that turn over can regenerate is correct, hippocampal injury should result in at least partial replacement of lost neurons.

Moving down the phylogenetic tree to reptiles (e.g., turtles and lizards), radial glial cells with GFAP-positive processes connecting to the pial surface are widely present in the brain and spinal cord of lizards (Lazzari and Franceschini 2001). Similar to birds, the reptilian periventricular region does not contain transit-amplifying cells (Fig. 3), and the number of differentiated ependymal cells appears reduced compared to birds (Garcia-Verdugo et al. 2002). NSC proliferation and DCX-positive cells migrating along radial glial fibers are observed in several regions of the adult lizard brain (olfactory bulbs, rostral forebrain, all cortical areas, anterior dorsal ventricular ridge, septum, striatum, nucleus sphericus, and cerebellum) and are believed to contribute to postnatal brain growth. However, differences in the extent of neuronal maturation have been reported (Font et al. 2001; Lopez-Garcia et al. 2002), which might be species-specific, age-related, or both (Delgado-Gonzalez et al. 2011). Furthermore, seasonal variations in NSC proliferation and migration exist from the ventricular region along radial and tangential migratory routes, with the latter giving rise to new neurons in the olfactory bulb. Analysis of

temperature effects on cell proliferation has revealed greater cell proliferation in spinal cords and brains, dorsal cortex, medial cortex, and diencephalon of juvenile turtles acclimated to warm temperature (27–30 °C) than in those acclimated to a cooler environment (5–14 °C) (Radmilovich et al. 2003). Finally, sex-specific differences in the rate of neurogenesis exist in the accessory olfactory bulb of *Podarcis* wall lizards, consistent with larger bulbs in males than in females (Font et al. 2012).

The functional role of adult neurogenesis in reptiles is not well understood, but many neurogenic structures in these taxa are related to chemoreception and may therefore represent an adaptive phenomenon. A reductionist explanation could be that most proliferative activity in the adult reptile brain, as well as in teleost fish and tailed amphibians (urodeles), is mainly associated with continuous growth.

There is no clear evidence that postnatal neurons are born in the lizard spinal cord, notwithstanding the persistence of radial glia-like cells and proliferative activity in the adult spinal cord. Some neurogenesis, however, has been proposed to occur in the juvenile turtle spinal cord (Duffy et al. 1992; Fernandez et al. 2002; Russo et al. 2004). Significantly, functional recovery in regenerated lizard tails is not due to spinal cord regeneration, because proliferating radial glial cells only form an ependymal tube, but to innervation of the regenerated muscle by neurons rostral to the amputation plane. In turtles, spinal cord transection evokes a radial glial response and axonal regrowth, which results in the reconnection of cord stumps and at least partial functional recovery. However, neurogenesis does not contribute to repair of the transected cord. Molecular analysis of spinal cord regeneration in turtles remains poorly understood, but an upregulation of Cx43 (Connexin43) following spinal cord injury, which is shorter in turtles than in mammals, has been proposed to reduce neural damage and favor regeneration (Garcia et al. 2012; Rehermann et al. 2011).

Reptiles are the only amniotes capable of regeneration following significant brain damage (Font et al. 2001). Intraperitoneal injection of 3-acetylpyridine in lizards specifically induces neurotoxic damage in the medial cortex, resulting in massive neuronal death. This is accompanied by a proliferation burst in the subjacent ependyma, as well as replacement of degenerated PSA-NCAM-positive cells and regeneration of the medial cortex (Lopez-Garcia et al. 1992; Molowny et al. 1995; Ramirez-Castillejo et al. 2002). In addition, following a stab injury to the dorsal telencephalic cortex in the lizard, a proliferative response is evoked in the SVZ. The lesion slowly repairs over months, with healthy-looking neurons present in the lesioned area (Romero-Aleman et al. 2004). However, according to current data, it is not possible to establish whether repair is due to the birth of new neurons or to the plasticity and migration/reorganization of surviving neurons; migration of mature neurons into the regenerating spinal cord has been observed in urodeles following tail amputation (Zhang et al. 2003). Neural regeneration in the turtle, an anoxia-tolerant vertebrate, is also observed in response to global ischemia. Lost neurons are replaced within 3 weeks after ischemic damage, as indicated by the presence of cells labeled with BrdU and the late neuronal marker NeuN (Kesaraju and Milton 2009). Although it is tempting to speculate that this may reflect a physiological adaptation for managing anoxia–reoxygenation, in-depth investigations are required to assess whether this may indeed be the case.

Adult Neurogenesis in Lower Vertebrates

Amphibians

Urodeles (newts and salamanders) and anurans (tailless amphibians, such as frogs and *Xenopus*) split off around 350 million years ago, and anurans rather than urodeles appear to represent the evolutionary branch from which reptiles and mammals emerged. Post-metamorphic anurans, as well as post-hatching reptiles, maintain a radial glia-like structure in the brain and spinal cord. We will refer to these cells as "radial glia-like" to distinguish them from the embryonic radial glia. In addition, given that in adult lower vertebrates the ventricular region is more akin to the embryonic-like structure than to the SVZ, we will refer to it as the ventricular zone (VZ).

Radial glia-like cells are present in the ventricular regions of both juvenile and adult frog brains, where proliferative activity, particularly in the lateral ventricle region, is significant, as indicated by BrdU labeling. At 2 days, no BrdU-positive cells were observed in the brain parenchyma, but at later time points they were detected in several brain regions, including the preoptic area, hypothalamus, and optic tectum, where they co-express neuronal or oligodendrocyte markers (Chetverukhin and Polenov 1993; D'Amico et al. 2011; Polenov and Chetverukhin 1993; Simmons et al. 2008). Whether the 3[H]-thymidine-positive small hypothalamic neurons identified by electron microscopy and immature neurons detected by immunocytochemistry in these studies become functional and play specific roles is not yet known. It will be important, for example, to thoroughly assess whether their number varies during the mating season and could be important in reproductive behavior. Some evidence suggests that acoustic social cues increase cell proliferation, which appears to be sex hormone independent. However, the extent of neuronal differentiation and possible changes in neuronal maturation and survival in response to different hormonal and environmental stimuli remain poorly understood (Almli and Wilczynski 2012).

Brain regeneration, as well as spinal cord regeneration, is restricted to larval stages in anuran amphibians (frogs), in contrast to urodeles, which can regenerate both structures in adulthood. The initial response to brain injury in adult frogs—ependymal cell proliferation—occurs similarly to animals that do regenerate, but these cells seem unable to leave the periventricular region and fill the gap. Whether this is due to intrinsic changes in radial glial properties in the adult brain, which becomes unable to support migration of neuronal precursors, or to extrinsic factors has not been resolved (Endo et al. 2007). However, the fact that the addition of cells to close the wound results in regenerative responses suggests that differences in the healing process may be critical for permitting or restricting the regenerative response. On the other hand, a paracrine effect of the grafted cells on the endogenous population is also possible, and the two hypotheses are not mutually exclusive (Prasongchean et al. 2012).

Urodele amphibians (newts and salamanders) continue to grow throughout life, which is most likely the basis for ongoing neurogenesis observed in the brain and

spinal cord. In the juvenile axolotl brain—a neotenic species that reaches sexual maturity without undergoing metamorphosis—extensive proliferation and neurogenesis occur in several brain regions. Shortly after BrdU injection, labeling is restricted to the ventricular regions throughout the axolotl CNS, but within 2 weeks most cells have migrated away from the VZ along the radial fibers. In addition, all BrdU-positive cells in the gray matter express NeuN (Maden et al. 2013). Under physiological conditions, proliferative zones in the adult newt brain appear to be restricted to the forebrain (Berg et al. 2010).

Both mechanical- and chemical-induced damages to the brain evoke a regenerative response in urodele brains. Removal of a large portion of the optic tectum in adult newts results in regeneration, with restoration of the retinotectal projection by 8 months (Okamoto et al. 2007). Given the extensive radial glia-like cell proliferation observed, it is most likely that the proliferating ependymal cells are the source of the regenerated tectum. The ventricular origin of the progenitors involved in brain regeneration following ablation of telencephalic segments has also been suggested by studies in axolotl (Maden et al. 2013). Neural ablation selectively triggers proliferation in the VZ adjacent to the damaged area and its restoration. Significantly, in experimental paradigms where the olfactory nerve is transected, a healing response is observed, but regeneration does not occur until the nerve regenerates.

Significant regeneration is observed also following chemical ablation of midbrain dopaminergic neurons with 6-OHDA (6-hydroxydopamine), which is used to model Parkinson's disease. Injection of 6-OHDA into the third ventricle of the adult newt induces death of dopaminergic neurons within 3 days; it also increases microglial numbers and activation, and interestingly, microglial inhibition improves regeneration (Kirkham et al. 2011). These results suggest an inhibitory role of the innate immune response in newts, but that remains insufficient to block regeneration of dopaminergic neurons. Following a neurotoxic insult, proliferation of radial glia-like cells is activated in the apparently quiescent midbrain; this is followed by neurogenesis, as well as replacement of dopaminergic neurons and behavioral recovery (Berg et al. 2010; Parish et al. 2007). This process appears to be mediated via Shh signaling, a pathway important also in mammals for the proliferation of embryonic and adult NSCs (Berg et al. 2010; Faigle and Song 2013). Furthermore, there appears to be an inhibitory feedback from dopaminergic neurons on proliferative activity of radial glia-like cells of the mesencephalon that express dopamine receptors (Berg et al. 2011). A role for neurotransmitter-dependent regulation of SVZ proliferation has been proposed in mammals (Young et al. 2011), which would suggest that inhibition of dopaminergic neuronal replacement may be downstream of induction of proliferative activity. Comparative analysis of the response to specific neuronal type ablation will be crucial for elucidating where replacement inhibition lays.

When carrying out such comparative studies, it is important to be well aware of the variables that could affect outcome and interpretation of results. For example, although it has been suggested that the uninjured newt midbrain is quiescent, there is proliferative activity in the VZ of the axolotl midbrain, albeit at lower levels than in the forebrain and cerebellum (Berg et al. 2010; Maden et al. 2013). To fully understand the basic mechanisms involved in the regenerative response, it will be

important to establish whether the response reflects age or species differences. In addition, it remains to be shown whether the level of proliferation in the normal newt midbrain is too low to be detected by currently utilized BrdU labeling protocols (Berg et al. 2010). In any case, whether induction of a fully quiescent population or activation of a slowly cycling one is taking place, the remarkable capability of adult newts to recruit cells to replace dopaminergic neurons makes them very valuable models for unraveling the cellular and molecular mechanisms underlying this phenomenon. A better understanding could possibly create a basis for inducing dopaminergic neuronal replacement in higher vertebrates.

The urodele spinal cord regenerates after transection and amputation of the tail, respectively. Spinal cord injury induces radial glia-like cell proliferation and formation of an ependymal tube that, unlike in the lizard, gives rise to a regenerated functional spinal cord containing both newborn neurons and glia (Benraiss et al. 1999; Nordlander and Singer 1978; Zhang et al. 2000, 2003). Injury triggers dedifferentiation/transdifferentiation of the radial glia-like cells, which, after a proliferative phase, differentiate into neurons and glia to form a new functional spinal cord (Ferretti 2004; Walder et al. 2003). FGF signaling plays an important role in neural progenitor cell proliferation following tail amputation and spinal cord transection, respectively (Moftah et al. 2008; Zhang et al. 2000, 2002), consistent with its known proliferative effect on mammalian NSCs. Shh signaling is important for dorsoventral patterning of the regenerating spinal cord, paralleling its role in development (Schnapp et al. 2005). Retinoic acid signaling also appears to be an important player in spinal cord regeneration, because RARβ-selective antagonists have been shown to inhibit ependymal outgrowth (Carter et al. 2011). Finally, spinal cord injury in urodeles has been shown to modulate expression of several molecules/pathways known to be involved in adult neurogenesis (Table 2) (Monaghan et al. 2007). Altogether, several signaling pathways implicated in the proliferation of mammalian NSC are found at play during CNS regeneration in urodeles. However, little is known about their precise roles and interplay. Differences in spatiotemporal deployment following injury between urodele and mammalian spinal cords may determine whether regeneration will succeed or fail.

Fish and Lamprey

Like urodeles, fish can grow and generate new neurons along the entire rostrocaudal brain axis, as well as the spinal cord, throughout life. Proliferative zones have been located in 16 distinct CNS regions along the entire anterior–posterior axis (Grandel et al. 2006). New neurons are also produced in the spinal cord, where proliferating cells have been detected in the ependymal layer and throughout the spinal cord parenchyma. Over the last 10 years, much information has emerged on neurogenesis and regeneration in adult teleost fish, particularly in zebrafish, and it has recently been extensively reviewed (Grandel and Brand 2012; Zupanc and Sirbulescu 2011; Kizil et al. 2012).

The NSC niche of the adult zebrafish exhibits intermediate features between adult mammals and birds and reptiles (Lindsey et al. 2012). It is more similar to the latter due to a lack of a continuous ependymal layer, which is found in mammals. Ependymal cells present in the zebrafish niche are even sparser than in nonmammalian amniotes. Similar to mammals, type C-like cells are present, although their organization within the niche differs. Adult fish brain NSCs are a heterogeneous population that exhibit both radial and non-radial glial cell phenotypes (Ganz et al. 2010; Zupanc and Sirbulescu 2011).

Tracking radial glia-like cells with GFP-expressing viruses has shown that these cells do exhibit NSC attributes; they are self-renewing and can generate different neural cell types in vivo (Rothenaigner et al. 2011). Furthermore, Notch signaling negatively regulates radial glia-like cell proliferative activity, whereas their fate appears to be Notch independent. Notch inhibition also increases proliferation in the transected spinal cord, and an increased number of motoneurons are generated in these animals (Dias et al. 2012). In addition, Bmp signaling seems to be a negative regulator of NSC growth (Kizil et al. 2012). In contrast, FGF signaling, which as discussed earlier stimulates NSC proliferation in urodeles and mammals, is also required for NSC proliferation in the fish brain. Interestingly, following spinal cord transection, FGF signaling is not only required for proliferation of radial glia-like cells but also for the formation of glial bridges across the lesion that allow rejoining of the stumps (Goldshmit et al. 2012).

Proliferating stem cells in the ventral subpallium generate rapidly dividing progenitors and neuroblasts that migrate to the olfactory bulb and differentiate into GABAergic and TH-positive neurons, similar to mammals. Importantly, dorsal and ventral neural progenitors in the adult telencephalon express a different combination of transcription factors compared to embryonic development (Adolf et al. 2006).

Functional CNS regeneration, which occurs in several teleost fish species and is associated with the generation of new neurons, has been studied particularly in the cerebellum, spinal cord, and retina (Anderson and Waxman 1985; Delgado and Schmachtenberg 2011; Hitchcock et al. 2004; Kaslin et al. 2009; Reimer et al. 2008; Sirbulescu et al. 2009; Zupanc and Ott 1999). Similar to amphibians, brain injury evokes a proliferative response in the ventricular region, and following a stab wound to the telencephalon, new neurons are generated by radial glia-like cells, as shown by genetic lineage tracing (Kroehne et al. 2011). Reactive gliosis and an inflammatory response are transient events, unlike in mammals where they resolve in the glial scar. Significantly, the inflammatory response appears to play a crucial role in activating the regenerative program in the injured zebrafish brain (Kyritsis et al. 2012). Inhibition of the inflammatory response in zebrafish reduces the proliferative response in the injured brain, whereas in urodeles its inhibition improves regeneration (Kirkham et al. 2011). Whether this is due to a difference in the ratio of "good" (e.g., M2 microglia) vs. "bad" (e.g., M1 microglia) components of the inflammatory response in urodeles and fish or to differences in the experimental conditions used and molecular pathways investigated has yet to be established. Such a comparative

study would shed much light on how one could attempt to modulate the inflammatory response in mammals to maximize a repair response.

The lamprey is the oldest vertebrate for which any information on neurogenesis is available. Lampreys are the descendants of some of the oldest known vertebrates and, together with the hagfish, are the only surviving jawless fish (cyclostomes) (Fig. 1). They have an eellike appearance, but unlike the eel, they have cartilaginous skeletons. After hatching, lampreys are almost blind larvae that undergo metamorphosis within 3–7 years.

The still limited information available on adult neurogenesis in lamprey suggests this is a relatively rare event (Vidal Pizarro et al. 2004). In the rhombencephalon and spinal cord, the highest mitotic activity is detected within the ependyma, with a higher mitotic index measured in the summer, although proliferative activity is observed also in the parenchyma. The proliferating cells are mainly gliogenic, and new neurons are seldom found in the lamprey CNS. There are no studies on brain regeneration in lamprey, and it should be noted that studies on spinal cord regeneration have been mainly performed in larval lamprey.

Following injury to the spinal cord, the adult lamprey recovers locomotory functions, although there is no evidence of neurogenesis. Furthermore, only approximately 50 % of the descending fibers regenerate. Recovery of presynaptic organization and morphology of giant reticulospinal synapses, although their numbers are lower than in controls, is observed when behavioral recovery is nearly complete (Oliphint et al. 2010). In contrast, regenerated giant reticulospinal axons produce very few synapses, with fewer vesicles and smaller active zones than observed in uninjured axons. Differences in axonal guidance molecules in neuronal populations may contribute to their different regenerative ability (Shifman et al. 2009). For example, in the lamprey, the poorly regenerating reticulospinal neurons express UNC5L, a member of the UNC5 receptor family (Barreiro-Iglesias et al. 2012). Interactions of these receptors and their ligand, netrin-1, play a role in axonal guidance, and increased UNC5 expression in the adult mammalian brain has been associated with reduced regenerative capabilities. Therefore, differences in UBNC5 expression in neuronal subsets may underlie the different regenerative abilities of neuronal populations in the lamprey. As in other species, spinal cord injury evokes neurite sprouting in the brain. However, it remains to be shown whether there is any occurrence of neurogenesis in the lamprey brain. Based on the overall more limited regenerative capability in lampreys than in fish, this seems unlikely (Lau et al. 2011). Surprisingly, though at the bottom of the vertebrate evolutionary tree, the lamprey CNS is not regenerated as efficiently as the urodeles or fish CNS.

Altogether, in adult lower vertebrates, extensive neurogenesis in the adult spinal cord appears to correlate with high regenerative capability, whereas the brain offers a more complex picture. There is not a simple relationship between the multiple variables involved: varying degrees of complexity, adult neurogenesis, and regeneration.

Conclusions

In-depth comparative analysis of the distribution, life span, and subtype of adult-born neurons, as well as analysis of the variables that can affect the regenerative response, such as anatomical location, injury paradigm, inflammatory response, and age, remains to be performed. It is hoped that the recently resurrected interest in evo-devo and nonmammalian models of regeneration will recruit more scientists to studying adult neurogenesis in the context of regenerative capability across vertebrates. A critical mass of scientists working on these complex issues is indeed required to dissect the cellular and molecular events underlying adult neurogenesis and to establish whether it is linked to an evolutionary selection of functional activities and regenerative capability. A better understanding of these mechanisms could be of value for improving neural repair in humans.

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Differential Intrinsic and Extrinsic Regulations of the Two Adult Neurogenic Regions

Weixiang Guo and Xinyu Zhao

Introduction

Neurogenesis is defined as the process of generating new neurons from neural stem and progenitor cells, followed by subsequent maturation and functional integration of these new neurons into the neural circuitry. Conventionally, this process was believed to be specific to embryonic development of the mammalian central nervous system (CNS). In the early twentieth century, the prominent histologist Ramon y Cajal made a statement that "Once the development was ended, the founts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult centers, the nerve paths are something fixed, ended, and immutable. **Everything may die, nothing may be regenerated**. It is for the science of the future to change, if possible, this harsh decree." This doctrine remained unchallenged until the 1960s and remained unchanged until the 1990s (Barinaga 1992). Only recently, the concept that new neurons are continuously added in specific regions of adult mammalian brains, including human brains, has gained wide acceptance (Alvarez-Buylla and Temple 1998; Ming and Song 2005; Temple and Alvarez-Buylla 1999; Weiss et al. 1996). Since its discovery, adult neurogenesis presents exciting opportunities and promises for the treatment of neurological and mental disorders with no effective cures (Ming and Song 2011). With accumulating findings in the past decade and

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active ongoing investigations, our understanding of adult neurogenesis is advancing at a surprising rate. For instance, we know increasingly more about the localization and identity of adult NSCs, molecular controls of NSC proliferation, fate specification and migration, regulation of morphological maturation, and functional integration of newborn neurons into adult CNS milieu (Aimone et al. 2006; Alvarez-Buylla and Lim 2004; Bonaguidi et al. 2012; Deng et al. 2010; Kriegstein and Alvarez-Buylla 2009; Zhao et al. 2008).

In the adult mammalian brain, neurogenesis takes place in two distinct neurogenic zones—the SGZ and SVZ—which are composed of an assortment of cell types, including NSCs and niche cells, soluble factors, membrane-bound receptors, and extracellular matrix molecules forming specialized microenvironments that are permissive to the production of new neurons (Alvarez-Buylla and Lim 2004; Fuentealba et al. 2012; Ming and Song 2011; Suh et al. 2009). In general, new neurons generated in the adult hippocampus remain in the DG, whereas new neurons generated in the SVZ migrate to the OB, the final destination for these new neurons. Neurogenesis in these two discrete neurogenic zones shares many similar features but also exhibits clear differences. Our understanding of the differences between the SGZ and SVZ has progressed significantly over recent years, but a great deal remains to be explored. Keeping these and other important questions in focus in this review, we will summarize the known differences between the SGZ and SVZ and conclude with a discussion on both known and proposed molecular mechanisms contributing to the differential regulation of these two niches.

Neural Stem Cells and Neurogenesis

Neural Stem Cells in Developmental and Adult Brains

NSCs define a type of cells that have the capacity to self-renew and to generate other neural cell types and tissues through asymmetric cell divisions. The concept of NSCs was established based on a large amount of experimental evidence. Initially, whether stem cells that give rise to neurons and glia during embryonic development belong to the same or distinct populations of stem cells was under debate. Until the 1990s, contrasting evidence supported both common and different precursors for neurons and glia (McKay 1997). More recent reports using genetic lineage tracing analysis confirmed that neurons and glia originate from common progenitors in the developing mammalian CNS, thereby confirming the concept of NSCs (Kriegstein and Alvarez-Buylla 2009). During early stages of CNS development, the neural plate emerges to subsequently form the neural tube, which primarily consists of neuroepithelial cells (NEPs) and is considered to be the most primitive form of NSCs (Gotz and Huttner 2005). From the emergence of the first NSCs, embryonic neurogenesis enters three distinct phases (expansion, neurogenesis, and gliogenesis) (Gotz and Sommer 2005). NEPs are the primary source of cells for the developing nervous system and give rise to all cells in the CNS, including radial glia-like cells (RGLs), which later assume the role of adult NSCs (Gotz and Barde 2005).

Even at the start of neurogenesis, NEPs are gradually replaced by radial glia (Gotz and Barde 2005; Kriegstein and Alvarez-Buylla 2009). Radial glia exhibit various astroglial properties and express markers of glial lineage, such as RC2, BLBP, and GFAP (Malatesta et al. 2008). Radial glia can divide symmetrically, generating more radial glia, and asymmetrically to give rise to both neurons and glia. Additionally, they also form the migration scaffold for new neurons in the developing brain. Similar to NEPs, radial glia also exhibit interkinetic nuclear migration during their cell cycle (Willardsen and Link 2011).

While there has been a consensus that NEPs and embryonic radial glia are considered to be NSCs, the identity of NSCs in adult brains was only recently clear. Ependymal cells have been the main candidates for resident adult NSCs (Chojnacki et al. 2009; Johansson et al. 1999), but this idea was largely abandoned (Spassky et al. 2005), and RGLs were determined to be the NSCs in the adult brain (Kriegstein and Alvarez-Buylla 2009; Malatesta et al. 2008). Ependymal cells appear to differentiate from RGLs during the first three weeks of life in the mouse brain (Tramontin et al. 2003). However, recent reports provide new evidence that ependymal cells may act as precursor cells following brain injury (Carlen et al. 2009). Identification of adult NSCs in vivo is based upon cell morphology and ultrastructure, expression of specific genes, and cellular mitotic activities (Bonaguidi et al. 2012; Chojnacki et al. 2009; Mirzadeh et al. 2008). However, the most common method of detection relies on several developmentally retained markers for RGLs, such as nestin (Lendahl et al. 1990), brain lipid-binding protein (BLBP) (Feng et al. 1994), glial fibrillary acidic protein (GFAP) (Doetsch et al. 1999), and Sry-related high-mobility group box transcription factor 2 (Sox2) (Ellis et al. 2004). Based on the expression patterns of these markers, several models have been proposed for the identity and lineage specification potentials of adult NSCs. One model suggests that GFAP-expressing RGLs are quiescent NSCs that give rise to new neurons in the OB, oligodendrocytes in the corpus callosum, and granule neurons in the adult hippocampus (Malatesta et al. 2003). In another model, Sox2-expressing non-radial cells are suggested to be the active NSCs that generate neurons and glia in the adult brain (Suh et al. 2007). These models may not be mutually exclusive, because they may represent multiple NSC subtypes with distinct morphologies and can differentially respond to physiological and pathological stimuli, as well as aging (Lugert et al. 2010).

Neurogenesis in Adults

Adult neurogenesis has been identified in all vertebrate species examined thus far, although it is gradually reduced during the course of evolution (Lindsey and Tropepe 2006). It has been assumed that complex brains require a high degree of stability and cannot cope with the degree of plasticity introduced by a large number of new neurons (Kempermann 2012). Indeed, adult neurogenesis in most mammals is mainly restricted to two specific regions: the SVZ and the SGZ. With extensive studies, models have addressed the process of cell genesis during adult mammalian neurogenesis (Alvarez-Buylla and Lim 2004). In the adult SGZ, RGLs (known as type 1 cells) are

activated from quiescence and proliferate into non-radial cells, both of which give rise to intermediate progenitor cells (IPCs, also known as type 2 cells) that are transient amplifying cells. IPCs give rise to neuronal lineage-committed proliferating progenitors or neuroblasts (type 3 cells), which in turn give rise to immature neurons. Immature neurons migrate to the inner granule cell layer and differentiate into mature granule neurons in the DG (Fig. 1a). In the adult SVZ, quiescent RGLs (type B cells) are activated and give rise to IPCs (type C cells), which in turn generate neuroblasts (type A cells). These neuroblasts form a migratory chain called the "rostral migratory stream" (RMS) toward the OB and differentiate into OB neurons (Fig. 1a).

In addition to the SVZ and SGZ, there have been several reports of ongoing neurogenesis in other adult mammalian brain regions, including the neocortex, striatum, amygdala, and the hypothalamus (Lindsey and Tropepe 2006). However, evidence for neurogenesis in these regions remains controversial. This review will focus on the two well-established neurogenic regions.

Differences Between SGZ and SVZ Neurogenesis

A large number of studies have shown that both SVZ and SGZ neurogeneses respond to physiological and pathological stimuli. However, most of these studies have only analyzed one region, not both [for a complete review, see (Ashton et al. 2012)]. Based on the limited literature, it is apparent that these two regions respond differently to certain physiological stimuli and pathological conditions. These differences are likely due to intrinsic differences in the NSCs and extrinsic differences in the stem cell niches residing in these two regions.

Different Neurogenic Destinations

NSCs in the SVZ and SGZ have distinct final destinations in both locations and cell fate. SVZ-NSCs give rise to neuroblasts that migrate long distances through the RMS to the OB, whereas SGZ-NSCs differentiate into immature neurons that remain local and migrate only a short distance from the SGZ to the granule cell layer upon maturation (Fig. 1). The types of mature neurons generated by these two neurogenic events are also distinct. The SVZ-NSCs eventually differentiate into inhibitory GABAergic and dopaminergic interneurons, whereas SGZ-NSCs differentiate into excitatory glutamatergic granule neurons (Ming and Song 2005). Whether these distinct cell fates are controlled by intrinsic or extrinsic mechanisms remains largely unknown. It is also possible that small numbers of other types of neurons are generated from NSCs in these two regions. For example, a recent study demonstrates that SVZ-NSCs can also generate glutamatergic neurons in the OB (Brill et al. 2009). Whether NSCs in the DG can produce interneurons in the hippocampus remains unknown.

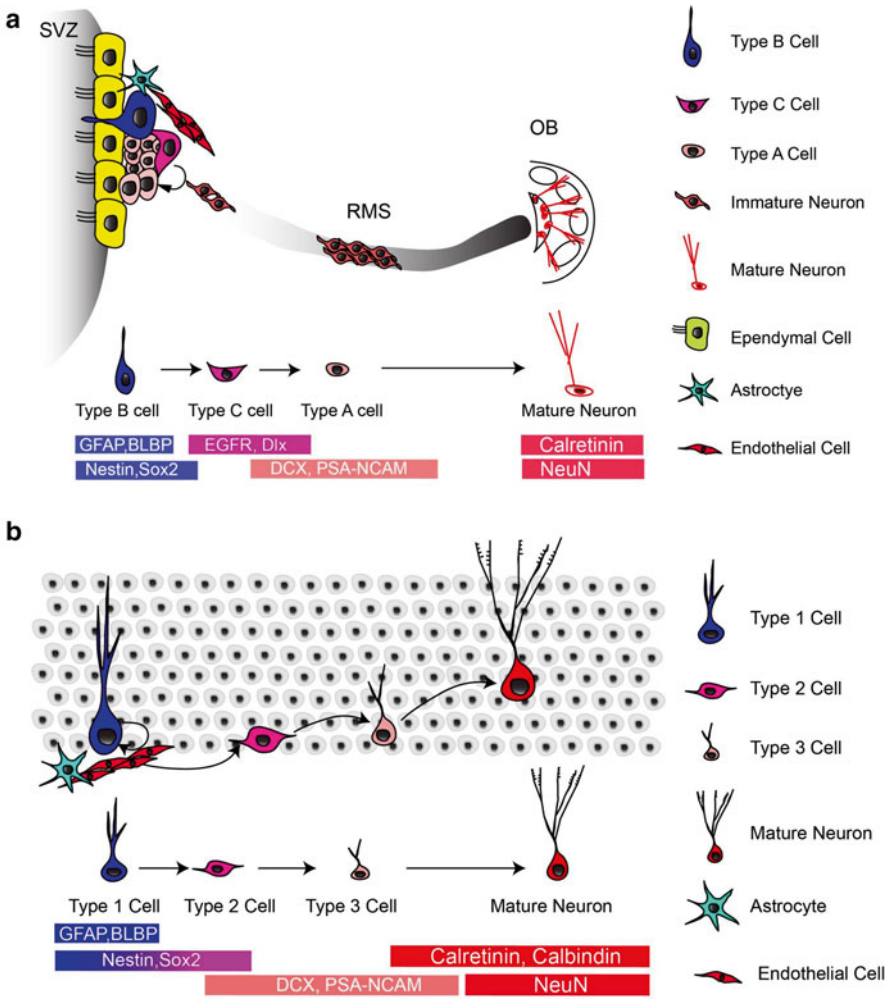

Fig. 1 (**a**) Schematic diagram showing developmental stages of SVZ neurogenesis. RGLs (type B cells) in the SVZ, when activated, divide asymmetrically with one daughter cell differentiating into IPCs (type C cells). IPCs actively proliferate and generate neuroblasts (type A cells). Neuroblasts migrate through the rostral migratory stream (RMS) and then become mature interneurons and integrate into the local neuronal circuitry in the olfactory bulb. Cells at different stages of SVZ neurogenesis express stage-specific markers, and the process of neurogenesis is regulated by niche cells, including ependymal cells, astrocytes, and vascular endothelial cells. (**b**) Schematic diagram showing developmental stages of SGZ neurogenesis. Activated RGLs (type1 cells) in the SGZ differentiate into IPCs (type 2 cells) via asymmetric cell division. IPCs actively proliferate and differentiate into neuroblasts (type 3 cells), which subsequently become immature neurons and then mature neurons. New mature neurons integrate into hippocampal circuitry and become excitatory granule neurons in the DG. The cells at different stages of SGZ neurogenesis express stage-specific markers, and the process of neurogenesis is regulated by niche cells, including astrocytes, vascular endothelial cells, and various types of neurons

Table 1 Differences in cytoarchitecture between SGZ and SVZ

	Adult neurogenic regions	
	SGZ	SVZ
Niche cells	Vascular epithelial cells Astrocytes Glutamatergic neurons Parvalbumin + interneurons	Ependymal cells Vascular epithelial cells Astrocytes
Stem cells and their progeny	Type 1 cells (RGLs) Type 2 cells (IPCs) Type 3 cells (neuroblasts) Mature glutamatergic neurons	Type B cells (RGLs) Type C cells (IPCs) Type A cells (neuroblasts) GABAergic interneurons Dopaminergic interneurons Glutamatergic neurons

Different Neurogenic Niches

Except for generating different subtypes of neurons, the cytoarchitecture of the two adult neurogenic regions is quite different (Table 1). There are four key cell types in the SVZ: ciliated ependymal cells that face the ventricle, which provide a barrier and filtration system for cerebrospinal fluid, type B cells, type C cells, and type A cells. Ependymal cells, forming pinwheel-like structures along the ventricular surface, surround type B cells. Type B cells also retain epithelial properties, with primary cilia protruding into the ventricle and a long basal process ending on the blood vessels. In contrast, the DG lies deep within the hippocampal parenchyma. Type 1 cells, type 2 cells, type 3 cells, mature neurons, and astrocytes are among the major cellular components of the DG neurogenic niche. The primary cilium of type 1 cells contact with blood vessels and astrocytes in the hilus region, and soluble factors and cell surface proteins from the blood, endothelial cells, and astrocytes act on the NSCs. Granule neurons, interneurons, and IPCs surrounding the NSCs also serve as niche components to regulate NSC fate. Adult neurogenic zones (especially the SVZ) are considered to be remnants of embryonic neurogenic niches, and multiple reports support that resident adult NSCs carry the same neurogenic potential (Alvarez-Buylla and Lim 2004). It remains to be determined whether these NSCs are indeed multipotent or whether they are restricted by spatial cues to differentiate into specific types of neurons. A recent investigation by Merkle et al. suggests that, in fact, a spatial code exists that is intrinsic to NSCs and it determines the types of neurons these NSCs can generate (Merkle et al. 2007). In the SGZ, a similar progenitor encoding by positional information remains to be discovered.

Distinct Response to Stimuli

SVZ neurogenesis has been proposed to play major roles in olfactory learning and memory, while SGZ neurogenesis contributes to hippocampus-dependent learning (Deng et al. 2010; Guo et al. 2011a; Zhao et al. 2008). These two neurogenic regions

Table 2 SVZ and SGZ respond differently to physiological stimuli and pathological conditions

	Adult neurogenic regions	
	SGZ	SVZ
Stimuli		
Olfactory associative learning	No effect	Promotes neurogenesis
Hippocampus-dependent learning and enriched environments	Increases neurogenesis	No effect
Nerve growth factor (NGF)	No effect	Increases cell proliferation
Sumatriptan (5-HT receptor 1B agonist)	No effect	Increases cell proliferation
GR127935 (5-HT receptor 1B antagonist)	No effect	Decreases cell proliferation
Pathology		
Cranial irradiation	Long-term effect	Recovers with time
Oxidative stress (xCT mutation)	Increases neurogenesis	No effect
Fragile X-like syndrome (FXR2 mutation)	Increases neurogenesis	No effect
Huntington's disease (R6/2 mouse model)	Decreases neurogenesis	No effect
Prenatal stress	Decreases neurogenesis	No effect

respond differently to various stimuli, including learning paradigms (Table 2). Olfaction-associated learning promotes SVZ neurogenesis in the OB, but exhibits no significant effect on hippocampal neurogenesis (Sultan et al. 2010). Conversely, environmental enrichment boosts neurogenesis in the SGZ, but not in the SVZ (Brown et al. 2003). Differential regulation of SVZ and SGZ neurogenesis by distinct stimuli suggests that the generation of new neurons in these two regions might be modulated by extrinsic cues produced by local stem cell niches, such as trophic factors and neurotransmitters. Neurotrophic factors are a family of molecules that play important roles in neuronal differentiation during development, as well as contribute to brain plasticity throughout life. A large number of studies have assessed the roles of trophic factors and neurotransmitters in adult neurogenesis [for comprehensive reviews, see (Suh et al. 2009; Zhao et al. 2004)]. Intraventricular administration of nerve growth factor (NGF) has been shown to increase cell proliferation in the SVZ (Fiore et al. 2002), while intra-hippocampal administration of NGF has no effect on cell proliferation in the SGZ (Frielingsdorf et al. 2007). In addition, neurotransmitters released by neurons can also influence neurogenesis.

Distinct Response to Pathological Conditions

Brain injuries and pathological conditions also have differential impacts on SVZ and SGZ neurogenesis (Table 2). Cranial irradiation, one of the most effective tools for treating malignant tumors, results in intellectual impairment and cognitive deficits through ablating proliferating cells in the adult brain. Although cranial irradiation represses cell proliferation in both the SVZ and SGZ, the SGZ suffers long-term effects, whereas the SVZ recovers with time (Hellstrom et al. 2009). Oxidative stress caused by mutation of the Na^+-independent cystine-glutamate exchange

antiporter (xCT) promotes neurogenesis in the DG, but not in the SVZ (Liu et al. 2007). Fragile X-related protein 2 (FXR2) belongs to a family of fragile X mental retardation proteins, and FXR2 mutant mice exhibit fragile X-like behavioral deficits, including hippocampus-dependent learning deficits (Bontekoe et al. 2002). FXR2 deficiency leads to impaired SGZ neurogenesis, but has no effects on SVZ neurogenesis (Guo et al. 2011b). In the R6/2 mouse model of Huntington's disease, SGZ neurogenesis is impaired, whereas SVZ neurogenesis is similar between R6/2 mice and their wild-type littermate (Gil et al. 2005). Furthermore, prenatal stress, a risk factor for the development of psychopathology, inhibits SGZ neurogenesis but spares SVZ neurogenesis (Belnoue et al. 2013).

Different Intrinsic Characteristics

Although early transplantation studies, including the transplantation of spinal cord-derived progenitors into the adult hippocampus, have shown the instructional role of a stem cell niche on NSC differentiation (Shihabuddin et al. 2000), accumulating evidence suggests that NSCs residing in different brain regions process intrinsic differences. For example, NSCs from the embryonic forebrain or cerebellum retain regional characteristics after homotopic transplantation (transplantation of isolated NSCs into the normal anatomical position in the recipient; e.g., adult DG-derived cells transplanted into the adult DG). However, when NSCs are heterotopically transplanted (transplantation of NSCs derived from one CNS region to another; e.g., DG-derived cells transplanted into the SVZ), NSCs from either neurogenic region exhibit a reduced capability to differentiate into neurons (Klein et al. 2005). Furthermore, homotopic transplantation of SVZ-NSCs into the SVZ consistently generates interneurons in the OB, whereas heterotypic transplantation into non-neurogenic regions exhibits limited generation of site-specific neurons (Herrera et al. 1999). In a similar condition, following homotopic transplantation, NSCs isolated from the DG generate granule neurons and integrate into local circuitry. However, when SVZ-NSCs are heterotopically transplanted into the adult DG, they fail to differentiate into granule neurons (Chen et al. 2011). Although the differentiation potential of heterotopically transplanted NSCs into the SGZ remains controversial, a better understanding of regional NSC characteristics may provide a more valuable outcome for cell therapy using NSC transplantation.

Molecular Mechanism Underlying Differences Between SGZ and SVZ Neurogenesis

A number of studies have focused on the molecular mechanisms of adult neurogenesis in the DG and the SVZ. Most of these studies have focused on either the DG or the SVZ [see Suh et al. (2009)]. This chapter will only review the studies that addressed differential regulation of these two neurogenic regions.

Extrinsic Factors

Neurogenic niches provide nurturing factors for NSCs, and the interactions between NSCs and their neighboring cells determine many vital properties of NSCs, including self-renewal, proliferation, differentiation, and fate determination. In this section, we will discuss how neurogenic niches differently regulate NSCs and neurogenesis in these two sites (Table 3).

Ependymal Cell Niche

The ependyma functions as a barrier, protecting the brain from noxious substances that accumulate in the cerebrospinal fluid. Therefore, ependymal cells in the SVZ form a unique niche for NSCs and can influence SVZ neurogenesis by blocking

Table 3 Distinct extrinsic and intrinsic regulations of neurogenesis in SVZ and SGZ

	Adult neurogenic regions	
	SGZ	SVZ
Extrinsic factors		
LRP2 (specifically expressed in ependymal cells)	No effect	Increases neurogenesis
Noggin	Granular neurons, RGLs, and IPCs are sources of Noggin	Ependymal cells are a source of Noggin
NTF (astrocyte-secreted factor)	NTF mutation leads to decreased glial differentiation of NSCs	NTF mutation leads to decreased neuronal differentiation of NSCs
PEDF (released from endothelial cells)	No effect on cell proliferation	Increases cell proliferation
GABA	PV + interneurons are a source of GABA GABA acts on type 2 cells (IPCs)	Neuroblasts are a source of GABA GABA acts on type B cells (RGLs) as a feedback regulator
Serotonin (5-HT)	Increases cell proliferation through 5-HT1a and 5-HT2b receptors	Increases cell proliferation through 5-HT1a and 5-HT2c receptors
Intrinsic factors		
Pax6 (transcriptional factor)	Maintenance of NSCs	Determines subtype interneuron differentiation
Mll1 (histone methyltransferase)	Increases cell proliferation and neuronal differentiation	Increases neuronal differentiation, no effect on cell proliferation
Querkopf (histone acetyltransferases)	NA	Increases neurogenesis
FXR2 (RNA-binding protein)	Decreases neurogenesis	No effect on neurogenesis
HuD (RNA-binding protein)	NA	Increases neurogenesis

BMP signaling in NSCs (Lim et al. 2000). However, Noggin has been shown to increase SGZ-NSC proliferation while exhibiting no effect on SVZ-NSCs (Bonaguidi et al. 2008). The sources of Noggin are different between the two neurogenic zones. Ependymal cells are the source of Noggin in the SVZ (Lim et al. 2000), while NSCs and granule neurons are the source of Noggin in the DG (Guo et al. 2011b). Therefore, an NSC intrinsic regulator of Noggin, such as FXR2, exhibits effect on DG neurogenesis, but has no effect on SVZ neurogenesis. The low-density lipoprotein receptor-related protein 2 (LRP2), a clearance receptor for BMP4, is specifically expressed in ependymal cells. Loss of LRP2 expression in the adult brain leads to reduced neurogenesis, which coincides with increased BMP4 expression and enhanced activation of the downstream mediator phospho-SMAD1/5/8 in SVZ-NSCs (Gajera et al. 2010). In contrast, LRP2 deficiency has no effect on hippocampal neurogenesis.

Astrocyte Niche

Emerging evidence suggests that glia, particularly astrocytes, have key roles in controlling multiple steps of adult neurogenesis within the niches (Ashton et al. 2012; Barkho et al. 2006; Ma et al. 2005; Song et al. 2002). However, astrocyte-derived neurotrophic factors may have different effects on neurogenesis between these two regions. For example, mice lacking neurotrophin-3 (NT-3) exhibit decreased gliogenesis in the SVZ (Kahn et al. 1999) but decreased neurogenesis in the DG (Shimazu et al. 2006).

Vascular Niche

The anatomy of the vasculature and nervous system is closely associated with the adult neurogenic zones (Palmer et al. 2000). The molecules mediating interactions between vessel and neural cells are also different in these two neurogenic regions. In the adult SVZ, pigment epithelium-derived factor (PEDF), released from endothelial and ependymal cells, stimulates neurogenesis. PEDF also acts as a critical mediator to convey endothelial influence on self-renewal of type B cells through activation of Hes1 and Hes5, two major effectors of the Notch signaling pathway (Ramirez-Castillejo et al. 2006). However, in the adult DG, exogenous expression of PEDF in the DG has no effect on cell proliferation (Namba et al. 2010). Further studies are needed to determine whether the PEDF receptor is differentially expressed between SVZ-NSCs and SGZ-NSCs.

Neuronal Circuitry

Neurogenic niches have a unique feature: they are heavily innervated by various types of neurons. Neurotransmitters are not only used to communicate between neurons but also serve as secreted molecules that control many aspects of

neurogenesis during embryonic development and in the adult brain. GABA (γ-aminobutyric acid) is an important regulator of embryonic neurogenesis (Suh et al. 2009). Remarkably, it also exhibits similar effects during adult DG neurogenesis, where it acts as an excitatory signal for NSCs and immature neurons but as an inhibitory signal for mature neurons (Tozuka et al. 2005). During GABA-mediated excitation, GABA interaction with its cognate receptor opens up the Cl^- channel. Subsequently, the concentration gradient across the membrane drives Cl^- flow outside of neurons, leading to depolarization. Parvalbumin-expressing (PV+) interneurons are a source of GABA, which promotes neuronal differentiation of IPCs in the adult DG and regulates the timing of synaptic integration of immature neurons (Ge et al. 2006; Song et al. 2012). In the SVZ, GABA also influences neurogenesis, but through a different mechanism (Liu et al. 2005). Type A cells (neuroblasts) are a source of GABA. Depolarized type A cells release GABA, which subsequently acts on type B cells in the SVZ through $GABA_A$ receptors and inhibits NSC proliferation. Therefore, GABA serves as a feedback regulator of neuronal production and migration (Bordey 2007). A lesion in serotonergic neurons or inhibition of 5-HT production by pharmacological treatment decreases cell proliferation and neurogenesis in both SVZ and SGZ (Brezun and Daszuta 1999). The lesion-mediated loss of cell proliferation can be restored by grafting fetal serotonergic neurons, which suggests that 5-HT is a positive regulator of NSC proliferation (Brezun and Daszuta 2000). However, accumulating evidence has demonstrated that 5-HT1a and 5-HT2c receptors mediate proliferation of SVZ-NSCs, whereas activation of 5-HT1a and 5-HT2b receptors promotes cell proliferation of SGZ-NSCs (Banasr et al. 2004). Therefore, the 5-HT receptor 1B agonist sumatriptan promotes cell proliferation in the SVZ, and its antagonist, GR127935, inhibits it. Nevertheless, neither of these agents effect cell proliferation in the DG.

Intrinsic Genetic and Epigenetic Controls

Extensive studies have demonstrated that transcriptional factors, epigenetic mechanisms, and posttranscriptional modifiers play critical roles in spatiotemporal regulation of neurogenesis in the adult brain. In this section, we will present several examples of how these factors differentially regulate adult neurogenesis (Table 3).

Transcriptional Regulation

Along the SVZ-RMS, the transcriptional factor Pax6 is expressed mainly by neuroblasts (type A cells) and is downregulated in differentiated GABAergic neurons of the granule cell layer. However, *Pax6* expression remains unchanged during differentiation of dopaminergic neurons of the glomerular layer (Hack et al. 2005). These results suggest that during SVZ neurogenesis, *Pax6* is required for specification of dopaminergic periglomerular cells. The number of dopaminergic periglomerular cells is enhanced by *Pax6* overexpression in adult neuronal progenitors and reduced

in *Pax6* heterozygous mutant mice, which suggests that a proper dose of *Pax6* is essential for differentiation and maintenance of various OB interneurons (Hack et al. 2005; Kohwi et al. 2005). However, in the adult DG, *Pax6* is mainly expressed by proliferating cells (NSCs and IPCs), and its expression is reduced to undetectable levels in DG neurons (Maekawa et al. 2005). Therefore, *Pax6* is required for maintenance of progenitor cells in the adult hippocampus. Future studies are needed to determine how Pax6 regulates proliferation or differentiation via different downstream effectors during neurogenesis in the DG and SVZ.

Upon BMP signaling activation, Smad proteins (Smad1/5/8), which are the BMP signal pathway mediators, are phosphorylated (pSmad1/5/8) and translocate with SMAD4 to the nucleus, where they act as transcription factors and induce ID1-4 expression (Miyazono et al. 2010). Activation of BMP signaling leads to NSC exit of cell cycle and differentiation into a glial cell fate (Bond et al. 2012). Cultured SGZ-NSCs seem to have higher levels of the endogenous pSmad1/5/8, which in turn leads to higher expression of ID proteins compared to cultured SVZ-NSCs (Bonaguidi et al. 2008). These results suggest that intrinsic differences in BMP signaling might underlie the proliferation differences between SGZ-NSCs and SVZ-NSCs. It remains to be shown whether BMP signaling activities or BMP receptors are different between SVZ-NSCs and SGZ-NSCs in vivo.

Epigenetic Machinery

Epigenetic regulations not only play an important role in embryonic development but also serve as important interfaces between genes and the environment during adulthood (Li and Zhao 2008). Epigenetic mechanisms carry out diverse roles in regulating adult neurogenesis throughout life in discrete regions of the mammalian brain. Mll1 (mixed-lineage leukemia 1), a member of the trithorax proteins, encodes H3K4 methyltransferase, and it is specifically required for neuronal differentiation from adult NSCs via binding to homeobox protein Dlx2 (Lim et al. 2009). However, Mll1 deficiency leads to decreased NSC proliferation in the adult DG, not in the SVZ. A genetic mutation in *Querkopf*, a member of the MYST family of histone acetyltransferases, leads to reduced NSC proliferation and neuronal differentiation in the SVZ (Merson et al. 2006), but its effect on SGZ neurogenesis has not yet been examined.

Posttranscriptional Regulation

Posttranscriptional regulation of critical regulatory mRNAs by RNA-binding proteins is likely to be a common mechanism during critical cellular processes, as well as in adult neurogenesis. During DG neurogenesis, the RNA-binding protein FXR2 regulates NSC proliferation and differentiation by binding to *Noggin mRNA* and repressing Noggin expression, an antagonist of BMP signaling (Guo et al. 2011b). In the SVZ, however, FXR2 has no effect on neurogenesis. Because Noggin expression is restricted to ependymal cells, where FXR2 is not expressed, Noggin

expression is not regulated by FXR2. Neuronal Hu protein, HuD, is an RNA-binding protein mainly expressed in neurons, although it is not expressed in DG neurons (Okano and Darnell 1997). HuD deficiency leads to increased cell proliferation and decreased neuronal differentiation during SVZ neurogenesis, which may be mediated through their mRNA targets, such as p21, p27, and N-Myc (Akamatsu et al. 2005). However, the functional role of HuD and its underlying mechanisms in hippocampal neurogenesis remain poorly understood.

Summary

Differential regulation of SVZ and SGZ neurogenesis is a largely unexplored research area in the field of neuroscience and stem cell biology. To understand the differences between SGZ and SVZ neurogenesis, first, both regions of adult neurogenesis must be assessed at the same time in animal models. Second, gene expression and biochemical analysis can be performed, by isolating NSCs from neurogenic regions, to decipher the differential underlying mechanisms. Previous methods for isolating NSCs from adult brain required at least two mice for each SVZ-NSC preparation (Reynolds and Weiss 1992; Seaberg and van der Kooy 2002) or six mice for each SGZ-NSC preparation (Babu et al. 2007). However, because direct comparisons between littermates are necessary, studies involving double and even triple transgenic animals, large numbers of mice would impose a major hurdle for experiments. We recently developed a more efficient method that allows for NSC isolation from both the DG and SVZ of a single adult mouse (Guo et al. 2012). With regard to cell culture, extended passaging of NSCs could modify properties of isolated cells by, for example, increasing chromosomal aberration and reducing the capacity for neuronal differentiation. Therefore, it is critical to compare NSCs and their progenies between these two neurogenic zones in vivo. Direct isolation of different cell populations by enzymatic dissociation, followed by fluorescence-activated cell sorting (FACS), has been used to isolate NSCs and its progeny (Bracko et al. 2012). However, such tissue dissociation leads to the disruption of three-dimensional relationships of multiple cell types, which may lead to changes in gene and protein expression. Genetically tagged ribosome proteins have been used to isolate RNA expressed in specific cell types without cell isolation (Doyle et al. 2008; Heiman et al. 2008), which may provide an excellent tool for identifying intrinsic factors differentially expressed between SVZ-NSCs and SGZ-NSCs and specific types of niche cells.

Since the landmark discovery by Eriksson et al. in 1998 (Song et al. 2002), many studies have focused on adult neurogenesis in human brains and the effect of neurological disorders on the germinal zones. In the human brain, NSCs in the DG and SVZ niches also respond differently to disease states (Curtis et al. 2012). Therefore, a better understanding of the differences in neurogenesis between these two neurogenic niches will not only address fundamental questions related to adult neurogenesis, stem cell biology, and plasticity but also provide new strategies for the treatment of neurological and psychiatric disorders.

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The Role of Adult-Born Dentate Granule Neurons in the Regulation of Mood

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Adult-Born Dentate Granule Neurons and Pattern Separation

Recent studies in rodents have implicated adult-born neurons in a range of hippocampal-dependent tasks, including processing of contextual information and spatial learning. Within the domain of contextual processing, there is growing evidence supporting a role for hippocampal neurogenesis in pattern separation, a mnemonic process by which similar perceptual patterns are separated or transformed into orthogonal neural representations. The proposed role for adult-born neurons in pattern separation was antedated by a foundation of computational and experimental studies implicating the DG in pattern separation (Rolls and Kesner 2006; Treves et al. 2008). Experimental evidence for the DG in pattern separation first came from lesion studies in rodents showing that colchicine-induced ablation of the DG impaired discrimination of two spatial locations based on distal contextual cues,

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especially when the overlap of the distal cues was large, i.e., spacing of the two locations was small (Gilbert et al. 2001). These findings were corroborated by studies using genetic approaches to specifically manipulate DG functions. For example, genetic disruption of synaptic transmission and plasticity at perforant path-dentate granule synapses was found to impair an animal's ability to differentiate between two similar contexts (McHugh et al. 2007). However, the capacity to distinguish between two distinct contexts was unaffected in these mice, suggesting that entorhinal inputs to CA3 were sufficient for this function. In concordance with previous computational modeling work, these studies suggested that the DG is required to minimize interference between overlapping spatial or contextual information. However, since these studies employed manipulations that affected the entire DG, they were unable to distinguish the contribution of adult-born neurons from those generated during DG development.

Electrophysiological analyses of adult-born neurons during their maturation have identified critical periods of heightened synaptic and structural plasticity relative to developmentally generated neurons, suggesting that young adult-born neurons contribute preferentially to DG functions in encoding (Ge et al. 2007; Saxe et al. 2006; Snyder et al. 2001; Zhao et al. 2006). Furthermore, young adult-born neurons may account for up to 10 % of the DG (Imayoshi et al. 2008; Snyder and Cameron 2012). Recent studies using loss-of-function and gain-of-function approaches have demonstrated that adult-born neurons are indispensable for pattern separation. For instance, mice with ablated adult hippocampal neurogenesis via targeted irradiation are less efficient than controls in detecting small, but not large, separations of maze arms or objects on a touch screen (Clelland et al. 2009). More recently, Sahay et al. showed that mice with genetically increased adult hippocampal neurogenesis are better at discriminating between two similar contexts than controls (Sahay et al. 2011a). During initial training in this task, both groups of mice generalized their conditioned responses (freezing) to both the aversive training (CS+) and safe unconditioned (CS−) contexts. Because mice were tested in CS+ and CS− contexts over several days with reinforcement of the CS+ context, but not the CS− context, the mice with more adult-born neurons were better at discriminating between the two contexts than controls. Conversely, the authors found that blockade of adult hippocampal neurogenesis by targeted x-irradiation resulted in impaired contextual fear discrimination learning. Similar results were obtained using a genetic approach to block adult hippocampal neurogenesis (Tronel et al. 2010). Paralleling these findings, Kheirbek and colleagues showed that genetic blockade of synaptic plasticity of just the young adult-born neurons, a manipulation that did not affect their survival, was sufficient to impair discrimination learning. Importantly, in these studies, genetic augmentation or abrogation of adult neurogenesis (or synaptic plasticity of young adult-born neurons) did not impact discrimination of distinct contexts, suggesting [as in the spatial radial arm maze task (Clelland et al. 2009)] that adult-born neurons were more likely required for reducing interference between two similar representations and that general mechanisms underlying behavioral inhibition were not altered. Together, these studies suggest that in the absence of adult-generated neurons, the vast majority of dentate granule neurons

fail to compensate in pattern separation and beg the question as to what functions the older neurons have in encoding. A recent study by Nakashiba and colleagues provides intriguing insight into this question (Nakashiba et al. 2012). These authors showed that transgenic mice with inhibited synaptic output of mature dentate granule cells, but not that of younger immature neurons, were better at pattern separation than controls. Furthermore, these mice were impaired in pattern completion, a process by which a full pattern is retrieved based on a partial or degraded cue. These observations suggest that young adult-born neurons and developmentally generated neurons may have opposing roles in mediating pattern separation and pattern completion, respectively, and that simply varying the number of new neurons may shift the balance between these two mnemonic processes. Given the pivotal role of CA3 in pattern completion (Marr 1971; Nakazawa et al. 2002; O'Reilly and McClelland 1994; Rudy and O'Reilly 1999), mature DG neurons may exhibit distinct patterns of connectivity relative to young adult-generated neurons.

Recent studies relying on functional magnetic resonance imaging (fMRI) and immediate early gene (IEG)-based circuit mapping have begun to examine the neural correlates of pattern separation in humans and in rodents. In rodents, Leutgeb et al. showed that dentate granule neurons, but not CA3 neurons, exhibit a remapping response to subtle morphing of contexts (Leutgeb et al. 2007). Interestingly, rather than exhibiting global remapping as observed in CA3, where nonoverlapping/independent ensembles of neurons are activated in response to different environments, the authors found the same population of DG neurons to be reactivated, albeit with different firing rates (also known as rate remapping). More recently, it has been suggested that adult-born neurons exhibit rate remapping during behavioral foraging and that the mature dentate granule neurons are relatively silent and fire only in a single location (global remapping) (Neunuebel and Knierim 2012). Although visualization of neuronal ensembles of activated neurons using IEG expression has consistently found sparse activation of the DG with 2–4 % of dentate granule neurons active in a given context, current IEG-based studies support a role for global, rather than rate, remapping in the DG (Schmidt et al. 2012) as a substrate for pattern separation.

Human studies using high-resolution fMRI have also supported a role for the DG in pattern separation. Bakker et al. had participants view a series of pictures of objects, which were either new, a repetition of a previously shown object, or a slightly different version of a previously shown object (lure). Although different regions of the temporal lobe were activated by a novel object, only the DG-CA3 circuit, but not other subregions, exhibited elevated levels of activity similar to first presentation of an object, in response to lures (Bakker et al. 2008). This lure response was interpreted as pattern separation, because it suggested recognition of a subtle difference in the object relative to previously observed objects. In contrast, an activation pattern similar to that of a repetition indicated pattern completion. To explicitly address pattern separation in the DG-CA3 circuit, the same authors used a similar encoding task, except that object similarity was parametrically varied (Lacy et al. 2011) akin to the context morphing used in in vivo place cell recording studies in rodents (Leutgeb et al. 2007). The authors found greater activation of DG-CA3

relative to CA1 for lures, with high similarity, suggestive of a pattern separation-like mechanism that transforms input similarity to generate divergent output. Using this task, the authors recently found that aged adults were less efficient than younger adults in pattern separation and that degradation of the perforant path, as well as putative changes in CA3 dendritic architecture, may underlie these differences in pattern separation efficiency (Yassa et al. 2011).

The preponderance of evidence favoring a role for adult-born neurons in pattern separation underscores the need to identify how a small number of adult-born neurons influence encoding functions in the DG. Although several models addressing this question have been developed (Aimone et al. 2011; Appleby et al. 2011; Becker et al. 2009; Myers and Scharfman 2008; Wiskott et al. 2006), we recently proposed a non-cell autonomous role for adult-born neurons as modulators of DG excitability (Lacefield et al. 2010; Sahay and Hen 2007; Sahay et al. 2011b) via connections with hilar interneurons and mossy cells (Toni et al. 2008). In such a model, the introduction of new adult-born neurons into the DG circuit increased the threshold for activation of the entire DG by recruiting feedback inhibition mediated by hilar mossy cells and interneurons, thereby generating a sparser pattern of firing, a feature thought to be conducive for orthogonalization of information and, consequently, pattern separation (Kesner 2007; Leutgeb et al. 2007; Treves et al. 2008). It may also constrain pattern completion functions of mature neurons when a similar environment is encountered, because mature neurons preferentially respond to previously experienced environments (Aimone et al. 2011; Tashiro et al. 2007). Conversely, a familiar environment will preferentially reactivate a specific cohort of old neurons, and this may inhibit the new neurons and favor pattern completion. In addition to feedback inhibition of the DG, young adult-born neurons may recruit feedforward inhibition through interneurons in the stratum lucidum to modulate pattern completion functions in the CA3. As stated earlier, unraveling the precise patterns of functional connectivity of adult-born and mature neurons with mossy cells, interneurons, and CA3 neurons will shed light on their functions in pattern separation and pattern completion, respectively. It is also important to address how adult-born neurons contribute to place cell remapping. Specifically, are adult-born neurons the primary substrates for rate remapping in a context morphing task? Do adult-born neurons contribute to global remapping under certain conditions such as when task demands are integrated into behavioral paradigms in which the context is morphed? Integration of in vivo recordings with visualization of the underlying cell types in mice, in which levels of neurogenesis are selectively modulated, will undoubtedly address some of these questions.

Adult-Born Dentate Granule Neurons and Mood Regulation

In addition to a role for adult-generated DG neurons in pattern separation, increasing evidence suggests a relationship between new neurons, stress, and mood regulation. Early studies from the Duman lab and others utilized growth factors and viral

expression systems implicated the DG in mood regulation (Adachi et al. 2008; Chen et al. 2001; Hunsberger et al. 2007; Lee et al. 2009; Son et al. 2012). For example, BDNF infusions into the DG, but not CA1, were sufficient to produce antidepressant-like behavioral effects in the learned helplessness paradigm and the forced swim test (Shirayama et al. 2002). Since the first observations that selective serotonin reuptake inhibitors increased neurogenesis in the DG of adult rodents (Malberg et al. 2000), it is now evident that this is true for all FDA-approved antidepressants. The first demonstration of a role for adult-born hippocampal neurons in mood regulation came from studies where mice received low-dose hippocampal irradiation with concomitant antidepressant treatment (Santarelli et al. 2003). The authors found that blocking adult hippocampal neurogenesis abrogated the response to antidepressants in the novelty-suppressed feeding (NSF) task and chronic unpredictable stress paradigm. Recent studies in mice obtained similar results using a chronic corticosterone mouse model of depression (David et al. 2009). Studies using genetic manipulations to modulate neurogenesis levels corroborated some of these findings. Li et al. showed that inhibition of adult DG neurogenesis through conditional deletion of the gene encoding the neurotrophin receptor, TrkB, in neural progenitor cells in the adult DG rendered mice insensitive to effects of chronic antidepressant treatment in behavioral tasks of anxiety or depression (Li et al. 2008). Antidepressants are not the only mood-promoting interventions that appear to require adult hippocampal neurogenesis; the adaptive behavioral responses conferred by environmental enrichment during recovery from stress were also shown to be dependent on this form of neural plasticity (Schloesser et al. 2010).

Although the precise mechanism(s) by which new neurons mediate these antidepressant behavioral responses remains largely unknown, stress and the main stress hormone system, the hypothalamo-pituitary-adrenal (HPA) axis, have emerged as a potential functional link between adult-born neurons, mood regulation, and antidepressant treatment responses. Stress plays a key role in mood and anxiety disorders, with abnormalities in HPA axis activity, including loss of inhibitory feedback, often associated with these disorders (Holsboer et al. 1987; Nemeroff et al. 1984). The hippocampus serves as a regulator of the HPA axis, modulating the activity of the paraventricular nucleus (PVN) of the hypothalamus via a polysynaptic pathway providing negative feedback (Herman et al. 1995; Sapolsky et al. 1984; Herman et al. 1998; Herman and Mueller 2006; McEwen 2001). To test the hypothesis that adult-born DG neurons play a critical role in the hippocampal regulation of the HPA axis and mood regulation, several groups have inhibited adult neurogenesis in rodents by different means while measuring downstream effects on HPA axis activity and behavior at baseline, as well as under stress conditions. Surget and colleagues found that ablating hippocampal neurogenesis by targeted x-irradiation did not lead to deficits in HPA axis regulation in the context of uncontrollable chronic mild stress, but rather impaired the ability of fluoxetine to reverse these deficits. The authors also showed that following dexamethasone injections into the ventral DG, in combination with IEG-based circuit activation mapping, fluoxetine treatment reversed stress-induced disinhibition (overactivation) of the PVN. However, whether the fluoxetine effect required intact adult hippocampal neurogenesis was not

assessed. Thus, chronic stress impaired hippocampal regulation of several components of the stress response, and intact neurogenesis was critical for restoration of behavioral responses by antidepressant treatment.

In contrast to chronic stress, the effects of acute stress on HPA reactivity appear to be modulated by levels of adult hippocampal neurogenesis. Schloesser et al. conditionally suppressed adult neurogenesis by utilizing an inducible genetic approach where dividing progenitors in the adult brain were rendered sensitive to the antiviral drug valganciclovir (Schloesser et al. 2009). Following exposure to a mild stressor, valganciclovir-treated mice, in which adult neurogenesis was inhibited, exhibited higher corticosterone levels than mice with intact neurogenesis (introduction to a novel environment), but not at baseline. These results support a role for new neurons in hippocampal inhibition of the HPA axis under conditions of mild stress. In a complimentary study, Snyder et al. (Snyder et al. 2011) employed both a genetic approach and hippocampal-specific x-ray irradiation, to inhibit adult neurogenesis, revealing that HPA axis activity was heightened following stress (thirty-minute acute restraint stress), as measured by corticosterone levels in mice with suppressed adult neurogenesis. Consistent with neuroendocrine dysregulation, neurogenesis-deficient mice displayed depressive and anxious phenotypes in several behavioral paradigms—NSF, FST, and a test of anhedonia, the sucrose preference test (SPT)—immediately following an acute stress. Surprisingly, in some tasks, such as the FST and SPT, neurogenesis-deficient mice exhibited baseline deficits, indicating that depending on the paradigm and task, neurogenesis may play a role that extends beyond stress response regulation. Together, through both focal irradiation and genetic approaches, the above studies support a role for hippocampal regulation of the HPA axis and disruption of negative feedback onto the HPA axis under conditions of acute stress. While antidepressants, environmental enrichment, and exercise all promote adult DG neurogenesis, stress and glucocorticoids inhibit production and survival of adult-born neurons (Dranovsky and Hen 2006; Mirescu and Gould 2006). Indeed, stress, by inhibiting adult hippocampal neurogenesis, renders the hippocampal-HPA axis increasingly responsive to future stress. In the short term, this may be adaptive by mediating appropriate behavioral responses to stressors. However, over time, this may become increasingly maladaptive and give rise to increased stress responsiveness and depressive behaviors in the absence of threatening or stressful events. Antidepressant treatment may improve this dysregulation by promoting neurogenesis, thereby restoring the imbalance that initiated the maladaptive cascade. Because antidepressants exert multiple effects on neural circuitry besides increasing adult hippocampal neurogenesis (Duman and Monteggia 2006; Maya Vetencourt et al. 2008), it remains to be seen whether increasing adult neurogenesis is sufficient to modulate HPA functions. In this context, it is worth pointing out that stimulating adult hippocampal neurogenesis may not always be beneficial. Blockade of adult hippocampal neurogenesis was found to inhibit social avoidance behavior, suggesting that the timing of pro-neurogenic interventions may be critical to the directionality of their impact (Lagace et al. 2010). Moreover, the heightened plasticity of young adult-born neurons may make them more modifiable by stress, thereby amplifying the effector (stress), rather than curbing its effects.

This may be why stressors, such as social isolation, drive symmetric division of stem cells, rather than asymmetric division, to generate more neurons (Bonaguidi et al. 2011; Dranovsky et al. 2011; Song et al. 2012). As with pattern separation and pattern completion, one might ask whether the adult-born and developmentally generated neurons (mature neurons) have similar or distinct functions in mood regulation. Although chronic antidepressant treatment has been shown to accelerate maturation of adult-born neurons (Wang et al. 2008), a recent study made the intriguing observation that antidepressants also induce dematuration of mature dentate granule neurons (Kobayashi et al. 2008). Kobayashi et al. found that chronic antidepressant treatment of adult mice shifted the molecular and functional properties of a large population of mature granule cells. Four to five weeks of fluoxetine treatment in mice resulted in reduced expression of molecular markers of granule cell maturity, decreased activity-dependent gene expression, and a shift in electrophysiological properties to resemble a more immature state. They further went on to show that the dematuration effect of antidepressants may be mediated in part via 5-HT4 receptor signaling. Additional molecular, genetic, and behavioral studies are needed to determine the precise mechanism(s) of dematuration as well as to determine what role this process plays in the antidepressant-mediated behavioral response.

Discussion

Anxiety disorders, such as PTSD, can be viewed as maladaptive responses involving dysregulation of neural circuitry that evolved to generate fearfulness. Healthy individuals are able to regulate activation of fear and neuroendocrine responses as a function of their environments. In contrast, individuals with PTSD exhibit heightened neuroendocrine (hyperarousal) and behavioral responses (fear, avoidance) to neutral stimuli, resembling the aversive event even in the presence of cues that convey safety. Efforts to delineate mechanisms underlying PTSD have implicated deficits in extinction learning, associative learning, extinction learning recall, fear inhibition, and consolidation (Johansen et al. 2011; Jovanovic and Ressler 2010; Kearns et al. 2012). However, much less is known about encoding mechanisms that underlie the overgeneralization of fear. We recently proposed that an imbalance in pattern separation and pattern completion may result in overgeneralization of fear (Sahay et al. 2011a, b). Specifically, failure to distinguish between perceptually similar environments may result in excessive pattern completion, whereby minimal cues may trigger full and unusually vivid recall of the traumatic episodic memory, together with the associated emotional responses. The overactivation of the stress response in a similar "safe" environment may arise from a failure to suppress the HPA axis once the previous aversive memory is retrieved. Short periods of intense stress or trauma, a known risk factor for PTSD, may modify connectivity of the DG-CA3 circuit so as to produce an imbalance between pattern separation and pattern completion. Thus, increasing the number of young adult-born neurons may enhance pattern separation to modulate the excessive generalization seen in PTSD (Fig. 1).

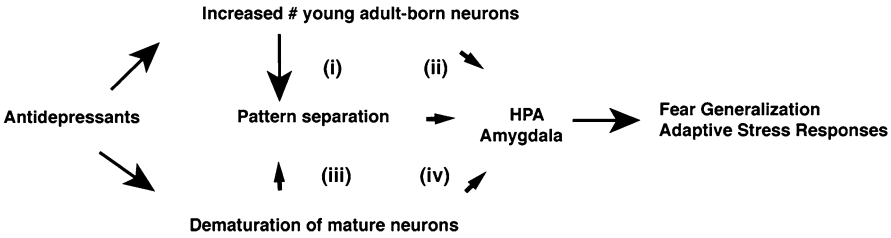

Fig. 1 Schematic showing the relationship between adult neurogenesis, antidepressants, dematuration, pattern separation, and mood regulation. Adult-born neurons may modulate fear generalization and adaptive stress responses through pattern separation-dependent and pattern separation-independent mechanisms (i and ii). The behavioral effects of antidepressants may require both increased adult hippocampal neurogenesis and dematuration of the mature DG neurons, and these effects may be mediated independent of pattern separation (iii and iv). *Large arrows* convey established links, whereas *small arrows* indicate relationships to be tested

In thinking about how antidepressants recruit adult-born neurons to mediate their behavioral effects, we must ask the following: How does the stimulation of neurogenesis by antidepressants give rise to changes in the PVN? It is tempting to speculate that new neurons might secrete growth factors that impinge upon interneurons (whose dendrites ramify across layers in the hippocampus), thereby affecting the properties of hippocampal subregions, such as the subiculum, to regulate HPA functions. Does increasing adult neurogenesis modulate activity or plasticity in the subiculum or other nodes in the stress regulation pathway, such as the bed nucleus of the stria terminalis or the septum? Does improving pattern separation also produce similar changes in the ventral subiculum to impact the HPA axis through these nodes? Given the differences in hippocampal connectivity along its septohippocampal axis with limbic structures, such as the amygdala, prefrontal cortex, and hypothalamus, do the effects of modulating neurogenesis on mood differ depending on whether the septal or temporal DG is targeted (Sahay and Hen 2007)? Furthermore, given that antidepressants, on the one hand, promote generation of new neurons, while at the same time causing dematuration of mature granule cells, it is interesting to consider whether these seemingly disparate processes might converge at the level of HPA axis regulation (Fig. 1). Should dematuration turn out to be a robust AD-induced effect, then one must ask whether dematuration of old (mature) dentate granule neurons makes them more similar to young adult-born neurons with regard to stress responsiveness and HPA modulation. The predicted effects of dematuration of mature neurons on pattern separation are not clear-cut. On one hand, dematuration may diminish pattern completion and enhance pattern separation by making the older neurons more juvenile-like. On the other hand, because dematuration results in decreased facilitation of mossy fiber-CA3 synapses, contextual encoding may be impaired. If so, antidepressants might engage pattern separation-independent mechanisms to produce changes in mood.

As these questions are addressed, results should be considered in the context of their relevance to depressive illness. For example, depression is not a hippocampal-specific disorder, but is associated with alterations in many brain regions, including

the prefrontal cortex, hypothalamus, amygdala, and nucleus accumbens (Krishnan and Nestler 2008), and it will be important to discern what role hippocampal neurogenesis plays in regulation of these regions. Additionally, in some individuals with depression, alterations in HPA axis regulation are not observed. Whether or not new neurons and antidepressant treatment play roles outside of stress response regulation to benefit these individuals will be important to determine. Despite these remaining questions, existing data provide insight into the role of adult-born neurons in regulation of mood and pinpoint the stress response pathway as one important means by which adult-born neurons may exert their effect. Understanding how adult-born neurons contribute to pattern separation and regulation of the stress response system is likely to provide a conceptual framework upon which to think about how the DG modulates mood. Gaining such an understanding holds the promise of development of novel therapeutic strategies to target depressive and anxiety disorders.

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Stem Cells and Neurogenesis in Relation to Dementia and Alzheimer's Disease Mouse Models

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Introduction

Dementia is a neurodegenerative disorder that results in progressive memory loss and cognitive deficits and affects millions of people in the Western world. The most common form is *sporadic* Alzheimer's disease (AD). It is characterized by a late onset and the gradual accumulation of β-amyloid peptide (Aβ)-containing senile plaques, which are derived from the amyloid precursor protein (APP). Also many neurofibrillary tangles (NFTs) are found that contain hyperphosphorylated tau protein and correlate well with cognitive decline. Early onset forms of AD exist as well, but these are rare and mostly familial. They are caused by mutations in the APP or presenilin 1 or 2 (PS1/2) genes, which eventually all result in the overproduction of the longer Aβ species. Alterations in intracellular APP processing by specific secretases are thought to cause accumulation of mainly the longer forms of beta amyloid (Aβ1-42/43) that are secreted and, over time, aggregate in extracellular amyloid plaques. As the toxic forms are most likely the oligomeric forms of Aβ that, prior to

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accumulation in extracellular plaques, may form already intracellularly, increasing attention is nowadays paid to a role for different forms of intraneuronal Aβ as well (Bayer and Wirths 2011, 2014; Gouras et al. 2010).

In addition to β-amyloid plaques, extensive numbers of neurofibrillary tangles are found in the AD brain. In AD, the neurofibrillary tangles are based on tau protein that is hyperphosphorylated at numerous sites by specific kinases. Protein tau is a microtubule-associated protein (MAP) involved in cytoskeletal stability and internal vesicle transport. Its hyperphosphorylation is thought to contribute directly to neuronal dysfunction. In support, the extent of neurofibrillary tangle pathology correlates well with cognitive decline in AD (Nelson et al. 2012), which is not the case for the amyloid plaque load, where considerable overlap exists between control subjects and AD patients (Musiek and Holtzman 2012; Perl 2010).

In addition to AD, frontotemporal dementia is a specific form of dementia that lacks amyloid deposits but is characterized by the select and abundant presence of neurofibrillary tangles. These "tangle-only" forms of dementia are caused by specific mutations in tau or tau-related proteins like progranulin (van Swieten and Heutink 2008; Seelaar et al. 2011). Together, the gradual accumulation in the brain of amyloid and tau neuropathology is thought to induce progressive neuronal dysfunction and degeneration, which eventually results in cognitive deficits, brain atrophy, and limited cell death in distinct subregions of the AD brain (Duyckaerts and Hauw 1997; Masters et al. 2006; Perl 2010; Ferrer 2012; Krstic and Knuesel 2013; Ferrer 2012).

AD is furthermore a very heterogeneous disorder with a wide variation in age of onset, disease duration, as well as in the extent of neuropathology between brain regions. In many instances, e.g., amyloid plaque load does not correlate well with the patients' symptoms, and despite considerable progress in understanding the biochemical, genetic, and molecular mechanisms underlying AD and despite promising trials aimed at inhibition of secretases or at vaccination against amyloid (Pul et al. 2011; Lambracht-Washington and Rosenberg 2013; Wisniewski and Goñi 2014), knowledge of the exact AD etiology is poor and effective treatment or prevention remains elusive.

While many of the current therapeutic strategies are aimed to slow down or stop the degenerative process of amyloid accumulation, novel strategies could focus on other substrates like tau and tangle pathology as well as on the regeneration of damaged tissue. This could take place by utilizing the therapeutic potential of stem cells that could in theory be introduced and/or recruited into damaged brain regions where they could be stimulated to differentiate into new neurons. The proper delivery of exogenous neural stem cells to restricted areas of the affected AD brain still remains a major challenge, but the discovery of ongoing neurogenesis and the presence of endogenous stem cells in the adult brain, and their regenerative potential, holds considerable promise for recruiting endogenous populations and potentially restoring neuronal populations and improving functional neural circuits.

As various studies have now shown that it is the local microenvironment that determines the neurogenic potential and properties of endogenous as well as exogenously transplanted stem cells, it will be critical to first obtain a better understanding of the effects of the different elements of the AD disease process and its

main mediators, on the endogenous stem cell population. In this chapter, we will therefore review the state of the art on stem cells in the brain and their responses in relation to Alzheimer pathology. We focus on hippocampal neurogenesis and available AD mouse models.

Hippocampal Neurogenesis

The hippocampus is well known for its involvement in cognitive processes, such as learning and memory (Morris et al. 1982; Jaffard and Meunier 1993), and is severely affected in the dementias. It is furthermore unique as it is one of the very few brain regions where neurogenesis continues to occur in adult individuals (Altman 1962). Stem cells located in the subgranular zone (SGZ) of the adult hippocampal dentate gyrus (DG) undergo extensive proliferation before they migrate into its granular cell layer. In young adult rats, approximately 9,000 new hippocampal cells are born per day. Many of these adult-generated cells die within the first few weeks (Dayer et al. 2003), due to a selection probably determined by local neuronal activity and trophic support (Deisseroth et al. 2004). Significant proportions of the new cells survive and differentiate in about 3–4 weeks into mature neurons. During this process, they are eventually incorporated into the adult hippocampal circuitry where they become functionally active and contribute to the properties of the DG network. Various molecular (Schouten et al. 2012) and epigenetic factors have been identified that modulate this process, e.g., in relation to Alzheimer-related factors (Fitzsimons et al. 2014; Mu and Gage 2011).

In addition to the DG, neurogenesis occurs in the subventricular zone (SVZ) of the lateral ventricle. Here, committed progenitor cells migrate via the rostral migratory stream (RMS) into the olfactory bulb (OB) where they differentiate into interneurons that are involved in olfactory discrimination learning (Gheusi et al. 2000; Alonso et al. 2006). The location of these two adult neurogenic zones in the hippocampus and the lateral ventricle wall, i.e., close to the corpus callosum and neocortex, indicates strategic positions for potential repair processes. However, the generation of new neurons is involved in cognitive function and could, therefore, also be influenced by disease pathology. Moreover, aberrant neurogenic responses or mechanisms could even be a part of the pathological events of neurodegenerative diseases, as will be discussed below.

Adult hippocampal neurogenesis is prominent in young rodents, but the amount of neuronal progenitors decreases over time (Kuhn et al. 1996; Bondolfi et al. 2004; Heine et al. 2004; Kronenberg et al. 2006; Montaron et al. 2006; Shetty et al. 2005; Jinno 2011) to low levels in middle-aged and particularly aged animals. Additional studies have shown that similar levels exist in older primates (Gould et al. 1999; Kornack and Rakic 1999). The very few studies on this subject indicate that the adult and elderly human brain is no exception in this respect: while the extent of neurogenesis and its different stages is difficult to study in a controlled manner in the postmortem human brain or under in vivo conditions, these numbers are likely

low and, as in rodents, appear to decrease with advancing age (Couillard-Després 2013; Manganas et al. 2007; Spalding et al. 2013; Knoth et al. 2010; Mirochnic et al. 2009; Eriksson et al. 1998; Curtis et al. 2011; Ho et al. 2013).

Regulation of Neurogenesis

The fact that neurogenesis occurs in many different animal species suggests an important functional role that is conserved throughout evolution. Interestingly, the process is not only changed with age but also highly susceptible to environmental- or experience-dependent modulation: voluntary exercise and environmental enrichment, e.g., are well known to change the in vivo fates of the newborn cells. Various studies have identified factors that can regulate production, maturation, and survival of the new hippocampal neurons during rodent adulthood. Some, like estrogen, environmental complexity, antidepressants, learning, physical exercise, and NMDA-related excitatory input, positively regulate neurogenesis, whereas factors like stress, cholinergic denervation, drugs of abuse, and aging decrease levels of neurogenesis (Marlatt and Lucassen 2010; Marlatt et al. 2012, 2013; Lee et al. 2012; Cooper-Kuhn et al. 2004; Mohapel et al. 2005; Lucassen et al. 2010; Schouten et al. 2012; Bruel-Jungerman et al. 2011; Zhao et al. 2008).

Although it seems somewhat counterintuitive, acute and chronic brain disorders or insults also stimulate the endogenous NSC population. For instance, head injury, epileptic seizures, and transient global and focal ischemia increase hippocampal proliferation and neurogenesis. The effects of insults on hippocampal circuit properties and characteristics of a slowly developing disorder like AD, however, are variable, depend probably on the stage of the disease, and are in general poorly understood (Winner et al. 2011; Coras et al. 2010; Gomez-Nicola et al. 2014; Perry et al. 2012). It is important to note that a variety of stimuli can affect different stages of the neurogenic process independently, e.g., each targeting specific populations of proliferating versus differentiating adult-generated cells.

Consistent with the important role of the hippocampus in cognition, many studies have found changes in adult neurogenesis to be paralleled by changes in hippocampal functional plasticity and/or cognition (Drapeau et al. 2007; Koehl and Abrous 2011). For example, housing rodents in an enriched environment, or allowing them access to a running wheel, not only increases the survival of progenitor cells, but also leads to an enhanced performance in the hippocampus-dependent water maze learning task (van Praag et al. 1999). Conversely, factors like stress, which are linked to a decrease in neurogenesis, impair behavioral performance on such tasks (Abrous et al. 2005; Brummelte and Galea 2010; Gould et al. 1999; Lucassen et al. 2010).

One of the most direct studies to address the relation between neurogenesis and cognition utilized an inducible transgenic model to ablate adult-born hippocampal neurons through expression of the proapoptotic gene *Bax* in hippocampal neural precursors. These mice showed impairment of spatial memory while less complex

forms of spatial memory were unaltered. Previous work has established that spatial learning requires different phases of cellular plasticity. Moreover, learning increases levels of newborn cells and their dendritic spines, which is associated with improved cognitive performance. Thus, cellular plasticity and neurogenesis contribute to various types of hippocampus-dependent learning and memory particularly when learning is challenging and difficult (Abrous et al. 2005; Curlik and Shors 2011; Dupret et al. 2007; Zhao et al. 2008; Cameron and Glover 2014).

One of the first demonstrations of a functional role for newly born cells was by a study that substantially reduced the number of newly generated granule cells using treatment with the antimitotic agent MAM, which also disrupted trace eyeblink conditioning and trace fear conditioning, both known to be hippocampus-dependent tasks (Shors et al. 2002; Anderson et al. 2011). Notably, the reduction in new neurons had no effect on learning during tasks that were hippocampus-independent. Additional studies showed a functional incorporation of adult-generated cells into the hippocampal circuit (van Praag et al. 2002), while hippocampal learning itself also increased neurogenesis, which depended on the difficulty of the task (Gould et al. 1999; Curlik and Shors 2011). Together, this directly implicates adult neurogenesis in hippocampal function. Subsequent studies utilizing irradiation or genetic modification aimed to eliminate adult neurogenesis in rodent models observed an impaired performance of the animals in spatial-navigation learning, spatial memory, spatial pattern discrimination, and fear conditioning tasks. Later studies revealed a reorganization of memory to extrahippocampal substrates, a role for adult neurogenesis in spatiotemporal learning and memory and the encoding of time in new memories with pattern separation as one of the most prominent tasks (Sahay et al. 2011; Clelland et al. 2009). Thus, neurogenesis is involved in several aspects of hippocampal function and may prove important in disorders associated with cognitive impairments, like AD (Abrous et al. 2005; Zhao et al. 2008; Oomen et al. 2014).

Regulation of Plasticity by Disease

In contrast to many of the stimuli mentioned above, and important for the context of this review, pathological alterations involving the hippocampal trisynaptic circuit can trigger changes in neurogenesis as well. Neurogenesis is e.g. modified by hippocampal and cortical damage. This includes acute excitotoxic, ischemic, or epileptic insults or by more gradual accumulation of aberrant proteins (Parent et al. 1997; Covolan et al. 2000; Blumcke et al. 2001; Jiang et al. 2001; Jin et al. 2001; Mu and Gage 2011). Whereas the extent and severity of the insult may modulate the extent of the neurogenic response, it is generally insufficient in functional terms as many of the newly formed cells turn into glial cells and/or contribute to the formation of scar tissue. In fact, in epilepsy, a popular concept even implicates neurogenesis in its etiology, assuming that the neurogenic response that occurs following the initial, and damaging, status epilepticus contributes to the occurrence of chronic epilepsy due to the fact that new neurons “rewire” inappropriately and form new contacts, such as new excitatory synapses on previously inhibitory synapses.

Thereby, neurogenesis may hamper the restoration of the damaged circuit and/or contribute to the emergence of chronic epilepsy and disease progression (Parent et al. 1997; Bielefeld et al. 2014), a possibility that may be of interest for AD etiology as well (Palop and Mucke 2010; Yan et al. 2012).

Some of the regulatory factors involved in the neurogenic responses could be growth-related peptides like BDNF, IGF-1, FGF-2, and VEGF, which are upregulated after ischemic damage (Kiyota et al. 1991; Gluckman et al. 1992; Plate et al. 1999; Schmidt-Kastner et al. 2001) and which are known stimulators of adult neurogenesis when studied in isolated conditions (Kuhn et al. 1997; Zigova et al. 1998; Aberg et al. 2000; Schänzer et al. 2004; Van Tijn et al. 2011; Marlatt et al. 2012, 2013). Much less is known about whether neurogenesis is also increased after *chronic* lesions, or during "slow" neurodegenerative processes, like those expected in AD, and in close relation to the neuropathology (Marlatt et al. 2014). To address the spatiotemporal characteristics of these responses, animal models have provided important tools, as will be discussed later.

Cell Cycle Markers in the Alzheimer Brain

Earlier studies had already suggested that cellular plasticity responses occur during AD. These were based on the presence of marker proteins selectively identifying specific stages of the cell cycle. Despite the fact that they were not expected to be present in the adult postmitotic brain, considerably more cells actively engaged in cell cycle were found in the AD hippocampus compared to control (Smith and Lippa 1995; Arendt et al. 1996; Kondratick and Vandré 1996; McShea et al. 1997; Nagy et al. 1997; Vincent et al. 1997; Busser et al. 1998; Yang et al. 2003). For instance, neurons containing neurofibrillary tangles in both the "dynamic" DG to which new neurons are added every day, as well as in the more "stable" cornu ammonis (CA) areas, co-express various cyclins, mitotic phosphoepitopes, and cyclin-dependent kinases (Smith and Lippa 1995; Arendt et al. 1996; Kondratick and Vandré 1996; McShea et al. 1997; Vincent et al. 1997; Busser et al. 1998). While on the one hand seen as endangered neurons that attempt to reenter the cell cycle (Smith and Lippa 1995; Kondratick and Vandré 1996; Busser et al. 1998; Herrup et al. 2004), cell cycle marker re-expression was on the other hand considered part of a more general regenerative process associated with neurogenic cells in the adult brain that depends on the "permissiveness" of the local environment.

Thus far, evidence from mouse models demonstrates that the induction of cell cycle changes after the onset of amyloid pathology is limited (Yang et al. 2006). This could depend on the limited age of the mice studied or on the artificial condition of transgene-controlled protein expression that is intrinsically different from the human situation. The re-expression of these markers in mature human neurons could also induce an abortive exit of the cell cycle or lead to cell cycle arrest, followed by lasting cellular dysfunction. Cell cycle protein expression in mature neurons is regarded as a maladaptive response of cells "stuck" in a cycle they cannot complete that is expected to precede cell death (Yang et al. 2003; Herrup et al. 2004).

Cellular Plasticity and Neurogenesis in the Human and Alzheimer Brain

After the initial studies using cell cycle markers, interest quickly turned to a possible role of stem cells and neurogenesis in the human brain. While BrdU pulse-chase and viral labeling have been instrumental in a better understanding of stem cell kinetics and regulation in rodents, such studies were not possible in living AD patients. Also, they were hampered by intrinsic difficulties with obtaining human postmortem tissue of sufficient quality but also by the lack of reliable makers to identify the different stages of the neurogenic process in postmortem tissue. Although specialized immunocytochemical markers, e.g., from the tumor research field, had been promising, the methodological issues of postmortem delay, specificity and fixation turned out to be not trivial. One example was doublecortin (DCX), a reliable and common marker to detect adult neurogenesis in rodents (Brown et al. 2003; Rao and Shetty 2004; Couillard-Despres et al. 2005). Unlike BrdU, detection of DCX does not require prior BrdU injections in living subjects, which made DCX a promising candidate marker. However, DCX, like many other microtubule-associated proteins (MAP) (Swaab and Uylings 1988), is very sensitive to degradation during postmortem delay (Boekhoorn et al. 2006a) and also labels subsets of astrocytes (Verwer et al. 2007). Hence, this marker has its drawbacks when used for detecting quantitative differences in neurogenesis in the human brain.

So far, only a few studies have reported changes in these and other young neuronal markers in human healthy or AD brain (Curtis et al. 2011; Winner et al. 2011; Zhao et al. 2008; Knoth et al. 2010). One report showed increases in various immature neuronal markers in a cohort of senile AD cases, suggesting that neurogenesis could be increased in AD (Jin et al. 2004b). In another study in younger, presenile patients, these results could not be replicated (Boekhoorn et al. 2006a). Although a significant increase in the number of Ki-67 positive, proliferating cells was found, quantification revealed that these cells were mostly located in nonneuronal compartments and associated with glial cells and the vasculature, while for proliferating cells located in neuronal layers, no differences were found between control subjects and AD patients. Additional causes for these discrepancies could be the age difference between the cohorts. Senile dementia is generally associated with a slower deterioration of cognition over time, whereas pathology in presenile dementia is often more severe, and reactions to hippocampal injury were thus expected to be more prominent in the younger group. Regardless of this, no indications were found for changes in cellular plasticity of neurons in presenile AD (Boekhoorn et al. 2006b) as was later confirmed for senile cases too (Marlatt et al. 2014).

Later studies have also used other markers like Musashi-1, nestin, and PSA-NCAM to show that neurogenic abnormalities in AD differ between phases and areas of neurogenesis and stages of AD: while hippocampal stem cells (Musashi-1) decrease, proliferation increases and differentiation/migration phase as well as axonal/dendritic targeting (DCX and β-III-tubulin) remain unchanged. This suggests an attenuation of stem cells together with compensatory increased proliferation that, however, does not result in an increased number of migratory neuroblasts and differentiated neurons in AD (Perry et al. 2012). Similar findings on microtubule-associated

protein isoforms showed that the mature high-molecular weight isoforms MAP2a and b were dramatically decreased in the AD dentate gyrus. The total amount of MAP2 protein, including expression of the immature neuronal marker, the MAP2c isoform, was less affected. These findings suggest that newly generated neurons in AD dentate gyrus do not become mature neurons, although proliferation is increased, confirming this general picture (Taupin 2009). Another study reported a decrease in DCX- and sex-determining region Y-box 2 (Sox2)-positive cells in human AD but an increase in bone morphogenetic protein 6 (BMP6) levels that was also found in APP transgenic mice, suggesting a role in defective neurogenesis in AD (Crews et al. 2010a, b).

In 1998, co-labeling of BrdU was shown with neuronal markers in human postmortem hippocampus in a unique patient cohort (Eriksson et al. 1998). Although this study was the first to show definite proof of adult neurogenesis in the human hippocampus, the methodology and cohort was unique and not suitable for experiments at larger scales in different populations. More recent methods used MRS spectroscopy (Manganas et al. 2007; Ho et al. 2013) or carbon dating in human postmortem tissue (Spalding et al. 2005, 2013). The latter technique takes advantage of the fact that during the 1950s and 1960s, radioactive atmospheric ^{14}C levels were increased due to nuclear testing and gradually declined after the ban in 1963. Similar to normal carbon, ^{14}C is stably incorporated into the DNA of dividing cells, and its level can accurately predict the age of any neuron derived from tissue. In this manner, it has been shown that in contrast to the cortex, a significant proportion of hippocampal neurons is born during adulthood (Spalding et al. 2013). In contrast to a common view in the field that adult hippocampal neurogenesis in the human brain should be a very limited phenomenon, this study demonstrated that about one-third of the hippocampal neurons present at birth is replaced during life and that the rate of neurogenesis in middle-aged individuals is comparable to that found in mice (Spalding et al. 2013). Also, extensive cell birth was identified in the human striatum (Ernst et al. 2014). So far, however, this technique has not yet been applied to AD material, but it could require definite proof as to how much neurogenesis is actually affected during AD.

Given the methodological pitfalls, the limited availability, presence of medication, and the variation between individual patients, together with the "end-stage" quality of human postmortem AD tissue, various research groups now focus on mouse models that overexpress AD-related proteins, like APP, PS, or tau (Crews et al. 2010a; Marlatt and Lucassen 2010; Mu and Gage 2011; Webster et al. 2014). Although aspects of redundancy, artifical overexpression of a transgene and indirect effects are of course important, these models recapitulate aspects of AD and FTD and provide a basis to address cause and effect and the temporal aspects of neurogenesis in response to AD neuropathology.

Neurogenesis and AD Mouse Models

The extent of neurogenesis may influence vulnerability to accumulating deleterious events, e.g., during aging, and as such may to some extent also reflect susceptibility to brain disorders like AD (Thompson et al. 2008; Zhao et al. 2008). Hippocampal neurogenesis is modulated by the expression of AD-related genes with the direction of the

effect often depending on the age and brain region under study, on the presence of neuropathology, and/or on the promoter used (Kuhn et al. 2007; Thompson et al. 2008; Crews et al. 2010a; Marlatt and Lucassen 2010; Mu and Gage 2011; Webster et al. 2014). In Alzheimer mouse models, APP, PS1, and APP/PS1 mutations generally cause reductions in neurogenesis when neuropathology is apparent, but also stimulatory effects have been reported but then mostly at earlier ages. Effects of mutated tau on neurogenesis are less well characterized or have been studied only postnatally and/or at young ages (Boekhoorn et al. 2006b; Sennvik et al. 2007).

In an attempt to link changes in neurogenesis to specific aspects of the disease, such as tauopathy or amyloid pathology, various mouse models of AD have been studied, either under naive conditions or under conditions when neurogenesis was modulated, e.g., by exercise, drugs, enrichment, or stress (Kronenberg et al. 2006; Hu et al. 2010; Cotel et al. 2012; Chadwick et al. 2011; Crews et al. 2010a; Marlatt and Lucassen 2010; Mu and Gage 2011; Marlatt and Lucassen 2010; Marlatt et al. 2010, 2013; Lazarov et al. 2005; Webster et al. 2014; Rodríguez et al. 2011). The majority of these have been APP- and/or PS1-based mouse models and, to a lesser extent, tau transgenic mice. Even though the exact cause of the age-associated decline in neurogenesis remains to be determined, the loss of growth factors from the local hippocampal microenvironment, such as FGF-2, IGF-1, BDNF, and VEGF, which are potent stimulators of adult hippocampal neurogenesis and neural stem cell growth in vitro, suggests a reduced neurogenic potential with age (Hattiangady et al. 2005; Shetty et al. 2005). This bears considerable relevance for AD itself, where many of these growth factors are reduced in their expression as well. Given the stimulatory effects of growth factors and of Aβ on stem cells in vitro, this could provide a putative mechanism for an impairment of neurogenesis in AD. Similar arguments hold for the prominent loss of cholinergic neurons and innervation in AD, which may contribute to impaired neurogenesis (Cooper-Kuhn et al. 2004; Mohapel et al. 2005; Bruel-Jungerman et al. 2011). Even though the exact function of APP remains elusive, PS1 has a well-established role in γ-secretase cleavage, and is also known for its prominent role in regulating β-catenin, a protein involved in Wnt signaling, which regulates hippocampal neurogenesis (Lie et al. 2005; Inestrosa and Varela-Nallar 2014). In addition, neurogenesis may be changed in AD mice due to a "loss of function" of normal APP and PS1.

In different transgenic models, adult neurogenesis is compromised in AD and generally precedes neuronal loss: dysfunctional neurogenesis, both decreased and increased, has been reported for AD transgenic models in both regions of adult neurogenesis, i.e., the SVZ and SGZ (Marlatt and Lucassen 2010; Mu and Gage 2011; Webster et al. 2014; Rodríguez and Verkhratsky 2011). Importantly, experimental conditions in these animal studies largely differ, depending on the use of transgenic expression of PSEN1, PSEN2, or of different APP single mutations, or knock-ins, or combinations thereof. Importantly, Aβ is not the only product of APP processing, and different APP metabolites may have different effects on different stages of neurogenesis (Mu and Gage 2011; Lazarov et al. 2010). For example, whereas the APP intracellular domain (AICD) negatively regulates proliferation and survival in the hippocampus, the soluble APP produced by α secretase may even stimulate neurogenesis (Ghosal et al. 2010; Demars et al. 2013; Winner et al. 2011). Therefore, the

net outcome will depend on the differential contributions of each APP metabolite produced within a certain model and how they are influenced by experimental conditions. In addition, BrdU regimens, doses, the time points analyzed after BrdU treatment, the genetic backgrounds of the mice, and the brain regions investigated vary considerably (Demars et al. 2010; Marlatt and Lucassen 2010; Lazarov and Marr 2010; Rodríguez and Verkhratsky 2011).

Furthermore, promoters determine expression in specific neuronal populations and thus the topographical distribution of the overexpressed transgene. For example, the use of the platelet-derived growth factor (PDGF) promoter results in the production of diffuse plaques, whereas prion protein and mouse thymocyte differentiation antigen 1 (mThy1) promoters favor plaque formation in the hippocampus and neocortex, etc. We will first discuss APP and PS1 mutant or deletion studies and then proceed to the discussion of tau mutant studies.

Amyloid Precursor Protein Transgenic Mice

Popular transgenic models of AD include mice expressing mutant APP. As single APP transgene, the FAD V717F (Indiana) mutation has negative effects on adult neurogenesis at an aged and symptomatic stage, mainly after amyloid deposition (Donovan et al. 2006). Double K670N M671L (Swedish) and triple (Swedish and Indiana) mutations of APP under many circumstances result in increased proliferation, and, in some cases, increased survival of the new neurons (Mirochnic et al. 2009; Haughey et al. 2002). Most earlier studies indicated that hippocampal neurogenesis is decreased in mice overexpressing the APP Swedish mutation that elevate Aβ (Donovan et al. 2006; Dong et al. 2004). In either an Aβ peptide injection model or in a mouse model expressing the APP mutant under control of the platelet-derived growth factor promoter (PDGF-APP), neurogenesis was found to be unaltered as long as Aβ pathology was absent, but its rate decreased as soon as plaque pathology developed (Haughey et al. 2002; Donovan et al. 2006). In contrast to the decreased number of dividing cells within the SGZ, PDGF-APP mice had significantly increased numbers of immature neurons in the outer portion of the granule cell layer. Whether the occurrence of these ectopic cells is due to abnormal APP function awaits further study, but changes in this subregion may at least explain some of the discrepancies with previous studies that combined all DG subregions in their quantitative analyses. Neurogenesis was also decreased in mouse models carrying three PS1 mutations (M146V, P117L, or A246E) (Wang et al. 2004; Wen et al. 2004; Chevallier et al. 2005). Also in a commonly used AD model, the triple transgenic AD mice (3xTg) harboring three mutant genes (PS1(M146V), APP(Swe), and tau(P301L)), decreased proliferation was found in male mice. This reduction in proliferation was directly associated with the occurrence of the first Aβ plaques and an increase in the number of Aβ-containing neurons in the hippocampus, which, in the case of 3xTg females, was directly correlated (Rodríguez et al. 2008).

In contrast to the abovementioned using PDGF-APP mice, Jin and colleagues have shown an increase in hippocampal neurogenesis in these mice that bear both the

Swedish and Indiana mutations (Jin et al. 2004a). These effects were found at 3 months of age, still in the absence of plaques, and at 1 year of age, at which time the hippocampus contained many plaques. Of importance, a considerable number of BrdU-positive cells in the hilus and molecular layer contribute to the total number of newborn cells that incorporate in the DG cell layer (Jin et al. 2004a; Donovan et al. 2006). These studies differ from others regarding the SVZ, where, at 3 months of age, no difference was found in the number of dividing cells; however, they detected a significant increase at 1 year of age (Jin et al. 2004a). These data suggest an opposite hypothesis, namely, that amyloid pathology, at least to the extent that it is derived from two different types of APP mutations, i.e., APPswe and APPind, increases neurogenesis. Alternative triggers, such as soluble or intraneuronal forms of amyloid, are still poorly studied in this respect.

In different strains of APPswe mutant mice, various cell cycle events were found to be increased (Yang et al. 2006), which resembles the situation in human AD where aberrant and ectopic expression of cell cycle markers has also been reported repeatedly. In contrast to the conclusion of Jin et al. (2004b), an alternative explanation is that expression of cell cycle markers selectively occurs in cells destined to die. In addition, this suggests that amyloid not only affects cell division but also survival of neurons.

Together, these studies suggest that the emergence of Aβ in the early stages of the pathology decreases, rather than increases, neurogenesis in mice (Jin et al. 2004a; Yang et al. 2006). It is important to note that changes in neurogenesis further depend on the pathological state of the AD-related protein, be it aggregated or mutated, or overexpressed (Haughey et al. 2002; Donovan et al. 2006), an assumption that was supported by Wen et al. who reported increases in hippocampal neurogenesis in a cohort of mice overexpressing the wild type but not mutated (P117L) form of PS1 (Wen et al. 2002).

Presenilin 1 Transgenic Mice

Interest in γ-secretase, the enzyme that generates highly fibrillogenic Aβ42, has led to the development of PS1 transgenic mice expressing mutant PS1. While PS1 is part of the γ-secretase complex, this pleiotropic gene also participates in mechanisms regulating cellular proliferation. PS1 is a key regulator in e.g., Notch and Wnt signaling mechanisms, but there is no direct evidence demonstrating that familial PS1 can influence proliferation or survival of NPCs in humans. PS1 signaling is responsible for the developmental maturation of glia and neurons. In Wnt signaling, PS1 is directly involved in β-catenin turnover, a mechanism responsible for proliferation of progenitor cells in the developing brain (Inestrosa and Varela-Nallar 2014). Normal PS1 facilitates phosphorylation of β-catenin, which leads to proteosomal degradation; mutant PS1 cells have an increased stability of β-catenin that leads to downstream nuclear signaling events. It is therefore not surprising that neuronal expression of mutant PS1 using a Thy1 promoter increased cell proliferation in the DG of 4-month-old transgenic mice. An increased cell proliferation did not result, however, in, an increased neuron survival in the hippocampus of these mice (Wen et al. 2002, 2004).

In a follow-up study, the same authors found decreased neurogenesis in mice overexpressing mutant PS1, whereas no effect was found of WT overexpression (Wen et al. 2004). The only difference with the previous study was that now older mice were used. Hence, the effects of WT PS1 on neurogenesis are either positive or neutral, whereas mutated PS1 had a neutral or negative effect on neurogenesis, with a clear age-dependency for the neurogenic effects of PS1.

Animal models for AD based on familial PS mutations (Elder et al. 2010) show elevated generation of Aβ42. Chevallier et al. (2005) used PS1 A246E mutant mice and determined an increased proliferation of subgranular progenitor cells in the DG, but only 25 % of the newly generated cells survived after four weeks. In the PS1 M146V knock-in mice, Wang et al. (2004) observed that neurogenesis was decreased as supported by decreases in proliferation, differentiation, and survival of precursor cells, while the P117L mutation also decreases neuronal differentiation of embryonic murine neural progenitor cells (Wen et al. 2004; Eder-Colli et al. 2009).

Mice deficient for both PS1 and PS2 were found to have increased proliferation and survival when evaluated at two ages (Chevallier et al. 2005). A study of PS1 expressed under the neuron-specific enolase (NSE) promoter found that cell proliferation was reduced by both wild-type and mutant P117L PS1 (Wen et al. 2004; Eder-Colli et al. 2009). Interestingly, wild-type PS1 mice had increased survival of immature neurons, whereas the mutants did not. A follow-up to this study incorporated groups with environmental enrichment and found that expression of the wild-type protein was sufficient to increase survival of immature neurons. Enriched environmental housing in these mice increased proliferation and newborn cell survival compared to the non-enriched group. This normal physiology was not preserved in mice expressing mutant PS1; enrichment increased proliferation, but there were no changes in Tuj1 expression and lower numbers were found of less surviving BrdU-positive cells. A more sensitive experiment was produced by crossing mutant PS1 M146V knock-in mice with PS1-deficient mice. Investigators generated mice with one mutant copy of PS1. Expression of mutant PS1 resulted in impaired learning in a contextual fear conditioning test. This impaired associative learning was positively correlated with impaired neurogenesis. The investigators, by comparing with the parental knock-in line, concluded that expression of wild-type PS1 can override the mutant PS1 gene. Although the expression of human PS1 transgenes does impact on neurogenesis, it is difficult to assess whether behavioral changes are due to increased neurogenesis or to the expression of the transgene per se (Wang et al. 2004; Elder et al. 2010).

APP/PS Bigenic Mice

As murine APP does not generate fibrillogenic peptides, typically bigenic mice are generated that express mutant PS1 and human mutant APP. As reviewed before, most APP and APP/PS1 mouse models show reductions in cell proliferation (Lazarov et al. 2010; Marlatt and Lucassen 2010; Mu and Gage 2011. A study evaluating mutant APPswe and APP-PS1L166P mice showed that APP mice had no difference in hippocampal neurogenesis when evaluated by BrdU incorporation at

5 months of age. At this age, the mice do not have amyloid deposits, but when evaluated at 25 months of age, APP mice exhibited significant increases in the number of BrdU- and DCX-positive cells (Ermini et al. 2008). A separate study utilizing different APP-PS1 mice at 8 months of age showed increased BrdU- and NeuN-positive cells compared to controls despite findings that APP-PS1x nestin-GFP mice exhibited decreases in nonproliferative, nestin-positive NPCs (Gan et al. 2008). Hence, endogenous neurogenesis appears to be elevated early in response to pathology, and to later decrease again. However, the molecular mechanisms and functionality of these new neurons remains unclear.

Aβ pathology reduces neurogenesis in several mouse lines (Haughey et al. 2002; Dong et al. 2004; Wang et al. 2004; Donovan et al. 2006), and, with the exception of in vitro data (Lopez-Toledano and Shelanski 2004), that lack true neuropathology (Feng et al. 2001; Caille et al. 2004; Yasuoka et al. 2004), many of these studies are consistent with the hypothesis that AD initially stimulates neurogenesis, and later decreases it, coinciding with the accumulation of amyloid pathology. Considering the roles of both APP and PS1 in embryonic development, e.g., as regulators of Wnt signaling (Caricasole et al. 2003; Chevallier et al. 2005; Wines-Samuelson and Shen 2005; Chen and Tang 2006), both genes are likely to be of general importance during both developmental and adult neurogenesis. Therefore, stimulatory effects of APP on increased neurogenesis could reflect a delayed or repeated developmental role rather than a pathological one. A developmental role is further supported by the fact that the total number of neurons was increased at 8 months of age (when no pathology is present) in the neocortex of APP23 mice overexpressing the Swedish mutation. However, at 27 months of age, these mice have developed a considerable plaque load that negatively correlated with the number of neurons (Bondolfi et al. 2002). In a related model, also cortical changes were found (Lemmens et al. 2011).

In other studies, Zhang et al. (2007) used APP, PS1, and both APP-PS1 mutants and only observed diminished neurogenesis in the double knock-in mice (Zhang et al. 2007). Jin et al. (2004a) observed an increased neurogenesis in PDGF-APP(Sw,Ind) mice, which express human APP isoforms APP695, APP751, and APP770 with the FAD's Indiana (V717F) and Swedish (K670N M671L) mutations driven by a platelet-derived growth factor promoter. They observed elevated neurogenesis in AD mice, which suggested that a compensatory mechanism may be active and that neurogenesis is increased in the early phases in response to emerging pathology, consistent with findings of others (Yu et al. 2009).

Tau Transgenics

Compared to the many studies on neurogenesis in relation to amyloid pathology, remarkably few studies have addressed a link with protein tau. This is striking since an extensive in vitro literature suggests a prominent role for tau during neuronal development, cytokinesis, neuronal maturation, and neuritic outgrowth (Gonzalez-Billault et al. 2002). Furthermore, tau phosphorylation occurs not only in AD but also during mitosis (Cross et al. 1996; Delobel et al. 2002). Moreover, many of the cell

cycle alterations seen in AD have been linked to tangle pathology (Arendt et al. 1996; Smith and Lippa 1995; Kondratick and Vandré 1996; Busser et al. 1998; Herrup et al. 2004). In vivo, the tau mutation P301S was found to be associated with overexpression of the cell cycle-dependent kinase inhibitors p21/Cip1 and p27/Kip1 (Delobel et al. 2006). Using a knockout–knock-in approach, it was further shown that expression of four repeats (4R) of tau reduces cell proliferation and increases differentiation and neuronal maturation, confirming an important role for tau in neuronal plasticity and differentiation (Sennvik et al. 2007). Moreover, the tau P301L mutation modulates cyclins, inducing cell cycle arrest in the G2 and M phases. In young tau P301L mutant mice, however, no effects were found on neurogenesis despite significant increases, in stead of possible decreases, in long-term potentiation and improved cognitive performance (Boekhoorn et al. 2006b). These data suggest that in the absence of age-related accumulation of tau phosphorylation, this familial tau mutation per se may not impair learning and memory, but rather improve cognition at young ages. Thus, tau protein may play an important beneficial role in hippocampal memory. Conversely, it is most likely not the mutation in tau, but rather the ensuing hyperphosphorylation, that is responsible for the cognitive decline observed in tauopathies (Sennvik et al. 2007; Fuster-Matanzo et al. 2009, 2012).

In a model utilizing human tau with two mutations, induction of hyperphosphorylation and NFTs was found in the hippocampus of in 3–6-month-old animals. Cell bodies of the DG are spared at this young age, but neurites in these areas were immunopositive for the antibody AT8, indicating aberrant phosphorylation of tau, similar to what is found in AD. Compared to non-transgenic mice, transgenic tau mice had twofold higher DCX levels and significantly higher expression of TUC-4 in the DG through 6 months. Mice overexpressing nonmutant human tau also show signs of proliferation; however, this proliferation was identified outside the SGZ and SVZ.

Taken together, the abovementioned data suggest that overexpression of wild-type or mutated tau is unlikely to promote neurogenesis. However, reduced tau expression may be associated with increased neurogenesis, at least within a specific postnatal period (Sennvik et al. 2007), and consistent with an inhibitory role of tau during mitosis, as suggested by others (Delobel et al. 2002, 2006). The fact that tau can inhibit neurogenesis does not imply that neurogenesis is inhibited in AD, where overexpression and aberrant expression of mutated forms of tau occurs. In AD, a large proportion of tau is thought to be hyperphosphorylated, which may lead to reduced microtubule binding and could, therefore, result in reduced tau functioning, which in turn, could lead to increased neurogenesis. While altered APP processing is likely to decrease neurogenesis, increased tau phosphorylation may actually result in the opposite effect. To test these hypotheses, it would be interesting to study the effect of tau phosphorylation on mitosis and neurogenesis in vitro and in vivo.

Environmental Stimulation of Neurogenesis in AD Models

The relationship between Aβ and neurogenesis has also been studied in combination with interventional studies. Environmental enrichment or wheel running in AD mouse models was expected to stimulate neurogenesis, parallel to behavioral

improvements and possible reductions in Aβ plaque load. In some models, environmental enrichment indeed increased newborn cell proliferation, survival, and neurogenesis. These changes corresponded to improved performance in a spatial memory task, but surprisingly, there was often no change in plaque load. Clearly, the neurogenic environment is preserved and permissible, which may allow options for functional recovery. Curiously enough, however, this recovery dissociates structurally from the functional pathology introduced in AD mice (Lazarov and Marr 2010; Marlatt et al. 2013).

Under normal conditions, environmental enrichment generally increases hippocampal neurogenesis; however, in one of the first kind of such studies, i.e., in PS1 knockout mice, which produce less amyloid, hippocampal neurogenesis could not be stimulated (Feng et al. 2001). Interestingly, this was paralleled by neuronal atrophy, increased astrogliosis, and associated with a reduced clearance of hippocampal memory traces. Enriched environment in APP and PSEN1 transgenic mice not only improved memory function but also reduced Aβ deposition (Lazarov et al. 2005), rescued impaired neurogenesis, and significantly enhanced hippocampal LTP in APPswe/PS1DE9 mice (Hu et al. 2010; Lazarov et al. 2005). However, this depends on the mouse models and ages used. The APP/PS1KI mouse model, e.g., failed to show significant improvement after 4 months of continuous enrichment (wheel running activity together with social enrichment), possibly because the mice were not exposed to the enriched environment until after disease onset (Cotel et al. 2012). Physical exercise alone improved cognitive performance in transgenic mouse models of AD (Nichol et al. 2007, 2008).

When both wheel running and enriched environmental housing were combined, the number of newborn granule cells in the DG of APP23 mice was increased and their water maze performance improved (Mirochnic et al. 2009; Wolf et al. 2006). However, environmental enrichment does not enhance neurogenesis in transgenic mice harboring FAD-linked PS1 variants or in forebrain-specific PS1 knockout mice, and it even suppresses neurogenesis in apolipoprotein E (ApoE) epsilon 4 transgenic mice. Taken together, these studies indicate that the effects of exercise and environmental enrichment on adult neurogenesis vary between the mouse models of AD (Lazarov et al. 2010; Lazarov and Marr 2010; Marlatt and Lucassen 2010).

In conclusion, different mouse models of different aspects of AD pathology have shown robust and transient increases in adult cytogenesis or neurogenesis, often parallel to the onset of pathology. The effect on neurogenesis of increased or mutated Aβ production appears to depend on two factors: the developmental stage of the animal and the presence or absence of pathology. Obviously, these two parameters are not independent of each other, since most APP or PS1 mutant mice show increased pathology with age. Altered APP or PS1 expression can increase neurogenesis in younger animals when Aβ pathology is still absent; however, it decreases neurogenesis in later stages when Aβ pathology is present.

Most increases in cytogenesis in AD mice are nonspecific and likely involved in gliogenesis and will at least not result in acute functional neuronal recovery, possibly because the microenvironment at the age when AD pathology becomes apparent is no longer permissive enough to support stem cell proliferation or neuronal differentiation (Doorn et al. 2014; Marlatt et al. 2014).

Utilizing neurogenesis for healthy aging would require its occurrence and stimulation over longer durations. Hence, it may perhaps be most beneficial if activity is established and maintained from midlife (or earlier) onwards to preserve adult neurogenesis prior to the onset of AD neuropathology or clinical presentation with dementia or AD. As neurons born in aged mammals are just as functional as the ones generated during developmental neurogenesis in young mammals, maintenance of stem cell proliferation and of the local microenvironment that enables a proper migration and connection is necessary to fully understand the dynamics of the neurogenic niche during aging and AD.

If we were to translate the data from animal models to the human familial AD situation, one would expect neurogenesis to be decreased rather than increased, with APP and/or PS1 as risk factors. Clearly, this contrasts from recent literature where plasticity markers were reported to be either increased or unaffected in a sporadic, senile cohort (Jin et al. 2004b) or in a presenile cohort (Boekhoorn et al. 2006a) respectively, possibly depending on the stage and severity of AD (Gomez-Nicola et al. 2014; Brain 2014; Enikomou et al. 2014; Biol Psych 2014). Aside from different methodologies, one obvious explanation could be that human AD pathology is more complex than altered APP expression alone, and, e.g., also other pathological changes such as in tau and, e.g., a longer disease duration and differences in metabolism between human and rodent brain are implicated. Moreover, although APP pathology may not directly stimulate neurogenesis, the resulting neuronal dysfunction, damage, and cell loss could later increase cell birth in an indirect manner, similar to brain injuries like ischemia.

Preventive Strategies for AD

Although there is currently no cure for AD, significant progress has been made in defining lifestyle conditions that promote healthy brain aging and, to some extent, delay the onset of AD. In clinical studies, poor social interaction, lack of physical exercise, malnutrition, and lack of cognitive stimulation have been singled out as risk factors of AD onset and progression (Laurin et al. 2001; Bennett et al. 2006; Scarmeas et al. 2006; Sitzer et al. 2006). In parallel, experimental studies found positive effects of enriched environment, physical exercise, and caloric restriction on accumulation of plaques in transgenic AD models (Adlard et al. 2005; Lazarov et al. 2005; Patel et al. 2005; Wolf et al. 2006). Although the functional link to neurogenesis and other forms of structural plasticity is not fully established, it is intriguing to note that most all of these lifestyle factors are prominent stimulators of adult hippocampal neurogenesis (Kempermann et al. 1997, 1998; van Praag et al. 1999; Lee et al. 2000) as well as other forms of plasticity (Rosenzweig 1966; Cotman and Berchtold 2002; Chen and Blurton-Jones 2012, Mattson et al. 2001, 2003). It is, therefore, crucial that patients are made aware of the beneficial effects of these lifestyle parameters on neuroplasticity and disease onset, even if a definitive proof of a role for neurogenesis in AD has not yet been provided.

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Hippocampal Neurogenesis in Neurodegenerative Movement Disorders

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Introduction

One of the characteristics of adult neurogenesis in the mammalian brain is the ability to generate new neurons throughout life. This cellular element of brain plasticity in adulthood was first reported by Altman and colleagues (Altman and Das 1965; Altman 1969) and extensively studied over the past 40 years in the brains of various mammalian species, in particular in rodents (Cameron et al. 1993; Kuhn et al. 1996) and humans (Eriksson et al. 1998; Curtis et al. 2003). These findings overturned the long-held dogma that the mammalian brain is incapable of generating new neurons. However, this process is not ubiquitous and occurs only in two distinct brain regions that harbor neural stem and precursor cells, primarily the subventricular zone (SVZ) adjacent to the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG). Adult neurogenesis involves several crucial steps, including asymmetric cell division of stem cells, resulting in daughter stem cells and cells with the potential to develop into neurons. These newly generated neuroblasts migrate to their final destination in the brain, and the new neurons mature and integrate by forming efferent and afferent connections with neighboring cells (reviewed in Zhao et al. 2008). These newly generated neurons exhibit electrophysiological properties of functional neurons, connect with neighboring cells, and

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integrate into existing neuronal circuits (Van Praag et al. 2002; Carleton et al. 2003). Neuroblasts from the SVZ migrate along the rostral migratory stream to the olfactory bulb (OB) and develop into mature and functional interneurons, specifically granule and periglomerular neurons, which contribute to specific olfactory learning processes (Alonso et al. 2012). Adult neurogenesis in the DG involves the proliferation of neural stem and precursor cells in the SGZ and the migration of newly generated neuroblasts into the granular cell layer of the DG, as well as the differentiation into mature neurons that functionally integrate into existing circuits of GABAergic and glutamatergic neurons in the hippocampal formation, in particular forming glutamatergic synapses on CA3 neuronal dendrites (Sandler and Smith 1991; Van Praag et al. 2002). Many aspects of modulation and regulation of adult neurogenesis have been identified, including a variety of transcriptional regulation and signaling pathways, as well as environmental factors, age, and acute and chronic diseases, such as neurodegenerative diseases (Ma et al. 2010; Mu et al. 2010).

Importantly, the hippocampus is critical for certain aspects of the formation of new memories and the acquisition of new skills (Mayford et al. 1996; Morgane et al. 2005; Bruel-Jungerman et al. 2007). A contribution of newly generated DG neurons to memory formation and spatial pattern separation appears very likely (Clelland et al. 2009; Sahay et al. 2011), and loss of hippocampal neurogenic function might have consequences on memory formation. In addition, increasing evidence has revealed a correlation between impaired generation of new neurons in the hippocampus and depression (Samuels and Hen 2011). The generation of new DG neurons is experience dependent, and social isolation impairs hippocampal neurogenesis (Dranovsky et al. 2011). Moreover, the ablation of adult neurogenesis increases anxiety-related behavior, while increased neurogenesis in the DG enhances contextual fear discrimination (Revest et al. 2009; Sahay et al. 2011). Therefore, several studies have proposed an important role of hippocampal neurogenesis in depression, based on data showing increased hippocampal neurogenesis by antidepressant treatment, as well as functional improvements (Malberg et al. 2000; Santarelli et al. 2003; Surget et al. 2008). Chronic treatment of several antidepressant agents was shown to increase DG neurogenesis (Santarelli et al. 2003; Miller et al. 2008), while the specific mechanisms remain elusive (Encinas et al. 2006; Marlatt et al. 2010). In particular, selective serotonin reuptake inhibitors (SSRI), or antidepressants that increase serotonin levels, were used to stimulate DG neurogenesis and ameliorate symptoms in models of depression-like behavior (Santarelli et al. 2003; David et al. 2009). A recent study described that some serotonin receptors are expressed in—or in close proximity to—the SGZ of the DG. In addition, hippocampal cell proliferation and survival of new neurons is differentially regulated by signaling via these serotonin receptors (Klempin et al. 2010). These findings suggest that dysregulation of the serotonergic system might contribute to depressive symptoms, as well as impaired hippocampal neurogenesis.

It has been suggested that compromised cellular plasticity in the hippocampus might, in part, be associated with various neurodegenerative diseases (Winner et al. 2011b). Interestingly, in Parkinson's disease (PD) and Huntington's disease (HD), specific alterations in neurogenic areas, such as the DG and SVZ/OB system, exist.

It is intriguing that early or premotor symptoms observed during the early stages of these diseases, such as depression, anxiety, cognitive impairments, or olfactory dysfunction, are related to impaired hippocampal neurogenesis (Simuni and Sethi 2008). Therefore, the mechanisms of neurodegenerative diseases are closely linked to brain plasticity. In slowly progressing neurodegenerative diseases, neurons are affected by neuronal dysfunctions involving synaptic transmission, synaptic contacts, and axonal and dendritic degeneration. Moreover, in different neurodegenerative diseases, neurite degeneration and neuronal loss occurs within specific neuron populations. In addition, the number of functional neurons in neurogenic regions, as well as adult neurogenesis, is altered or decreased. Brain regions differ in their vulnerability to different disease processes; the DG of the hippocampus is a region that is very susceptible to neurodegenerative mechanisms (Braak et al. 2003; Pavese et al. 2010; Carlesimo et al. 2012). In age-related hereditary and sporadic neurodegenerative diseases, it might sound like a paradox to study endogenous cellular plasticity that physiologically declines with age. Nevertheless, it has become apparent that disease processes in PD, as well as in HD, start early and many years prior to the occurrence of typical motor symptoms (Stout et al. 2011; Hinnell et al. 2012). In addition, during nervous system development, the turnover of axons, dendrites, and synapses is a common response to acute or chronic injury (reviewed in Luo and O'Leary 2005). Similarly, the generation and death of newly generated cells play important roles in development and maintenance in the adult brain, and alterations in these processes are observed in neurodegenerative diseases. In general, chronic neurodegeneration exhibits different impacts on stem cell maintenance, proliferation, survival, and functional integration. In particular, the fate of newly generated neurons 'in a diseased brain' is an interesting model system to study early cellular and neuritic alterations in neurodegenerative diseases, hopefully leading to a better understanding of the initial pathogenic events in these diseases. Therefore, we will review studies that have analyzed adult hippocampal neurogenesis in two important sporadic and hereditary movement disorders, and will describe recent findings.

Hippocampal Neurogenesis in Parkinson's Disease

Nonmotor Symptoms in PD

Parkinson's disease (PD) is the second most common neurodegenerative disease and affects approximately five million people worldwide, with rising prevalence due to increased life expectancy (de Lau and Breteler 2006). The neuropathological hallmark of PD is the accumulation of α-synuclein (α-syn) (Spillantini et al. 1998). Although a physiological role for α-syn has been proposed in developmental plasticity and synaptic remodeling (Nemani et al. 2010), α-syn accumulates as intracellular deposits in Lewy bodies and Lewy neurites in PD (Spillantini et al. 1998). Mutations in the human α-syn gene (e.g. A53T, A30P, and E46K) (A53T, A30P, and

E46K) are observed in rare autosomal-dominant forms of PD (Polymeropoulos et al. 1997; Kruger et al. 1998; Zarranz et al. 2004). Motor symptoms in PD, i.e., bradykinesia, rigidity, resting tremor, and postural instability, are predominantly linked to the degeneration of dopaminergic neurons in the substantia nigra.

The occurrence of nonmotor symptoms, such as depression, cognitive deficits, and autonomic dysfunction, is likely associated with other neurotransmitter systems (serotonergic, noradrenergic, and cholinergic). These profound nonmotor symptoms occur early in the disease course and have a strong impact on quality of life (Hinnell et al. 2012). In particular, depression is an important nonmotor symptom and present in up to two-thirds of all PD patients (Gallagher et al. 2010). Depression severely affects quality of life measures in PD patients, even exceeding the impact of motor symptoms (Tolosa and Poewe 2009; Hinnell et al. 2012). Moreover, large-scale studies found specific early cognitive impairments in up to 20 % of untreated PD patients (Aarsland et al. 2009), and in advanced stages, more than 80 % of patients exhibited cognitive disturbances (Hely et al. 2008). Therefore, it is important to note that recent imaging studies in PD patients reveal early affection of the hippocampus in the disease process (Weintraub et al. 2011; Carlesimo et al. 2012). As these important nonmotor symptoms of PD were attributed to cortical and hippocampal α-syn pathology (Mattila et al. 2000; Ferrer et al. 2011), it became even more important to study adult hippocampal neurogenesis in transgenic models of PD.

Cellular Plasticity and Neurogenesis in the Hippocampus of Transgenic Animal Models of PD

While toxin-induced PD rodent models focus mainly on dopaminergic pathology (reviewed by Blandini and Armentero 2012), transgenic animals became an important tool for studying nonmotor symptoms and the impact of potential disease modifiers. Transgenic mice with mutated or overexpressed PD genes are of particular interest for these purposes. Specifically, the genes encoding for α-syn, leucine-rich repeat kinase 2 (LRRK2), parkin, PINK1, and DJ-1 have been studied in transgenic animals (for review see Dawson et al. 2010). The majority of these models display progressive neuropathology and exhibit specific functional, nonmotor deficits observed in PD, although no significant nigrostriatal degeneration has been obtained. Nevertheless, the α-syn models provide important insights into cellular and molecular mechanisms associated with α-syn and its relevance for neuronal dysfunctions in PD (reviewed by Rockenstein et al. 2007; Kahle 2008).

Adult neurogenesis analysis in different PD animal models revealed distinctly impaired proliferative activity and survival of newly generated neurons (reviewed in Winner et al. 2011b, and Marxreiter et al. 2012). Results from adult hippocampal neurogenesis studies provide an interesting approach for studying disease mechanisms in PD on the cellular level, in particular for nonmotor symptoms like depression and cognitive impairment. Because adult hippocampal neurogenesis was primarily characterized in transgenic α-syn and LRRK2 models, we will focus on these models (Table 1).

Table 1 Adult hippocampal DG neurogenesis in transgenic PD models

Genotype/transgene	Promoter	Age	Proliferation (SGZ)	Neuroblasts	Neuronal differentiation	Survival	Cell death	References
α-synuclein hWT	PDGF	4 M	⇔ PCNA + cells	⇓ DCX + cells	⇔ % BrdU+ / NeuN + cells	⇓ BrdU+ / NeuN + cells	⇑ TUNEL + cells	Winner et al. (2004), Crews et al. (2008)
α-synuclein mutant A53T	PDGF	4 M	⇓ PCNA + cells	⇓ DCX + cells	⇔ % BrdU+ / NeuN + cells	⇓ BrdU+ / NeuN + cells	⇑ TUNEL + cells	Crews et al. (2008), Kohl et al. (2012)
α-synuclein hWT inducible	CaMK	4 M	⇔ PCNA + cells	⇓ DCX + cells	⇔ % BrdU+ / NeuN + cells	⇓ BrdU+ / NeuN + cells	N.D.	Nuber et al. (2008)
LRRK2 G2019S mutant	BAC	4 M	⇓ BrdU + cells	⇓ DCX + cells	⇔ % BrdU+ / NeuN + cells	⇓ BrdU+ / NeuN + cells	N.D.	Winner et al. (2011c)

BAC bacterial artificial chromosome, *BrdU* bromodeoxyuridine, *CaMK* calcium/calmodulin-dependent protein kinase II alpha, *DCX* doublecortin, *M* months, *NeuN* neuronal nuclear antigen, *N.D.* no data, *PCNA* proliferating cell nuclear antigen, *PDGF* platelet-derived growth factor-ß, *TUNEL* Tdt-mediated dUTP-biotin nick end labeling

α-syn has been identified in several species, indicating an evolutionary conserved role for this molecule. The modulation of α-syn has been specifically attributed to neuronal remodeling, namely, songbird learning. Presynaptic localization of α-syn further supports a direct physiological role in neuronal plasticity. Although not essential for cell survival or synapse formation, α-syn plays an important role in presynaptic dopamine recruitment and synaptic transmission (Iwai et al. 1995; Abeliovich et al. 2000). To investigate the impact of α-syn on adult neurogenesis, it was important to analyze physiological functions during the generation of new neurons in the adult brain. Physiologically, α-syn and β-synuclein (ß-syn) co-localize in presynaptic nerve terminals and are highly co-expressed, indicating redundancy (Li et al. 2002). Recently, we used double α/ß-syn knockout mice (Chandra et al. 2004) to investigate adult neurogenesis. The downregulation of α/ß-syn resulted in an increased percentage of new neurons in the hippocampal DG. These results suggested increased neuronal differentiation, although all other parameters studied (total number of dividing cells, proliferation, and cell death) were not altered (Winner et al. 2012). To elucidate the mechanisms of α-syn-mediated neurodegeneration in PD, transgenic α-syn models, exhibiting intensive overexpression of α-syn in the cortex and/or hippocampus, were used (Masliah et al. 2000; Hashimoto et al. 2003; Nuber et al. 2008).

The first transgenic mouse model carrying human wild-type (wt) α-syn under the control of the human platelet-derived growth factor-β (PDGF) promoter exhibits typical accumulation of human α-syn in various brain regions, including the cortex and the hippocampal formation (Masliah et al. 2000). These animals exhibit spatial memory deficits by the age of 12 months (Masliah et al. 2011). In the hippocampal DG, co-expression of human wt α-syn and neural progenitor markers is found as early as Sox2 expression but also later in developing doublecortin (DCX)-expressing neuroblasts and mature neurons (Winner et al. 2012). Overexpression of wt α-syn led to decreased hippocampal neurogenesis, reflected by a reduced number of neuroblasts and surviving newborn neurons, paralleled by increased cell death. At the same time, neural stem cell proliferation was not affected (Winner et al. 2004; Crews et al. 2008). To analyze neurite outgrowth, newly generated neurons in the DG of PDGF wt α-syn animals were labeled with an enhanced green fluorescent protein (eGFP) retrovirus (Winner et al. 2012). Dendrite outgrowth of newly generated neurons was significantly impaired in wt α-syn mice. In addition, increased spine density in new neurons was observed in the wt α-syn mice, possibly indicating a compensatory effect in early pre- and postsynaptic pathology due to reduced connectivity. Reduction in mushroom spines, indicating a lack of maturation, were observed in wt α-syn mice, and most likely overexpression of α-syn hinders normal levels of presynaptic activity. To further distinguish cell-autonomous from non-autonomous effects, we investigated selective overexpression of wt α-syn in newly generated neurons. Again, we observed decreased dendritic length and impaired branching (Winner et al. 2012). When wt α-syn levels were selectively increased in the newly generated neurons but not increased in their surroundings, spine numbers decreased. The α/β-syn knockout model described above was consecutively used to distinguish the impact of endogenous and overexpression of α-syn in newly

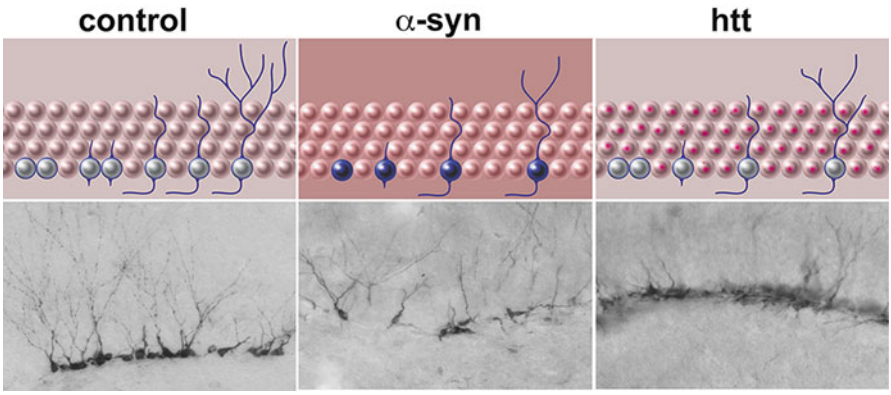

Fig. 1 Impaired adult neurogenesis in the dentate gyrus of animal models expressing α-synuclein (α-syn) and mutant huntingtin (htt). Intracellular expression of transgenic α-syn (*blue*) and intranuclear expression of truncated mutant htt (*red*) in newly generated neurons lead to decreased number of doublecortin-expressing neuroblasts and impaired dendritic branching (*lower row*). (adapted from Kohl et al. 2007; Winner et al. 2012)

generated neurons. When endogenous mouse α/β-syn was absent, dendrite development of GFP-labeled newly generated neurons was not altered. However, dendrite length reduction following selective overexpression of α-syn in newly generated neurons, by injecting a retrovirus overexpressing human α-syn, was no longer present in the α/β-syn knockout mice. These experiments showed that cell-autonomous overexpression of wt α-syn induced a reduction in dendrite length, which was modulated by changes in concentration of endogenous α-syn levels, even if detected at low levels. Spine numbers also decreased in new neurons overexpressing human α-syn in α/β-syn knockout mice (Winner et al. 2012), which strongly suggested differential effects of cell-intrinsic wt α-syn and α-syn overexpression in surrounding DG cells on hippocampal neural stem cells, as well as the maturation of newly generated DG neurons in this transgenic α-syn model (see Fig. 1).

In addition, a conditional model expressing human wt α-syn under the control of the calcium/calmodulin-dependent protein kinase II alpha (CaMK) promoter exhibited widespread distribution of α-syn in the brain, including the hippocampus, which was partly reversed by shutting down the transgene (Nuber et al. 2008). These animals exhibited motor impairments and spatial learning deficits by 12 months of age. Interestingly, decreased DG neurogenesis, without changes in adult neural stem cell proliferation, was observed. More importantly, this detrimental effect on DG neurogenesis was reversible by conditional cessation of transgene expression, illustrated by an increased number of DCX-expressing DG neuroblasts (Nuber et al. 2008).

Interestingly, expression of human mutant A53T α-syn under the PDGF promoter led to a more severe neuropathological phenotype, compared to the wt α-syn animal models (Hashimoto et al. 2003), and was paralleled by an even more severe

decrease of hippocampal neurogenesis. While the number of newly generated neurons was significantly reduced, even neural stem cell proliferation in the DG of these transgenic mice was decreased (Crews et al. 2008; Kohl et al. 2012). This effect was paralleled by a decreased number of Sox2-expressing neural stem cells in the SGZ of the DG (Kohl et al. 2012), and transgenic A53T α-syn was detected in neural stem cells expressing Sox2, as well as in developing neuroblasts and mature neurons. In addition to the direct contribution of α-syn to impaired proliferation, differentiation, and survival of newly generated DG neurons, it is likely that differentiation of serotonergic and dopaminergic modulatory inputs in PD plays a role in decreased DG neurogenesis. In particular, a lack of site-specific integration of newly generated cells might be a result of reduced modulatory inputs.

Animal models with an LRRK2 transgene containing the most frequent G2019S mutation presented with increased anxiety-related behavior (Melrose et al. 2010). In addition, LRRK2 was highly expressed in the hippocampus, in particular in PSA-NCAM-positive neuroblasts (Melrose et al. 2007). Importantly, transgenic G2019S LRRK2 mice exhibited decreased proliferation of neural stem cells, which ultimately led significantly to decreased survival of newly generated neurons in the DG (Winner et al. 2011c). Moreover, LRRK2 overexpression resulted in severely impaired dendritic branching in primary neuronal cultures (MacLeod et al. 2006) as well as in newly generated neurons in the adult hippocampus (Winner et al. 2011c).

Hippocampal Neurogenesis in Human PD

To date, there are only a few studies on neurogenesis in human PD patients. A presumed impairment consisting of decreased SVZ progenitor proliferation in PD patients is still under debate (Hoglinger et al. 2004; van den Berge et al. 2011). Data on neurogenic activity in the hippocampal DG of PD patients is limited. Höglinger et al. observed a decreased number of DG cells expressing nestin and beta-III-tubulin in three PD cases and five patients suffering from PD with dementia (PDD) compared to three controls (Hoglinger et al. 2004). Interestingly, PDD showed a more severe decreased number of nestin-expressing cells in the human DG. In addition, in dementia cases with Lewy bodies (DLB), the number of Sox2-expressing cells in the DG was reduced (Winner et al. 2012). In the DG of DLB patients, there was a decreased number of proliferating cells labeled with PCNA and immature neurons expressing the marker Musashi; however, the number of DCX- and nestin-expressing cells in the DG remained unchanged (Johnson et al. 2011). The current findings are of particular interest because decreased hippocampal neurogenesis was also described in patients suffering from depression. Specifically, the number of proliferating cells in the human DG expressing the marker MCM2 was significantly reduced in depressed patients ($n = 10$), compared to ten healthy controls (Lucassen et al. 2010), but increased in patients treated with SSRI or tricyclic antidepressants (Boldrini et al. 2009, 2012).

Possible Underlying Mechanisms of Impaired Hippocampal Neurogenesis in PD

To date, the mechanisms underlying impaired DG neurogenesis in PD and PD models remain elusive. Nevertheless, recent data on the impact of overexpressed α-syn on dendritic development and spine formation of new DG neurons (Winner et al. 2012) revealed the significance of α-syn protein on the integration of new neurons in the DG, a crucial step in hippocampal neurogenesis (Van Praag et al. 2002). Moreover, the deleterious effect of mutant A53T α-syn on the proliferative capacity of neural stem cells in the DG, in contrast to wt α-syn (Crews et al. 2008; Kohl et al. 2012), suggests a differential effect of α-syn species, as well as different structural states of α-syn (i.e., oligomers, fibrils, and aggregates), on proliferating and maturing hippocampal neurons (Winner et al. 2011a). These data are paralleled by the fact that overexpressed α-syn has been detected in mature, as well as developing neurons and in Sox2-expressing neural stem cells. In this context, impairments of cell cycle-related mechanisms by specific α-syn species might play an important role for altered Notch1 signaling in hippocampal neural stem cells in the presence of α-syn in vitro (Desplats et al. 2012) and in vivo (Crews et al. 2008).

Further possible mechanisms are related to altered expression of pro-neurogenic growth factors. Recently, decreased expression of brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) was detected in A53T α-syn mice compared to non-transgenic littermates (Kohl et al. 2012). BDNF has been described to promote differentiation and survival of newly generated DG neurons in the DG (Waterhouse et al. 2012). Although serum BDNF levels are reduced in PD patients (Scalzo et al. 2010), impaired trophic support of newly generated DG neurons might play a role in decreased neurogenesis in the A53T α-syn transgenic model. To date, data from human postmortem PD studies remain limited and can not proof yet that mechanisms affecting DG neurogenesis in animal models also occur in human PD.

Interventions for Hippocampal Neurogenesis in PD

After recognizing specific impairments of hippocampal neurogenesis in PD models, initial attempts were made to rescue the generation deficit of new neurons in the DG to analyze neural regeneration using in vivo models. We studied the effect of a chronic oral treatment of the SSRI fluoxetine on hippocampal neurogenesis in transgenic mice expressing the human mutant A53T α-syn mutation in developing and mature neurons. Decreased proliferation of adult neural stem cells and adult neurogenesis was reversed in A53T α-syn mice (Kohl et al. 2012). In addition, the fluoxetine effect primarily resulted from increased cell proliferation in the DG, together with a shift toward neuronal differentiation, leading to a greater than threefold

increase of newly generated newborn neurons (Kohl et al. 2012). To elucidate possible underlying mechanisms in vivo, we tested growth factor expression levels in the hippocampi of A53T α-syn mice. Compared to non-transgenic littermates, the A53T mice exhibited significantly reduced brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF), which was reversible after fluoxetine treatment, with the expression levels greater than the respective controls (Kohl et al. 2012). Decreased proliferation of neural stem cells in mice expressing the mutant A53T α-syn (Crews et al. 2008; Kohl et al. 2012) led to the assumption that α-syn impairs cell cycle-related mechanisms. In this context, alterations in Notch1 signaling were observed in hippocampal neural stem cells in the presence of α-syn in vitro (Desplats et al. 2012). Together with data from transgenic A53T α-syn mice (Crews et al. 2008), interventions affecting Notch1 signaling (Wang et al. 2010) might be another promising approach to ameliorate impaired hippocampal neurogenesis in PD.

The notion that specific compounds are able to increase hippocampal neurogenesis recently came into focus. Several antidepressant agents, such as fluoxetine, are able to increase DG neurogenesis after chronic treatment (Santarelli et al. 2003; Miller et al. 2008), while the specific underlying mechanisms remain elusive, particularly in PD. However, a possible role of serotonergic and dopaminergic signaling, as well as effects of neurotrophic factors, remains probable. Nevertheless, new specific chemicals that specifically enhance DG neurogenesis (Pieper et al. 2010) were not evaluated yet in transgenic tested in PD animal models. Interestingly, exposure to environmental enrichment or a running paradigm, factors known to strongly increase neurogenic activity in the hippocampus (Kempermann et al. 1997; van Praag et al. 1999a), has not yet been evaluated in transgenic α-syn PD models. In the transgenic LRRK2 G2019S mouse model, impaired hippocampal neurogenesis was partially restored by a chronic running paradigm, as determined by increased number of DCX-expressing DG neuroblasts (Winner et al. 2011c). Moreover, the withdrawal of overexpressed α-syn in a conditional mouse model led to the observation that impaired DG neurogenesis was primarily attributable to α-syn expression, because it was reversed after cessation of the transgene, as determined by a rescue of the numbers of DCX+neuroblasts and newly generated neurons (Nuber et al. 2008).

In summary, successful approaches to rescue hippocampal neurogenesis in PD remain limited. Interventions affecting serotonergic signaling in the DG seem to be quite promising, while extensive analysis of the underlying mechanisms is crucial to develop more specific strategies for enhancing hippocampal neurogenesis in PD.

Hippocampal Neurogenesis in Huntington's Disease

Nonmotor Symptoms in HD

HD is the most common hereditary, hyperkinetic movement disorder, occurring in 3–10 subjects per 100,000 individuals in Europe and North America (for review see Pringsheim et al. 2012). It is a devastating, autosomal-dominant disease caused by

a CAG trinucleotide repeat expansion within the huntingtin gene, encoding an extended polyglutamine tract (>39) in the huntingtin (htt) protein (The Huntington's Disease Collaborative Research Group 1993). The mutant protein htt is ubiquitously expressed in the organism. However, the brain is most severely affected by the disease process. Neuronal degeneration occurs mainly in the striatum and certain cortical areas (Vonsattel et al. 1985; Vonsattel and DiFiglia 1998), including the hippocampal formation (Spargo et al. 1993). The important neuropathological hallmark of the disease is the accumulation of the mutant protein in neurons, leading to the formation of insoluble ubiquitinated intranuclear inclusions and protein aggregates in neurites (Trottier et al. 1995). Emerging evidence suggests that the mutant protein leads to neuronal damage via a gain of toxic function inducing neuronal loss and gliosis (de la Monte et al. 1988; Trushina et al. 2004).

The progressive clinical phenotype consists of involuntary choreic movements, as well as nonmotor symptoms, such as cognitive decline and psychiatric symptoms, including personality changes (Review in Walker 2007). The age of onset in most cases ranges from 35 to 50 years, and the disease is invariably fatal 10–30 years after the occurrence of the first symptoms. The disease can be diagnosed years before onset of initial symptoms, but there is no disease-modifying therapy for HD. Typical early, nonmotor symptoms are cognitive deficits, and cognitive function decreases over time (Stout et al. 2011). Moreover, depressive symptoms often precede the onset of motor symptoms for many years and have a strong impact on quality of life (reviewed by Epping and Paulsen 2011). Nevertheless, controversy remains as to the nature and origin of depression in Huntington disease, while alterations in neuronal plasticity and the consequent disruption in neural circuitry, in particular of the hippocampus, are suggestive of the depressive phenotype observed in HD. Interestingly, imaging studies in early HD point to an early involvement, not only of basal ganglia but the hippocampal formation as well (Rosas et al. 2003).

Hippocampal Neurogenesis in Transgenic Animal Models of HD

Following detection of the HD gene, several transgenic mouse lines have been developed to investigate disease mechanisms. These mouse models differ according to various aspects, e.g., promoter used, size of the expressed htt fragment, number of trinucleotide repeats, background strain, etc., leading to a variety of different phenotypes exhibiting various important aspects of disease pathology and mechanisms (Crook and Housman 2011; Brooks et al. 2012). These models exhibit distinct aspects of human HD, in particular motor deficits, accumulation and aggregation of transgenic mutant htt, and behavioral abnormalities. Nevertheless, in the context of hippocampal function and adult neurogenesis, only some models have been analyzed in more detail (Table 1).

The first transgenic animal models for HD were the R6/1 and R6/2 lines, with the introduction of exon 1 of the human HD gene carrying expanded CAG repeats into the mouse germ line. They differ in their number of CAG repeats, age of onset, and survival (Mangiarini et al. 1996), and the mice develop a progressive neurological

phenotype that includes severe motor impairments, as well as non-movement disorder components. R6/1 mice exhibit memory impairments at the age of 3–4 months (Murphy et al. 2000; Brooks et al. 2012), but R6/2 develop spatial memory deficits much earlier at the age of 4–5 weeks (Lione et al. 1999). Moreover, female R6/1 mice exhibit depression-like behavior prior to onset of motor symptoms (Pang et al. 2009), and in 4-week-old R6/2 animals, early behavioral abnormalities are paralleled by decreased hippocampal serotonin and its metabolites (Mochel et al. 2011). These results lead to the assumption that hippocampal neurogenesis might be involved in the pathogenesis of disease-related depression in HD. The R6/2 line exhibits early impaired hippocampal neurogenesis at the age of 5.5 weeks, including a deficit in stem cell proliferation as early as 2 weeks of age, determined by PCNA and Ki-67 stainings (Gil et al. 2005). While some conflicting data were presented by Phillips et al., where the number of DCX-expressing neuroblasts remained unchanged in R6/2 and non-tg mice without kainic acid treatment (Phillips et al. 2005), severely impaired DG neurogenesis, including reduction in stem cell proliferation and number of neuroblasts and immature neurons, was confirmed (Kohl et al. 2007; Peng et al. 2008; Fedele et al. 2011). Moreover, the proliferative capacity of DG-derived neural stem cells from R6/2 mice in vitro was impaired (Phillips et al. 2005). The slower-progressing R6/1 line exhibited impaired hippocampal neurogenesis as early as 10 weeks of age, with decreased numbers of DCX-expressing neuroblasts and reduced survival of newly generated neurons (Lazic et al. 2006). In contrast, decreased cell proliferation in the DG was described earliest in R6/1 mice by 22 weeks (Lazic et al. 2004), leading to the assumption that survival of newly generated neurons is more strongly affected than stem cell proliferation in this model. Interestingly, in the R6/2 model, the percentage of neuronal differentiation remained unchanged (Gil et al. 2005; Phillips et al. 2005; Kohl et al. 2007), while the R6/1 mice exhibited impaired maturation into a neuronal phenotype later in life (Grote et al. 2005; Lazic et al. 2006; Hannan and Ransome 2012). In particular, data discrepancies from different studies addressing cell proliferation and cell survival may be attributable to differences between markers used for labeling cell proliferation (endogenous markers, such as Ki-67 and PCNA vs. exogenous labeling of proliferating cells with BrdU), as well as BrdU administration protocols for survival studies, quantification methods for labeled cells, and various mouse colonies. Nevertheless, R6 mice have been intensively used in the evaluation of potential therapeutic strategies to ameliorate disease progression (review in Gil and Rego 2009).

Another model carrying a truncated fragment of human htt (exons 1, 2, part of 3), which contains 82 CAG repeats under the control of the mouse prion protein promoter (the N171-82Q model), exhibits motor deficits and spatial learning deficits by 3 months of age, as well as a reduced life span (Schilling et al. 1999). Moreover, they develop depressive-like behavior (evaluated in the forced swim test) from 3 months of age (Chiu et al. 2011). This is paralleled by decreased hippocampal neurogenesis in 4-month-old N171-82Qs, while neural stem cell proliferation in the DG remains unaltered (Duan et al. 2008).

The absence of a full-length mutant htt protein and the very rapid disease progression, at least in the R6/2 line, led to the development of animal models that mimic the human disease by carrying a full-length human mutant htt: The BACHD mouse model expresses a transgene with 97 glutamine repeats on a bacterial artificial chromosome (BAC) in an FVB/N background (Gray et al. 2008). In the YAC128 model, a yeast artificial chromosome (YAC) was used to introduce 128 glutamine repeats into the human full-length htt gene (Hodgson et al. 1999). While BACHD mice exhibit impaired hippocampal learning by the age of 9 months (Gray et al. 2008; Abada et al. 2012), cognitive dysfunction typically precedes motor abnormalities in the YAC128 mouse model (Van Raamsdonk et al. 2005). Moreover, the YAC128s exhibit early depressive-like behavior and anhedonia at 3 months of age (Pouladi et al. 2009). Importantly, 9- and 12-month-old YAC128 mice exhibit significantly decreased hippocampal neurogenesis, with a reduced survival of newly generated BrdU + cells in the DG and a reduced proportional differentiation into a mature neuronal phenotype (Simpson et al. 2011). In addition, younger YAC128 animals exhibit a slight, but significant, reduction in DG cell proliferation, as well as in the number of neuroblasts after 3 months of age (Simpson et al. 2011).

Moreover, several knock-in strains were developed with an enlarged CAG repeat expansion ranging from 72 to 150, which was introduced into the mouse huntingtin gene (Shelbourne et al. 1999; Wheeler et al. 2002). These mice primarily exhibit early behavioral abnormalities with hyperactivity and hypoactivity but exhibit a normal life span and do not present with cell loss or gliosis. Nevertheless, the Hdh Q111 knock-in mouse exhibits early depressive-like behavior by the age of 15 weeks and spatial memory deficits by 8 months (Giralt et al. 2012; Orvoen et al. 2012). However, analysis of DG neurogenesis in 15-week-old Hdh Q111 animals reveals no difference in cell proliferation or number of DCX + neuroblasts, although male mice exhibit a reduced number of mature DCX + neuroblasts (Orvoen et al. 2012).

Finally, a rat HD model has been established that carries a truncated htt cDNA fragment encoding for 51 CAG repeats under control of the rat htt promoter (von Hörsten et al. 2003). These rats exhibit slowly progressive motor dysfunction, an adult onset of reduced anxiety, but only subtle cognitive impairments (Fielding et al. 2012; Zeef et al. 2012). In addition, 8- and 12-month-old transgenic HD rats were extensively analyzed for the effect of transgene expression in DG neurogenesis. A reduced number of hippocampal progenitor cells was accompanied by an expanded quiescent stem cell pool (characterized by BrdU and Sox2 co-expression) and diminished cAMP-responsive element-binding protein (CREB) signaling (Kandasamy et al. 2010).

In summary, DG neurogenesis has been analyzed in several of the many different transgenic HD animal models. Impaired hippocampal neurogenesis was observed long before development of motor symptoms, which was often paralleled by early cognitive symptoms and depression-like behavior. Nevertheless, it seems to be crucial to evaluate the different models carefully for future studies on hippocampal neurogenesis in HD.

Table 2 Results of DG neurogenesis analysis at various ages in different rodent models of HD

Genotype/ transgene	Age	Proliferation (SGZ)	Neuroblasts/ immature cells	Neuronal differentiation	Survival	Cell death	Citation
R6/2 mouse	2–12 weeks	⇓ BrdU+, PCNA+, Ki-67+ cells	⇓ DCX+cells	⇔ % BrdU+ / NeuN+cells	⇓ BrdU+ / NeuN+cells	N.D.	Gil et al. (2004, 2005)
	5/9/10/13 weeks	⇓ BrdU+, PCNA+cells	⇓ DCX+cells ⇔ Pax6+, Tbr2+ cells ⇓ NeuroD+, Calretinin+cells	⇔ % BrdU+ / NeuN+cells	⇓ BrdU+ / NeuN+cells	⇑ TUNEL+cells	Phillips et al. (2005), Kohl et al. (2007), Peng et al. (2008), Fedele et al. (2011)
R6/1 mouse	7–10 weeks	⇔ BrdU+cells	⇓ DCX+cells	⇔ % BrdU+ / NeuN+cells	⇓ BrdU+ / NeuN+cells	⇔ cl. caspase3+ cells	Lazic et al. (2004, 2006)
	19/22/25/33 weeks	⇓ BrdU+cells, ⇔ Ki-67+ cells	⇓ DCX+cells	⇔ / ⇓ % BrdU+ /NeuN+cells	⇓ BrdU+ / NeuN+cells	⇔ cl. caspase3+ cells	Lazic et al. (2004, 2006), Grote et al. (2005), Walker et al. (2011), Hannan and Ransome (2012)
N171-82Q mouse	16 weeks	⇔ BrdU+cells	N.D.	⇓ % BrdU+ / NeuN+cells	⇓ BrdU+ / NeuN+cells	N.D.	Duan et al. (2008), Potter et al. (2010)
YAC128 mouse	3–18 months	⇓ BrdU+, Ki-67+ cells ⇔ PCNA+cells	⇓ DCX+, NeuroD+cells	⇓ % BrdU+ / NeuN+cells (9/12 months)	⇓ BrdU+ / NeuN+cells (9/12 months)	N.D.	Simpson et al. (2011)
Hdh Q111 mouse	15 weeks	⇔ BrdU+cells	⇔ DCX+cells ⇓ "late" DCX+cells (only in males)	N.D.	N.D.	N.D.	Orvoen et al. (2012)
HD transgenic rat	8 months 12 months	⇔ BrdU+, PCNA+cells ⇓ BrdU+, PCNA+cells	⇑ DCX+cells ⇓ DCX+cells	⇓ % BrdU+ / NeuN+cells ⇓ % BrdU+ / NeuN+cells	⇓ BrdU+ / NeuN+cells ⇓ BrdU+ / NeuN+cells	N.D. N.D.	Kandasamy et al. (2010)

BrdU bromodeoxyuridine, *DCX* doublecortin, *N.D.* no data, *NeuN* neuronal nuclear antigen, *NeuroD* neurogenic differentiation factor, *Pax6* paired box protein 6, *PCNA* proliferating cell nuclear antigen, *SGZ* subgranular zone, *Tbr2* T-box brain protein 2, *TUNEL* Tdt-mediated dUTP-biotin nick end labeling

Hippocampal Neurogenesis in Human HD

In contrast to these data from animal models, one recent postmortem study on DG neurogenesis in human HD patients could not confirm data obtained from HD animal models. While Curtis et al. described increased cell proliferation in the SVZ of human HD patients (Curtis et al. 2003), the same group could not detect significant differences in cell proliferation in the SGZ of HD patients compared to healthy controls (Low et al. 2011) using PCNA as a proliferation marker. It is important to emphasize that the degree of cell proliferation in the human hippocampus is very low—much lower compared to the human SVZ. In addition, the patient samples used in this study were at a late stage of the disease. Moreover, the patients might have received antidepressant treatment, such as SSRIs, which increases DG neurogenesis in human patients suffering from depression, even though these findings remain under debate (Lucassen et al. 2010; Boldrini et al. 2012).

Mechanisms Underlying Impaired Hippocampal Neurogenesis in HD

The possible mechanisms underlying a deleterious effect on hippocampal neurogenesis in HD remain still elusive. To date, it remains under debate whether small oligomers or larger inclusion bodies are the deleterious species of mutant htt (Arrasate et al. 2004; Legleiter et al. 2010). Moreover, in the context of hippocampal neurogenesis, it seems interesting to note that in R6/2 transgenic mice, mutant htt aggregates were only present in mature, NeuN-expressing neurons but not in immature neuroblasts expressing DCX (Kohl et al. 2007) or immature neurons expressing NeuroD (Fedele et al. 2011) (see Fig. 1). This is in contrast to data showing that even the proliferative capacity of neural stem cells in the DG of the R6/2 transgenic model is disturbed (Gil et al. 2004; Phillips et al. 2005; Kohl et al. 2007). In addition to the possibility that small oligomers of htt affect adult neural stem cells even stronger than larger aggregates, non-cell-autonomous mechanisms might also play an important role. Unfortunately, a detailed temporal expression analysis of htt in adult neural stem cells in any transgenic HD model is lacking. Moreover, a possible function of wild-type htt in adult neurogenesis was not investigated, although it is known that physiological htt is required for cortical neurogenesis during development (Tong et al. 2011). In addition, htt interacts with elements required for cell cycle and cell division, as well as microtubule dynamics (Gutekunst et al. 1995; Godin et al. 2010). Because these functions are essential for rapid-proliferating neural stem cells, a role for the mutant htt in proliferation disturbance is conceivable.

One possible factor related to decreased DG neurogenesis in HD animal models is the absence of pro-neurogenic trophic factors, in particular BDNF. BDNF is known to promote synaptic plasticity, as well as differentiation and survival of newly generated neurons in the DG (Waterhouse et al. 2012), and plays an important role in hippocampal function (Bekinschtein et al. 2008). Importantly, while normal htt promotes

expression and vesicular transport of BDNF, mutant htt interferes with this function (Zuccato et al. 2003; Gauthier et al. 2004). Moreover, reduced BDNF levels have been described in various HD models and even in human HD cases (Canals et al. 2004; Spires et al. 2004; Strand et al. 2007; Zajac et al. 2010). Therefore, downregulation of this neurotrophic factor might contribute to neurogenesis deficits in HD. In line with impairments of neurotrophic factors, in particular BDNF, dysregulation of neurotransmitter systems might play a role in decreased DG neurogenesis in HD.

Antidepressants, in particular SSRIs, have also been shown to rescue depressive-like symptoms and hippocampal neurogenesis in rodent HD models (see section "Interventions to restore hippocampal neurogenesis in HD"). Moreover, in transgenic R6/1 and R6/2 mice, serotonin levels and serotonin receptor expression were reduced (Reynolds et al. 1999; Pang et al. 2009; Mochel et al. 2011). These findings suggest that a dysregulation of the serotonergic system might contribute to the disease process, as well as to impaired hippocampal neurogenesis. Furthermore, alterations in dopaminergic signaling have been implicated in some animal models of HD (Hickey et al. 2002; Petersen et al. 2002; Mochel et al. 2011). Because dopaminergic input into the DG has been suggested to influence generation and function of new DG neurons (Hoglinger et al. 2004; Mu et al. 2011), this transmitter system might also be involved.

Finally, some data have provided evidence that energy metabolism and mitochondrial function in particular are disrupted in HD (reviewed in Mochel and Haller 2011). To date, these HD aspects have not been analyzed in the context of impaired hippocampal neurogenesis. In summary, a large variety of mechanisms are involved in the impairment of hippocampal neurogenesis in HD models, suggesting several opportunities to overcome these deficits. Nevertheless, for further analysis of DG neurogenesis in HD, it is crucial to describe the different effects of mutant and normal htt in the neurogenic niche in more detail.

Interventions to Restore Hippocampal Neurogenesis in HD

Because specific cognitive impairments and depression are early symptoms of HD in humans, hippocampal function in particular was linked to decreased hippocampal neurogenesis in HD transgenic mouse models. Several approaches have been employed to increase DG neurogenesis to increase cognitive function or even slow disease progression by endogenous cell replacement strategy.

Environmental enrichment and voluntary physical activity are important factors that stimulate DG neurogenesis. In particular, environmental enrichment has been evaluated in detail in the context of hippocampal neurogenesis. These enriched conditions, including various tactile, social, and exploration stimuli, together with more physical activity, are known to increase DG neurogenesis in rodents (Kempermann et al. 1997). These paradigms have been transferred to rodent HD models, showing that enrichment paradigms are able to improve motor symptoms and, in part, slow disease progression in R6/1, R6/2, and N171-82Q mice (van Dellen et al. 2000; Hockly et al. 2002; Schilling et al. 2004). Using the R6/1 mouse line, environmental

enrichment was analyzed by Lazic and colleagues. They observed increased cell survival and number of neuroblasts in the DG at 25 weeks of age but not in presymptomatic 10-week-old animals. In addition, there were no changes in SVZ progenitor proliferation (Lazic et al. 2006). These findings might lead to the assumption that increased DG neurogenesis contributes at least in part to beneficial effects of environmental enrichment in this model. Moreover, further studies showed that hippocampal-dependent learning was improved by environmental stimulation in this model (Nithianantharajah and Murphy 2009). Interestingly, a study testing the beneficial effects of environmental stimulation in HD patients showed improvements in physical and mental functions (Sullivan et al. 2001).

One important aspect of enrichment paradigms for rodents is physical activity, in particular in the form of voluntary wheel running. Increased physical exercise is known to be a strong enhancer of hippocampal neurogenesis (van Praag et al. 1999a, b). We used a wheel running paradigm to rescue DG neurogenesis in the R6/2 mouse model of HD. Interestingly, DG neurogenesis was not rescued in 9-weeks-old R6/2 mice (Kohl et al. 2007) or in 12-week-old R6/1 animals (Renoir et al. 2012). In addition, in the N171-82Q model, a similar running paradigm did not rescue DG neurogenesis (Potter et al. 2010). A recent study analyzed the impact of wheel running on serotonergic signaling but failed to rescue impaired DG cell proliferation in the R6/1 model (Renoir et al. 2012). This is in contrast to studies where cognitive deficits in R6/1 mice were ameliorated by a running paradigm, while hippocampal BDNF levels remained unchanged (Pang et al. 2006). A more recent study observed increased BDNF levels as a result of running but only in R6/1 females (Zajac et al. 2010). Despite these conflicting data, BDNF might indeed be an important factor in the transfer of beneficial effects of physical exercise and environmental enrichment on DG neurogenesis.

Because transgenic HD models display depressive-like behavior (Pang et al. 2009; Pouladi et al. 2009), several studies have been conducted to determine the effect of antidepressant treatment on behavioral symptoms, as well as effects on hippocampal neurogenesis. In the R6/1 model, a 10-week treatment with the SSRI fluoxetine increased the number of new DG neurons by increasing neuronal differentiation of newly generated progenitors (Grote et al. 2005). Another SSRI, sertraline, effectively rescued proliferation deficits and reduced survival of newly generated DG neurons in the R6/2 model, which was paralleled by increase hippocampal BDNF levels (Peng et al. 2008). Sertraline was also sufficient to increase cell proliferation and cell survival in the N171-82Q HD mouse model, together with increased cortical BDNF levels and improved motor function (Duan et al. 2008). Further studies used different antidepressants to analyze effects on depressive behavior, cognitive function, or serotonergic signaling without investigation of hippocampal neurogenesis (Duan et al. 2004; Pang et al. 2009). Taken together, SSRIs that increase serotonin levels represent an efficient treatment option to rescue hippocampal neurogenesis in HD.

One study evaluated the possible effect of cytokine asialo-erythropoietin administration on hippocampal neurogenesis in R6/2 mice but did not find beneficial effects on cell proliferation in the DG or motor deficits and neuropathology (Gil et al. 2004). More recently, testosterone treatment was evaluated in male R6/1 mice without showing rescue effects on DG neurogenesis (Hannan and Ransome 2012).

In conclusion, compounds affecting serotonergic signaling in the hippocampus are a promising approach to rescue hippocampal neurogenesis deficits in HD. Nevertheless, there is a need to describe the underlying mechanisms in more detail, as well as the development of additional treatment strategies. In addition, exogenous environmental stimuli represent an interesting approach, even if some paradigms have failed to rescue DG neurogenesis in HD models. Most importantly, more specific compounds increasing DG neurogenesis need to be tested in future studies.

Conclusion

Both PD and HD are neurodegenerative diseases characterized by a complex variety of motor signs, cognitive impairments, and neuropsychiatric symptoms. In particular, impaired hippocampal function may result in early symptoms of both diseases, which is highly relevant for the quality of life in affected patients. Since the emergence of knowledge about the link between hippocampal function and the generation of new neurons in the hippocampal DG, several studies have been conducted to describe adult neurogenesis in PD and HD in more detail. Transgenic animal models exhibit early disturbances of hippocampal neurogenesis, even before motor symptoms occur. The relevance of α-syn for the generation and integration of new DG neurons in PD models were described in rodents and humans recently (Winner et al. 2012), and possible interventions to rescue neurogenesis deficits in PD are just beginning to develop (Kohl et al. 2012). In HD animal models, hippocampal neurogenesis has been extensively evaluated. Nevertheless, the function of mutant and normal htt in adult neurogenesis needs to be further investigated. Potential interventions have focused on neurotrophic support, in particular for BDNF, and the use of compounds affecting serotonergic signaling in the DG.

For both diseases, the transfer of preclinical treatment strategies into clinical application for PD and HD patients will bear further challenges to improve cognitive function and mood changes. Nevertheless, further insight into the generation of new neurons in the adult brains of PD and HD patients will help to define novel targets to improve therapeutic approaches in particular for nonmotor symptoms of both movement disorders.

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Linking Adult Neurogenesis to Epilepsy Through Epigenetics

Kyungok Cho and Jenny Hsieh

Introduction

Neurogenesis, defined as the generation of new neurons from resident neural stem/progenitor cells, persists throughout life in the subgranular zone (SGZ) of the hippocampal dentate gyrus and subventricular zone (SVZ) of the lateral ventricle (Ming and Song 2005; Altman and Das 1965). Although it is controversial whether neurogenesis occurs in areas other than SGZ and SVZ (Gould 2007; Rakic 2002), neurogenesis is known to participate in various brain functions including cognition, mood, and stress regulation (Zhao et al. 2008). In addition to these physiological activities, pathological stimuli, such as stroke, ischemia, traumatic brain injury, and epilepsy, can also affect adult neurogenesis as they increase proliferation of neural stem cells (Parent 2003; Richardson et al. 2007). However, unlike the functional role of neurogenesis under physiological conditions, where new neurons appear to participate in antidepressive functions and enhance learning and memory, a debate still remains as to whether the role of adult neurogenesis under pathological conditions is beneficial or not. For example, after ischemia and traumatic brain injury, where extensive neuronal loss is a key pathological feature, transplantation of neural stem cells may be therapeutically beneficial to replace damaged neurons and

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improve functional recovery (Richardson et al. 2007; Hallbergson et al. 2003; Miljan and Sinden 2009). Moreover, stimulation of environmental signals to mobilize and recruit endogenous neural stem cells into damaged areas is also considered a potential treatment strategy, suggesting that addition of new neurons, by promoting endogenous neurogenesis, may be advantageous (Hallbergson et al. 2003).

In contrast, in the case of epilepsy, new neurons are considered to aggravate the occurrence of subsequent spontaneous seizures (Parent and Lowenstein 2002). Epilepsy, in particular, is distinct from other brain disorders, because seizure activity directly targets neuronal populations that initiate a cascade of pathological events, affecting other subsequent cellular populations in the brain. However, in other brain disorders, such as stroke, ischemia, and traumatic brain injury, neurons are influenced by nearby blood vessels or mechanical destruction, which compromises their function. Therefore, in this chapter, we will first focus on epilepsy and highlight the seizure-induced morphological abnormalities in hippocampal neurogenesis. In the second half of the chapter, we will describe epigenetic mechanisms—such as DNA methylation, histone modification, and noncoding RNAs—linked to changes in hippocampal neurogenesis associated with the pathogenesis of epilepsy (Qureshi and Mehler 2010; Pitkanen and Lukasiuk 2009; Lubin 2012).

Epilepsy and Aberrant Seizure-Induced Hippocampal Neurogenesis

Epilepsy, defined as recurrent unprovoked seizures, is one of the most common neurological disorders worldwide, affecting approximately 50 per 100,000 people (Kotsopoulos et al. 2002; Hirtz et al. 2007). Epileptic disorders are comprised of heterogeneous diseases in nature and are classified according to clinical signs, such as age at onset, specific electroencephalography (EEG) characteristics, or seizure types, implying that the root cause of epilepsy remains elusive (Berg et al. 2010). Indeed, currently available pharmacological approaches focus mainly on suppression of acute convulsive seizures, and none of them are disease-modifying or classified as anti-epileptogenic drugs.

Despite heterogeneous clinical features, general epilepsy is derived from an acute seizure event, often caused by high fever, brain infection, hypoxia, or traumatic brain injury (Jensen and Baram 2000). After the initial insult, there is a silent (latent) period lasting several months to years before the onset of spontaneous recurrent seizures. During this latent period, a variety of cellular changes occur in the brain including neurodegeneration, neurogenesis, gliosis, axonal sprouting, and angiogenesis, contributing to the development of epilepsy, which is clinically defined as two or more unprovoked seizures (Pitkanen and Lukasiuk 2009). This process is called epileptogenesis, where a neuronal network acquires the capability to generate recurrent seizures without a causative stimulus (Rakhade and Jensen 2009).

Among a wide variety of alterations caused by seizures, emerging evidence suggests the importance of seizure-induced neurogenesis in the epileptogenic

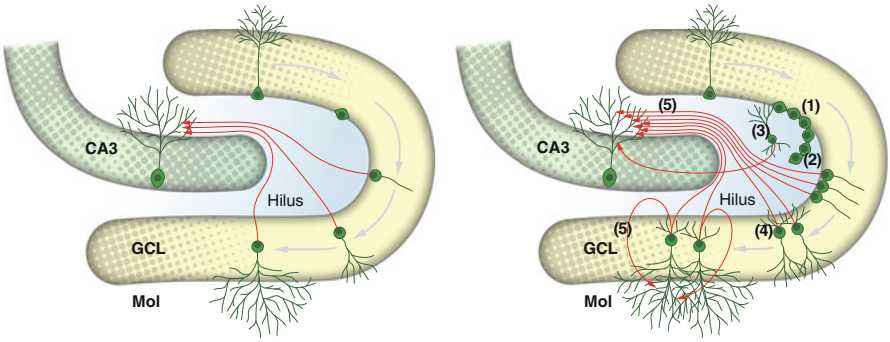

Fig. 1 Seizure activity induces changes in adult hippocampal neurogenesis. (**a**) In the adult mammalian dentate gyrus, neural stem/progenitor cells in the subgranular zone proliferate to generate neuroblasts, which subsequently differentiate into immature and mature granular neurons. (**b**) Seizures facilitate proliferation of neural stem/progenitor cells (1), chain migration of neuroblasts (2), generation of ectopic granule cells (3), formation of hilar basal dendrites (4), and mossy fiber sprouting (5). Modified from (Hsieh and Eisch 2010). *GCL* granule cell layer, *Mol* molecular layer

process (Jung et al. 2004, 2006; Parent and Murphy 2008) (Fig. 1). Studies using rodent models of mesial temporal lobe epilepsy, a common and often intractable form of epilepsy, showed that proliferation of hippocampal stem/progenitors dramatically increased after status epilepticus (SE), a prolonged initial seizure activity (Parent et al. 1997). Cell proliferation returned to basal levels by 4 weeks after SE and was maintained for 6 months, although neuronal differentiation decreased at chronic epileptic stages, leading to declined hippocampal neurogenesis over time (Parent et al. 1997; Hattiangady and Shetty 2010; Hattiangady et al. 2004). Moreover, after SE, these dentate gyrus-generated cells migrate inappropriately into the hilus and the molecular layer, possibly exacerbated by depletion of reelin-producing interneurons (Parent et al. 1997; Gong et al. 2007). The persistence of hilar basal dendrites (HBDs) on granule neurons (Ribak et al. 2000) and axonal outgrowth of granule cells, called mossy fiber sprouting (Parent et al. 1997), are other characteristic features in the epileptic hippocampus. Interestingly, dentate granule cells born after SE, or immature dentate granule neurons at the time of SE, developed HBDs and mossy fiber sprouting 10 weeks later, whereas fully mature neurons were unaffected by SE (Kron et al. 2010; Jessberger et al. 2007a).

The functional importance of seizure-induced cellular abnormalities involves ectopic granule cells, HBDs, and mossy fiber sprouting, which may contribute to epileptogenesis (Parent and Kron 2012). Hilar ectopic granule cells are thought to be hyperexcitable, receiving excitatory inputs from CA3 pyramidal cells (Scharfman et al. 2000). Moreover, SE increases the number of granule cells with HBDs, which form recurrent excitatory circuitry with hilar mossy fiber collaterals (Ribak et al. 2000). In addition to HBDs, mossy fiber sprouting is also proposed to be pro-epileptogenic by making synaptic contacts with GABA-negative dendritic spines, presumably adjacent granule cells (Buckmaster et al. 2002). The role of mossy fiber sprouting in epileptogenesis is further supported by several studies showing that

pharmacological inhibition of granule cell axonal sprouting reduces the number of spontaneous seizures (Zeng et al. 2009; Huang et al. 2010). In line with these data, ablation of neurogenesis after SE attenuates frequency and duration of spontaneous recurrent seizures, with decreased ectopic granule cells in the hilus (Jung et al. 2004, 2006). Moreover, using a genetic model to specifically ablate dividing neural progenitors, we have evidence that newborn granule cells prior to and shortly after SE could contribute to epileptogenesis (unpublished data). However, a recent study utilizing irradiation to modulate neurogenesis in epilepsy showed no effect on the ability of seizures for subsequent development of epilepsy, suggesting complex mechanisms underlie the process of epileptogenesis (Pekcec et al. 2011). Although these results remain controversial, these data support the notion that aberrant neurogenesis after SE can be responsible for the development of abnormal hippocampal circuit formation and spontaneous recurrent seizures, suggesting that aberrant hippocampal neurogenesis may be a therapeutic target for the prevention and treatment of epilepsy.

Epigenetic Regulation of Hippocampal Neurogenesis in Epilepsy

During the latent period in epileptogenesis, seizures induce a variety of gene expression changes, which may contribute to abnormal hippocampal neurogenesis and altered network function. One of the candidate regulatory mechanisms that control long-lasting changes in gene expression is epigenetics, which refers to changes in gene expression that do not accompany changes at the DNA sequence level (Goldberg et al. 2007). This can be achieved by covalent modification of histone proteins or DNA itself, leading to altered chromatin structure and gene expression. The basic chromatin structure is called the nucleosome, composed of 147 base pairs of DNA wrapped around the octameric core histones H2A, H2B, H3, and H4 (Luger et al. 1997). Histone proteins have free N-terminal tails that can be modified by methylation, acetylation, phosphorylation, ubiquitination, ribosylation, and SUMOylation via histone-modifying enzymes, such as histone acetylases/deacetylases (HATs/HDACs) and histone methylases/demethylases (HMTs/HDMTs), providing a platform to recruit transcription factors and other binding proteins (Strahl and Allis 2000). These posttranslational histone modifications mediate conformational changes of the nucleosome, either exposing or masking DNA to facilitate and repress gene transcription, respectively (Qureshi and Mehler 2010). In addition to histone proteins, DNA can be epigenetically modulated by methylation, occurring especially at the cytosine residue of CpG dinucleotides (Suzuki and Bird 2008). Unlike histone methylation, DNA methylation is suggested to silence gene transcription (Lubin 2012).

Epigenetic mechanisms act as an interface between genomic information and the environment by modulating specific genes within cells to exquisitely respond to diverse environmental stimuli. Recently, altered epigenetic chromatin modifications were reported in many neurological disorders, including autism, Alzheimer's disease, schizophrenia, brain tumor, Huntington's disease, and epilepsy (Lubin 2012;

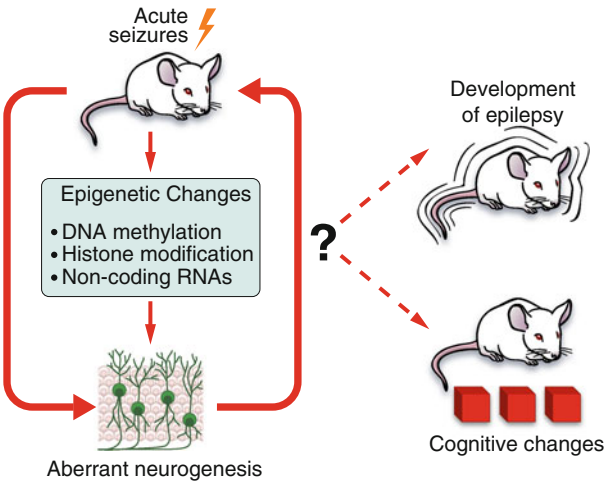

Fig. 2 Epigenetic mechanisms modulating seizure-induced aberrant neurogenesis and epileptogenesis. Acute seizures induce alterations in epigenetic mechanisms, including changes in DNA methylation, histone modification, and noncoding RNAs. In turn, epigenetic dysregulation of gene expression influences aberrant hippocampal neurogenesis, which may lead to the development of epilepsy and cognitive decline

Dubuc et al. 2012; Coppieters and Dragunow 2011; Miyake et al. 2012; Narayan and Dragunow 2010). In particular, epilepsy has a variety of spatiotemporal gene expression changes during the latent period, possibly contributing to the development of epilepsy (Rakhade and Jensen 2009). For example, after seizure activity, transcription factors, such as c-Fos (Tsankova et al. 2004; Sng et al. 2006), c-Jun (Sng et al. 2006), and CREB (Tsankova et al. 2004), and neurotrophic factors, including brain-derived neurotrophic factor (BDNF) (Tsankova et al. 2004; Huang et al. 2002) and glutamate receptor 2 (GluR2), a calcium-impermeable subunit comprising ionotropic glutamate receptors (Huang et al. 2002), are perturbed in association with histone modification. Moreover, seizure-induced decreased expression of GluR2, which can be mediated by histone deacetylation, results in enhanced epileptogenesis (Huang et al. 2002; Sanchez et al. 2001), suggesting that the epigenetic machinery may play crucial roles in the pathogenesis of epilepsy (Fig. 2). Among various potential pro-epileptogenic factors, epigenetic dysregulation of seizure-induced gene expression and aberrant neurogenesis is discussed in the following section.

DNA Methylation

DNA methylation of CpG islands is catalyzed by DNA methyltransferases (DNMTs) (Goll and Bestor 2005). A recent study reported that the expression of DNMTs was increased in human temporal lobe epilepsy (Zhu et al. 2012). Interestingly, DNMT1

was shown to negatively influence mRNA expression of reelin (Noh et al. 2005), a well-known extracellular matrix molecule, which regulates proper migration of hippocampal progenitor cells into the dentate gyrus granule cell layer under physiological conditions (Gong et al. 2007). Furthermore, increased DNA methylation at the reelin promoter was found in the epileptic hippocampus, which correlated with granule cell dispersion (Kobow et al. 2009). These results correlated with previous studies showing that reelin deficiency in epilepsy is responsible for granule cell dispersion, which is a migration defect of granule neurons (Haas and Frotscher 2010; Muller et al. 2009). Taken together, high methylation rates at the reelin promoter after initial seizures, perhaps mediated by DNMTs, may reduce reelin expression in the epileptic hippocampus, thereby allowing neural progenitor cells to migrate into locations where granule cells are not normally located.

With technological advances of next-generation sequencing methods, genome-wide analysis of DNA methylation status in the dentate gyrus has revealed that electroconvulsive seizures induce dynamic changes in de novo DNA methylation and active demethylation (Guo et al. 2011). Specifically, Gadd45b, a member of DNA demethylases, was upregulated by electroconvulsive seizures, promoting proliferation of hippocampal neural stem/progenitor cells and dendritic development of newborn neurons (Ma et al. 2009). This increased Gadd45b expression correlated with CpG demethylation of brain-derived neurotrophic factor (BDNF) and fibroblast growth factor 1 (FGF1) promoters—growth factors well known to influence neurogenesis and synaptic plasticity (Ma et al. 2009). These studies illustrate that epigenetic control of hippocampal neural stem cells may be linked to the pathophysiology and development of epilepsy.

Histone Modifications

Accumulating evidence suggests alterations in histone modifications in experimental models of status epilepticus and human temporal lobe epilepsy (Sng et al. 2006; Huang et al. 2002, 2012; Crosio et al. 2003; Hsieh and Eisch 2010). One of the best-studied histone modifications is acetylation and deacetylation of the histone proteins, controlled by HATs and HDACs, respectively. Following kainate-induced status epilepticus, histone H4 acetylation was rapidly induced in the hippocampus, where pretreatment with curcumin, a HAT inhibitor, resulted in reduced hyperacetylation of histone H4 (Sng et al. 2006). In addition, results from our group showed that the HDAC inhibitor valproic acid (VPA) normalized seizure-induced proliferation of hippocampal stem/progenitor cells, as measured by the immature neuronal marker doublecortin (DCX) and the cell proliferation marker Ki-67, respectively. Moreover, VPA treatment after initial seizures dramatically reduced the formation of HBDs, along with improved cognitive impairment (Jessberger et al. 2007b). Because VPA is one of the most commonly used antiepileptic drugs, which has subsequently been shown to effectively block HDAC (Gottlicher et al. 2001), it is

noteworthy that epigenetic intervention during epileptogenesis may normalize aberrant hippocampal neurogenesis, thereby preventing comorbidities related to epilepsy, such as cognitive function.

Noncoding RNAs

Noncoding RNAs are functional RNA molecules that are not translated into proteins. These molecules are receiving widespread attention as an important class of epigenetic regulators that interact with chromatin modifiers and transcription factors to regulate gene expression (Costa 2005). Among them, microRNAs (miRNAs), 21–25 nucleotides in length and known to control posttranscriptional gene expression, are being extensively investigated with regard to biogenesis, expression profiles, and functional significance (Bartel 2004; Sayed and Abdellatif 2011). Several recent epilepsy studies used miRNA array analysis to identify dozens of hippocampal miRNAs that were altered by SE (Song et al. 2011; Jimenez-Mateos et al. 2011; Hu et al. 2011). In all three studies, miR-132 was consistently upregulated in the hippocampus. Considering that miR-132 is required for dendritic development of newborn neurons in the dentate gyrus (Magill et al. 2010), it will be interesting to determine the functional roles of miR-132 in seizure-induced aberrant hippocampal neurogenesis, such as HBD formation.

Another interesting candidate is miR-134, which was increased by SE in two of the three studies (Song et al. 2011; Jimenez-Mateos et al. 2011; Hu et al. 2011). MiR-134 is a brain-specific miRNA that regulates dendritic spine morphology in hippocampal neurons (Schratt et al. 2006). In addition to its role in spine size determination, miR-134 promotes proliferation of neural stem/progenitor cells and inhibits neuronal migration (Gaughwin et al. 2011). Intriguingly, a recent study demonstrated that miR-134 silencing following SE resulted in near-complete suppression of spontaneous recurrent seizures, pointing out a potential role of miR-134 as an anti-epileptogenic agent (Jimenez-Mateos et al. 2012). Therefore, future studies are warranted to further establish the mechanistic link between miR-134 and its seizure-suppressive effects, possibly in relation to hippocampal neurogenesis.

In contrast to the upregulated miRNAs mentioned above, the three aforementioned studies showed no overlap in miRNAs downregulated by SE (Song et al. 2011; Jimenez-Mateos et al. 2011; Hu et al. 2011). However, because we are focusing on neural stem cells, it is interesting to note that the lethal-7 (let-7) family has attracted attention due to its association with neural differentiation (Song et al. 2011; Sempere et al. 2004). In addition, let-7 overexpression in neural stem cells reduced cell proliferation and facilitated cell cycle exit, thereby accelerating neural differentiation, whereas let-7 inhibition increased neural stem cell proliferation (Zhao et al. 2010). Because SE decreases let-7 expression, which then promotes neural stem/progenitor cell proliferation, and because increased hippocampal cell proliferation following SE is one of the key pathological features in epilepsy,

uncovering the links between miRNAs, epileptogenesis, and seizure-induced aberrant neurogenesis may shed light on the underlying mechanisms involved in the development of epilepsy.

Future Perspectives

Exploring the epigenetic control of seizure-induced neurogenesis has immense potential for the development of novel therapeutic and preventative strategies in epilepsy. Here, we described recent studies demonstrating the role of epigenetic mechanisms in seizure-induced aberrant neurogenesis and epileptogenesis. Although these initial investigations give us insight regarding a possible cellular target for epilepsy, the exact mechanism by which seizure activity is relayed to different neural stem/progenitor cell and neuroblast populations, ultimately leading to epilepsy development, remains to be shown. Because adult neurogenesis is a multi-step process (e.g., stem cell activation, proliferation, migration, and dendritic and axonal maturation), it is likely that seizure activity may affect each step of neurogenesis via various mechanisms. In future studies, it will be important to determine the stages of adult neurogenesis that most specifically correlate with epileptogenesis.

Scientific approaches using transgenic mice, such as cell-type-specific Cre driver lines, in combination with genetic ablation tools could provide useful information to define the cell populations and/or neurogenesis stage most important for epilepsy development. Once the cell population is better defined (e.g., type-3 DCX-positive cells), transcriptome and epigenome analysis (e.g., methylome, chromatin immunoprecipitation with histone-specific modifications combined with deep sequencing) of FACS-sorted cells from experimental rodent seizure models could give insight into seizure-induced signaling pathways and molecular mechanisms (e.g., epigenetic regulation of the production of ectopic granule cells and mossy fiber sprouting, or the role of miRNAs in seizure-induced hippocampal neurogenesis). Because seizures induce massive hippocampal cell death, it is possible that the amount of freshly isolated cell populations could be a limitation for performing current genome-based techniques. To circumvent this issue, a complementary approach may be to use an in vitro seizure model to expand the various neural stem/progenitor cells.

Through the use of available cell-specific transcriptome and epigenome datasets, researchers may be able to search for potential pathways that mediate the cellular and molecular changes associated with seizure-induced neurogenesis and epileptogenesis, which could lead to the development of novel classes of anti-epileptogenic drugs/compounds. Considering the fact that current antiepileptic drugs are merely anticonvulsants, and none can ultimately cure epilepsy, it will be important to understand the genetic and epigenetic regulation of hippocampal neurogenesis, during the process of epileptogenesis, to explore novel therapeutic avenues.

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Activity-Based Maintenance of Adult Hippocampal Neurogenesis: Maintaining a Potential for Lifelong Plasticity

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Most studies of adult hippocampal neurogenesis focus on regulation based on a baseline, and the general assumption is that more new neurons correlate with improved functionality, whereas reduced levels are linked to pathology and reduced function. The contribution of new neurons is implicitly or explicitly accepted to be central to the general role of the dentate gyrus, the hippocampus, or even "the brain." While this generalization remains to be fully explored and the implicit acceptance of any claim is problematic, not least because the devil will always lie in the detail, there is indeed growing evidence that neurogenesis fundamentally contributes to hippocampal function (Aimone et al. 2010; Kempermann 2011). Immature newborn neurons, with a particular ratio of inhibitory vs. excitatory input that facilitates responsiveness to incoming stimuli from the entorhinal cortex, appear to increase synaptic plasticity in the dentate gyrus (Marin-Burgin and Schinder 2011; Marin-Burgin et al. 2012). These newly formed neurons are lastingly integrated into the network, so that their functional contribution appears to be dual: one related to a transient period of increased plasticity and one based on the fact that they add a new node to the mossy fiber system network between the dentate gyrus and CA3. At the behavioral level, these two functionalities have not yet been fully distinguished, if they are ultimately distinguishable at all. Nevertheless, new neurons are involved in pattern separation, a critical function of the dentate gyrus (Aimone et al. 2009; Clelland et al. 2009), by presumably preventing catastrophic interference within the

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CA3 region (Wiskott et al. 2006). In addition, they add flexibility to learning by allowing integration of novel information into established contexts and representations (Garthe et al. 2009). Moreover, they might facilitate independence of learned information in the hippocampus, as well as the encoding of temporal and contextual information. If adult neurogenesis is essential to dentate gyrus functions, then the idea that increasing neurogenesis is beneficial does not seem farfetched. And if this is true, adult hippocampal neurogenesis might be a quantitative trait that provides an advantage in an animal's or a species' adaptability in an evolutionary sense (Kempermann 2012).

Importantly, the regulation of adult neurogenesis is function and activity dependent. Situations with increased functional demand also induce adult neurogenesis, which has been shown in animal studies utilizing learning paradigms and exposure to enriched environments (Gould et al. 1999; Kee et al. 2007; Kempermann et al. 1997; Tashiro et al. 2007). Unless regulation is impaired by pathology, neurogenesis should always be at the appropriate level, if the relationship between function and regulation is indeed close. Newborn neurons not only preferentially respond to novel stimuli, but stimulated neurons are also more likely to be lastingly recruited (Kee et al. 2007; Tashiro et al. 2007). This implies that in the absence of appropriate stimuli and learning challenges, such integration should not take place. If appropriate stimuli did not arise, then the potential for neurogenesis would go unused. This potential, however, which is represented by proliferative activity of precursor cells in the adult subgranular zone of the hippocampus, can indeed be boosted by presumably nonspecific stimuli, most notably locomotion and physical activity (Van Praag et al. 1999; Kronenberg et al. 2003). Surprisingly, with some contradiction to the mentioned hypothesis, by increasing the potential for adult neurogenesis through physical activity, the number of cells recruited increased following exposure to an identical enriched environment (Fabel et al. 2009). The size of the potential apparently determines how many cells are actually recruited from that pool, despite the fact that only a small fraction is recruited (Kempermann et al. 2003), which means that a large reserve of recruitable cells exists. The two effects, nonspecific and specific, are additive.

The baseline of adult neurogenesis changes over time, in particular during the first few weeks of life in a rodent, which corresponds to the early years in a human. Subsequently, adult hippocampal neurogenesis remains fairly stable at a very low rate (Kempermann et al. 1998; Kuhn et al. 1996; Encinas et al. 2011; Ben Abdallah et al. 2010), which appears to be sufficient to allow for the abovementioned functional contributions. An explanation for this surprising fact is that activity in the dentate gyrus is very sparse, which means that few excitable young cells can have a large impact. The other explanation is that there is a massive convergence of input between the dentate gyrus and CA3. Adult neurogenesis takes place in a network bottleneck, so even a small number of added cells can have a large impact due to this strategic position. Long-term stimulation maintains adult neurogenesis at an increased level, even if started at an older age; in addition, after stimulation withdrawal, precursor cell proliferation remains high (Kempermann et al. 2002; Kempermann and Gage 1999). Sustained physical activity had the same effect—increasing precursor cell proliferation, which, presumably in the absence of relevant

cognitive stimuli, was not translated into the generation of new neurons (Kronenberg et al. 2006). These results indicated that even relatively generic or perhaps moderate physical or cognitive stimuli are sufficient to maintain a level of adult neurogenesis. Notably, in these experiments, the stimuli were chronic and lacked particular challenges, suggesting that even though acute regulatory peaks wear off fairly soon, a sustained positive effect of the stimuli (via increased neurogenesis and perhaps linked improved behavioral performance) might remain afterwards (Kronenberg et al. 2006). Consequently, over time, acute peaks of activity might matter less than the overall level of activity. Long-term enrichment and similar manipulations affect the baseline potential of neurogenesis, as well as the level of neurogenesis.

With this mechanism, activity contributes to what one might call a "neurogenic reserve" (Kempermann 2008). By leading an active life, one is able to maintain neurogenesis and its potential at high levels, allowing for better coping mechanisms with increased cognitive demand at older age. However, this increase is relative; at a very young age, the stimulation of adult neurogenesis due to stimulation is an order of magnitude stronger. If the stimulation begins later in life, the effect is very large in relative terms, but very small in absolute numbers(Kempermann et al. 1998) and presumably much lower than in cases where the maintenance effect had been present earlier and for longer periods of time. Future studies are needed to determine the latter direct comparison, but individual studies performed to date suggest so. The neurogenic reserve is, thus, not built de novo, but to a large degree dependent on what has happened before. It might still be beneficial to become active at a later time point, but it is definitely more beneficial to start early. Activity would reduce the difference between projected age-related decline, as worst-case scenario, and the potential best case. This best case is presumably always less than what could be achieved in childhood and adolescence. Maintaining adult neurogenesis by providing the appropriate stimulation and challenges would allow for the best personal trajectory in the aging process.

This idea, although applied currently to the neurogenic reserve hypothesis, was derived from related ideas on more general theories of neural or cognitive reserves. These hypotheses describe the brain's range for compensation in the face of aging and neurodegeneration, but they do not comprise specific mechanisms. This is different in the case of adult neurogenesis, where maintenance of the functional potential provided by new neurons is not only a highly specific mechanism but also leads to highly specific functions. The neurogenic reserve is also more concrete than other postulated reserves; Ulman Lindenberger has cautioned that they should not be misunderstood as mechanisms of compensation without a preexisting functional basis (Lindenberger et al. 2013; Nyberg et al. 2012). The widely held idea, for example, that ischemic stroke-spared brain regions could take over the function of damaged areas is not generally correct and occurs possible only in rather special cases that require premorbid functional involvement of the new region. Such involvement might not been previously obvious. In this case, functional MRI images might be deceiving, because they show relative, not absolute, activities. Lindenberger and colleagues thus propose that "maintenance" is the more appropriate term than "compensation" (Lindenberger et al. 2013; Nyberg et al. 2012). Brain reserves,

unlike the last liters in a tank of gas or the liquid reserves in a bank account, are not set aside in the brain sets for harder times yet to come. Rather, activity maintains the entire system at a level of plasticity and flexibility corresponding to a much younger age. This reserve would be integrated into the overall functionality, much like a trained muscle, where fiber numbers do not have to increase to allow for sustained functional benefits, as long as atrophy is prevented.

Future studies involving adult neurogenesis that focus on factors that maintain, rather than only acutely boost, neurogenesis over longer periods of time might be more interesting and medically relevant. In the first case, one might focus on neurogenic "control," but rather proper "regulation" in the latter. Both terms are related, but not identical. Control applies to a baseline, from which acute regulation can depart. To date, most studies identifying individual "neurogenic factors" have focused on acute regulation. Identifying the mechanisms that underlie successful maintenance of adult neurogenesis across the lifespan is an entirely different question.

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Neural Stem Cells and Demyelinating Disease

Abbe H. Crawford and Robin J.M. Franklin

Functions of the Myelin Sheath

To understand the pathological significance of myelin loss, it is important to appreciate the functions of the myelin sheath. The oligodendrocyte is responsible for generating CNS myelin sheaths, which consist of lipid-rich membrane layers arranged concentrically around the axon. Their presence alters the electrical properties of the ensheathed region of the axon by creating an area of high resistance and low capacitance. The action potential effectively jumps across these regions of high resistance to the intervening regions of exposed axolemma in a process known as saltatory conduction—a form of rapid and efficient neuro transmission. Therefore, a primary function of myelin is to optimize electrical transmission (Kotter et al. 2011).

In addition to enabling saltatory conduction, there is growing evidence that the myelin sheath is required for long-term axonal survival. Transgenic mice lacking the integral myelin proteolipid protein (PLP) show long-term axonal degeneration, despite the presence of functional myelin that harbors only subtle ultrastructural alterations in compaction (Boison and Stoffel 1994; Klugmann et al. 1997). Axons were shown to develop focal accumulation of organelles at distal paranodal regions (Griffiths et al. 1998), indicative of impaired fast axonal transport (Edgar and Nave 2009). Similar degenerative changes were found in transgenic mice lacking other single myelin proteins, such as myelin-associated glycoprotein (MAG)

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(Pan et al. 2005) or 2′,3′-cyclic-nucleotide 3′-phosphodiesterase (CNP) (Lappe-Siefke et al. 2003), and in patients with Pelizaeus–Merzbacher disease, caused by mutations in the *Plp* gene (Edgar and Nave 2009). These studies revealed that minor perturbations of the oligodendrocyte can have significant long-term consequences for the axon (Franklin et al. 2012).

Currently, studies are examining the mechanisms by which the oligodendrocyte supports the neuron and its axon, and these mechanisms are likely to be varied. Ultrastructural observations suggest that myelinating glia can sequester aged axonal organelles (Spencer and Thomas 1974) and transfer trophic factors to the axon via direct transport (Novotny 1984) or secreted exosomes (Kramer-Albers et al. 2007; Wilkins et al. 2003). Furthermore, the oligodendrocyte appears to provide direct support to the axon by conveying glycolysis products to fuel oxidative phosphorylation (Nave and Trapp 2008; Rinholm and Bergersen 2012). A recent study demonstrated that the myelin sheath provides the axon with lactate via the monocarboxylate transporter 1 (MCT1) located in the myelin sheath; this provision is essential for neuronal survival (Lee et al. 2012).

A growing body of evidence for the role of the oligodendrocyte in preserving axonal integrity, independent from the ability to generate the myelin sheath, provides an explanation for the progressive atrophy observed in chronically demyelinated axons. It would also suggest that the therapeutic promotion of remyelination could prevent the neurodegenerative component of demyelinating disease (Franklin and Ffrench-Constant 2008).

Demyelination

Demyelination is the pathological loss of myelin sheaths from around axons. Sometimes referred to as "primary demyelination" it is the loss of myelin as a direct consequence of injury to the oligodendrocyte, and should be distinguished from Wallerian degeneration, in which secondary demyelination occurs as a consequence of primary axonal loss. From a clinical perspective, there are two major causes of primary demyelination in the central nervous system (CNS): genetic abnormalities that affect glia (leukodystrophies) and inflammatory damage to myelin and oligodendrocytes (Franklin and Ffrench-Constant 2008; Popescu and Lucchinetti 2012). Regardless of its cause, demyelination impairs neuronal function.

Chronic demyelination is the hallmark feature of multiple sclerosis (MS), an inflammatory demyelinating disease of the CNS, which remains among the most prominent and devastating disorders in contemporary neurology. Autoimmune-mediated attacks lead to oligodendrocyte destruction and the development of multiple diffuse foci of primary demyelination and acute axonal damage within the CNS. During the early stages of disease, the demyelinated regions are effectively remyelinated, but over time this process becomes progressively less effective (Fig. 1). Ultimately, areas of chronic demyelination develop in which exposed axons are susceptible to degeneration, leading to the secondary progressive neurological dysfunction characteristic of the later stages of MS (Peru et al. 2008; Ruckh et al. 2012).

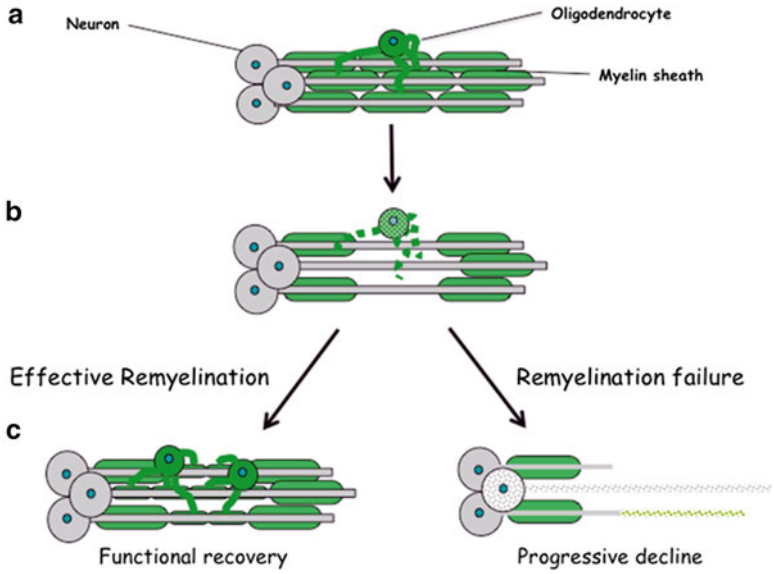

Fig. 1 Schematic representation of the CNS response to demyelination. (**a**) Oligodendrocyte extending processes to provide myelin internodes to ensheath multiple axons. (**b**) Demyelinating insult causes oligodendrocyte death, myelin sheath destruction, and axonal exposure. (**c**) Effective remyelination can replace lost myelin sheaths and enable functional recovery. However, in various demyelinating diseases, including multiple sclerosis, the remyelination response is incomplete, and axons are left exposed and susceptible to degeneration, with resultant clinical neurological decline

The etiology of MS remains poorly understood and the pathogenesis has been incompletely depicted. Genetic susceptibility is likely to be a major contributing factor, with additional influences from environmental factors and, potentially, as yet unidentified pathogens (Sotgiu et al. 2004). MS is a clinically heterogeneous disorder, and symptoms may manifest themselves in a relapsing–remitting or a progressive fashion, with the ultimate trend toward deteriorating neurological function and disability (Lublin et al. 2003). In cases of failed myelination, stimulation of remyelination could alleviate the major underlying causes of disability: impaired conduction by demyelinated neurons and axonal degeneration (Ruckh et al. 2012).

Understanding Developmental Myelination and Regenerative Remyelination

The developmental process of myelination and the adult regenerative process of remyelination share the common objective of ensheathing nerve axons with myelin. They may, therefore, be expected, as a consequence of evolutionary efficiency, to use the same mechanisms (Fancy et al. 2011). The "recapitulation hypothesis" of myelin regeneration states that mechanisms that underlie remyelination following injury are essentially the reinstating of a developmental myelination program

(Franklin and Hinks 1999). Therefore, a better understanding of this developmental program is an important objective, which could provide a crucial baseline from which to study remyelination and the reasons for its failure.

In the developing spinal cord, oligodendrocyte precursor cells (OPCs) arise in the motor neuron progenitor (pMN) domain of the ventral neuroepithelium from embryonic day 12.5 (E12.5) under the control of the ventral midline signal Sonic Hedgehog (Shh) (Richardson et al. 1997; Rowitch 2004). A later population of OPCs develops in the dorsal spinal cord, in dorsal neural progenitor domains 1 to 5 from E15.5 (Cai et al. 2005; Vallstedt et al. 2005). Soon after their generation, the OPCs migrate into the surrounding gray and white matter, reaching all regions of the developing CNS. Some OPCs identify axonal targets and differentiate into myelinating oligodendrocytes, while others remain reserved as immature cells throughout the parenchyma.

Given the widespread distribution of oligodendrocytes throughout the CNS, it is somewhat surprising that the founder cells of the oligodendrocyte lineage are highly localized in the developing neural tube while also raising the question of the functional rationale of producing oligodendrocytes at several dorsoventral positions (Miller 2005). Potentially, multiple origins are needed to produce a sufficient number of oligodendrocytes for effective myelination of all axons. Alternatively, separate embryonic origins could signify different functional subgroups of oligodendrocytes that have developed to meet specific needs. Different classes of neurons develop from different regions of the neural tube, so it is possible that glial products also differ (Richardson et al. 2006). Recent data suggests that both dorsal and ventral OPC populations contribute to remyelination (Zhu et al. 2011), but further work is needed to quantify and compare their roles in remyelination and to determine whether both populations are equally affected by disease and aging. Whether one population proves to be more (or less) effective at remyelinating particular tracts, or at remyelinating in general, could influence strategic approaches to myelin regeneration (Tripathi et al. 2011). Furthermore, if oligodendrocytes could be generated using multiple methods, then the potential for harnessing that capacity to promote recovery in the adult CNS would be significantly enhanced (Miller 2005).

OPC Development in the Forebrain

In a similar fashion to the spinal cord, OPCs are generated from multiple locations within the developing forebrain. Their initial production begins in the Nkx2.1-expressing ventral territories, beginning around E12.5. These cells disperse into all regions of the developing brain and are followed by a second wave of developing OPCs emerging from the Gsh2-expressing lateral ganglionic eminence. A final wave of Emx1-positive oligodendrocyte precursors originates dorsally in the cortex itself. Selective destruction of separate waves of OPCs via expression of a diphtheria toxin transgene resulted in neighboring populations spreading into the vacant

territory to restore normal oligodendrocyte distribution. Ultimately, mice in this study developed normally, suggesting that any functional difference between the three OPC populations was not crucial. Perhaps this compensatory redundancy serves to ensure rapid, fail-safe myelination and remyelination of the complex mammalian brain (Kessaris et al. 2006; Ventura and Goldman 2006).

Adult OPCs

Cultures of perinatal rat optic nerve cells lead to the first identification of glial progenitors (Raff et al. 1983). By exploiting the expression of a range of molecular markers, including nerve/glial antigen 2 (NG2) and platelet-derived growth factor receptor alpha subunit (PDGFRa), the natural history of glial progenitors was revealed (Ffrench-Constant and Raff 1986; Miller 1996; Richardson et al. 2006). Analysis of the adult CNS subsequently confirmed the presence of multiprocessed glial progenitor cells dispersed throughout the white and gray matter at a density similar to microglial cells, creating a continuous network of cells and cell processes (Chang et al. 2000; Dawson et al. 2003). The existence of these adult progenitors was immediately recognized to have important implications for the repair of demyelinating damage.

The relationship between OPCs in the perinatal CNS and in the adult was not immediately obvious, and it was not until the advent of genetic fate mapping approaches in transgenic mice that it was formally demonstrated that adult cells descended by lineage from their perinatal counterparts (Richardson et al. 2011; Zawadzka et al. 2010). The adult cells do, however, differ from perinatal OPCs in various ways, including specific antigenic markers and growth factor responsiveness. Additionally, the adult cells exhibit a slower basal motility rate and a longer basal cell cycle time (Fancy et al. 2011).

Speculation continues as to whether adult OPCs are, in fact, latent neural stem cells. One study demonstrated that OPCs purified from early postnatal rat optic nerves could be reprogrammed into multipotent stem cells. Primary treatment with fetal calf serum or bone morphogenetic proteins (BMPs) was followed by basic fibroblast growth factor (bFGF) to generate free-floating balls of cells (neurospheres) containing neural stem cells. Individual cells from these neurospheres could give rise to colonies containing a mixture of oligodendrocytes, astrocytes, and neurons, sparking hope that OPCs could be a regenerative resource for neurodegenerative diseases that involve neuronal and glial loss (Kondo and Raff 2000). However, further studies have shown that by far the most common differentiation products of parenchymal OPCs are oligodendrocytes, both in the normal and injured adult CNS. Some Schwann cell and astrocyte generation has also been documented (Richardson et al. 2011; Zawadzka et al. 2010).

It remains to be determined whether OPCs can generate neurons in vivo. Some PDGFRα-positive OPCs have been shown to accumulate in the piriform cortex and

acquire NeuN reactivity and morphology resembling piriform projection neurons (Rivers et al. 2008). However, further fate mapping of OPCs in the corpus callosum, motor cortex, and anterior piriform cortex failed to identify neuronal generation (Clarke et al. 2012). Therefore, OPCs may have an underlying potential to generate neurons but may not readily reveal that potential in the environment of the developing or damaged CNS. Future studies are needed to determine whether pharmacological interventions could redirect cells toward a neuronal fate (Richardson et al. 2011).

Defining Adult OPCs

Is there a case for referring to OPCs as adult stem cells? They are self-renewing and multipotent—criteria that are used by some to assign the term "stem cell." However, the absence of asymmetric cellular division in these cells and their rapid proliferative response to injury indicate that they have much in common with progenitor cells that amplify in transit in other stem cell-containing tissues, such as skin or bone marrow (Franklin and Ffrench-Constant 2008). As a result, the slightly ambiguous term of adult progenitor/stem cell is frequently used.

OPCs are abundant within the adult CNS, suggesting that they may have housekeeping roles beyond their function as a cell source for lesion repair, potentially by contributing to the maintenance of CNS homeostasis. An intriguing finding has been the identification of two distinct electrophysiological populations of OPC. One population expresses voltage-gated sodium and potassium channels, generates action potentials, and senses its environment by receiving excitatory and inhibitory synaptic input from axons while the other lacks action potentials and does not receive synaptic input (Karadottir et al. 2008). These two groups, however, do not correspond to dorsal and ventral developmental origins (Tripathi et al. 2011). Axonal-OPC communication has been shown to affect OPC proliferation and differentiation (Barres and Raff 1993; Li et al. 2010), and blocking action potentials retards myelination (Demerens et al. 1996). Thus, there is a great deal of cross-talk between axons and OPCs to potentially control myelination and remyelination, which could provide novel therapeutic targets (Bergles et al. 2000; Karadottir et al. 2008).

Germinal Niches

In the postnatal forebrain, "germinal niches" of neural stem cells have been identified that represent the remaining sites of neurogenesis that persist into adulthood. These niches are harbored in the sub-ventricular zone (SVZ) lining the lateral ventricles and in the subgranular zone (SGZ) of the dentate gyrus in the hippocampal formation, providing new neurons to the olfactory bulbs and hippocampus, respectively. In addition to neurogenesis, germinal niches act as a cortical source of astrocytes and

oligodendrocytes during postnatal development (Kazanis 2009). At present, the extent to which mobilization of adult neural stem cells contributes to myelin repair after injury remains poorly understood (Peru et al. 2008). Demyelination in nearby white matter regions enhances SVZ proliferation, and these cells migrate toward the area of demyelination and differentiate into oligodendrocytes (Nait-Oumesmar et al. 1999). Recent data has demonstrated that the human SVZ becomes reactivated in MS and contains early glial progenitor cells expressing Olig2 and Sox10 transcription factors (Nait-Oumesmar et al. 2007). However, whether these cells can travel to distant and diffuse lesions and contribute to significant repair remains to be seen.

The Biology of Remyelination

Injuries involving oligodendrocyte loss with axonal preservation have the potential to undergo complete cytoarchitectural reconstruction and restoration of the glial compartment, albeit with a thinner and shorter myelin sheath than would be expected for a given axonal diameter. Remyelination involves the generation of new, mature oligodendrocytes. OPCs are the source of remyelinating oligodendrocytes, as shown by genetic fate mapping (Zawadzka et al. 2010), and transplantation studies in which OPCs have been shown to remyelinate areas of demyelination with great efficiency (Groves et al. 1993).

Experimental demyelination studies, involving toxin injection directly into the CNS to create a focal area of demyelination, have revealed characteristic age-dependent patterns of remyelination, which begins with the initiation of an inflammatory reaction in response to toxic damage. Local astrocytes and microglia recognize the disturbance in tissue homeostasis and release a plethora of inflammatory mediators that activate local cells, including OPCs. Upon activation, OPCs switch from a quiescent state to an activated phenotype, becoming sensitive to chemoattractants and mitogens, such as PDGF and fibroblast growth factor (FGF) released by microglia and astrocytes (Murtie et al. 2005; Woodruff et al. 2004). The activation process involves changes in OPC morphology (Levine and Reynolds 1999; Reynolds et al. 2002), as well as the upregulation of several genes. Many of the upregulated genes are also active during developmental oligodendrocyte generation, such as the transcription factors Olig2, Sox2, and Nkx2.2 (Franklin and Ffrench-Constant 2008).

Following activation, the recruitment phase of remyelination begins. OPCs proliferate and migrate into the damaged area to ensure rapid repopulation of the lesion. Once in situ, the OPCs differentiate into mature oligodendrocytes and begin the process of generating a myelin membrane to ensheath exposed axons. The oligodendrocytes must first establish contact with the axon to be remyelinated, before then generating the myelin protein membrane. The membrane is concentrically wrapped and tightly compacted to form the myelin sheath, which is typically thinner and shorter than the pre-lesion sheath, as shown in Fig. 2. However, despite its smaller dimensions, the newly formed myelin sheath appears sufficient to ensure full functional recovery of the axon (Franklin and Ffrench-Constant 2008).

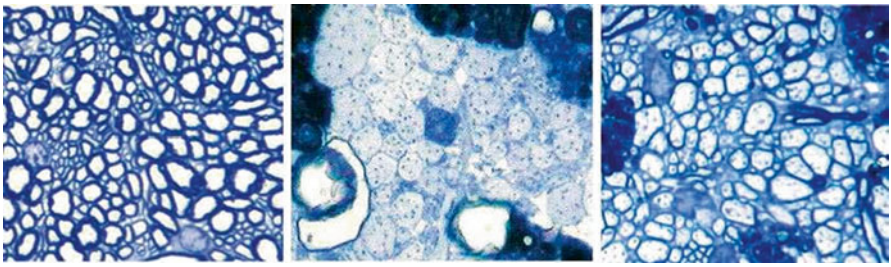

Fig. 2 Remyelination can be identified in resin-embedded tissue sections, as examined by light microscopy. The images in this series are transverse sections from the adult rat cerebellar white matter. Cross sections of normally myelinated axons of various diameters can be seen in the left-hand panel. The middle panel shows demyelinated axons, with debris-filled macrophages, 5 days after injection of ethidium bromide. In the right-hand panel, remyelinated axons with typically thin myelin sheaths 4 weeks after demyelination can be seen

Ultimately, remyelination serves to reinstate rapid axonal conduction, resolve functional deficits, and promote axon survival. Studies using OPC transplantation into demyelinated lesions have shown that remyelination protects axons (Irvine and Blakemore 2008), and analysis of MS autopsy samples have demonstrated that axonal survival is greatest in areas that have been remyelinated (Kornek et al. 2000). Future studies will determine whether therapeutic manipulation of remyelination can minimize or even reverse neurodegenerative changes.

The Impact of Aging on Remyelination

Almost every tissue studied has shown age-related decreases in the rate and/or efficiency of normal cellular turnover and regeneration in response to injury, strongly suggesting an age-related decline in stem cell function. The question arises as to whether age-associated declines in tissue regeneration are a consequence of intrinsic aging of stems cells or of impaired stem cell function in the aged tissue environment (Lombard et al. 2005; Park and Gerson 2005; Rando 2006).

The decline in remyelination that occurs with advancing age, compounded by an age-associated increase in the vulnerability of demyelinated axons to atrophy (Irvine and Blakemore 2008), poses a significant barrier to CNS therapy, particularly for long-term demyelinating diseases, such as MS. Although remyelination occurs more slowly in the aged CNS, it can nevertheless proceed to completion. This suggests that it is the rate of remyelination that is affected, rather than the extent (Shields et al. 2000). This declining rate is due to both a decreased efficiency of OPC recruitment into the demyelinated area, and of OPC differentiation into mature, remyelinating oligodendrocytes (Doucette et al. 2010; Shen et al. 2008).

What causes decreased OPC recruitment and differentiation with aging? Studies suggest that intrinsic determinants of OPC behavior and extrinsic environmental signals to which OPCs are exposed are altered in the aged CNS (Franklin and Ffrench-Constant 2008). For example, in vitro studies have revealed age-associated changes in growth factor responsiveness of adult OPCs (Tang et al. 2000; Zhao et al. 2006), while in vivo studies have demonstrated slower recruitment into precursor-depleted white matter of transplanted old adult precursor cells than of young adult cells (Chari et al. 2003). Furthermore, Shen et al. (2008) identified a critical age-associated change in the epigenetic regulation of OPC differentiation during remyelination. OPC differentiation is associated with recruitment of histone deacetylases (HDACs) to the promoter regions of differentiation inhibitors (Marin-Husstege et al. 2002). In aged animals, HDAC recruitment is impaired, resulting in prolonged expression of these inhibitors, delayed OPC differentiation and slower remyelination.

There are many extrinsic environmental signals that change with aging, but the altered inflammatory response is one of the most intensively studied at present. An impaired macrophage response in the aged animal, which is associated with delayed expression of inflammatory cytokines and chemokines, leads to poor clearance of myelin debris and, therefore, to persistent myelin debris-associated differentiation inhibitory proteins (Kotter et al. 2006; Zhao et al. 2006). Changes also occur in the expression of remyelination-associated growth factors, such as IGF1 and TGF-β1, resulting in delayed OPC recruitment and differentiation (Hinks and Franklin 2000).

A key question relating to the development of remyelination therapies is the extent to which age-associated changes can be reversed. Intriguing experiments on skeletal muscle regeneration, using the technique of heterochronic parabiosis, provided clear proof of principle that poor regeneration in aged animals could be rejuvenated (Conboy et al. 2005). A more recent study utilized heterochronic parabiosis to show that remyelination in an aged animal was improved by exposure to a young systemic circulation. The study demonstrated that monocyte recruitment from the young parabiotic partner into the aged mouse lesion contributed to improved remyelination efficiency by enhancing myelin debris clearance (Ruckh et al. 2012). Thus, despite their intrinsic alterations, aged OPCs remain responsive to exogenous pro-differentiation signals and retain their competence for efficient repair. These results provide hope that the endogenous adult OPC is a valid therapeutic target for remyelination-enhancing therapies.

Remyelination Failure

Remyelination is the default repair process following loss of myelin sheaths. However, failure to remyelinate is a major feature of several neurological diseases. While the most profound factor affecting remyelination is aging, as discussed above, other factors play important roles. Theoretically, remyelination could fail

due to a primary deficiency in precursor cells, a failure of precursor cell recruitment, or a failure of precursor cell differentiation and maturation. Data from experimental studies has disproved the first theory by demonstrating that OPCs are remarkably efficient at repopulating regions from which they have been depleted (Chari and Blakemore 2002). In addition, repeated episodes of focal demyelination in the same area neither deplete OPCs nor prevent subsequent remyelination (Penderis et al. 2003). OPC density in chronic lesions is, on average, lower than in normal white matter. However, OPC density can be as high as in normal white matter or remyelinated lesions, suggesting that OPC availability is not a limiting factor for remyelination (Kuhlmann et al. 2008). Nevertheless, the situation might be different when an area of tissue is exposed to a sustained demyelinating insult; in this case remyelination impairment seems to be due, at least in part, to decreased OPC availability (Mason et al. 2004).

In the second proposed mechanism, remyelination may fail due to inadequate OPC recruitment into the lesioned area, which involves proliferation, migration, and repopulation. Large lesions require a more protracted recruitment phase, and it has been calculated that it could take up to five months for a 2-cm lesion to become repopulated with OPCs (Chari and Blakemore 2002). OPC recruitment into areas of demyelination may fail owing to disturbances in local expression of OPC migration guidance cues, such as semaphorin 3A and 3F (Williams et al. 2007). This is likely to be more significant in aging, because older OPCs seem to be intrinsically less responsive to recruitment signals (Chari et al. 2003).

Most evidence, however, points to the importance of the third mechanism, whereby OPC differentiation and maturation is the rate-limiting step. OPCs unable to differentiate into mature oligodendrocytes were initially identified in MS lesions using the oligodendrocyte lineage marker O4 (Wolswijk 1998). This was subsequently verified with the OPC marker NG2 and most recently with Olig2 and Nkx2.2 (Chang et al. 2002; Kuhlmann et al. 2008). One possible explanation for failed differentiation is that chronically demyelinated lesions contain factors that inhibit precursor differentiation. The first factor to be implicated was the Notch–jagged pathway, a negative regulator of OPC differentiation (Wang et al. 1998). LINGO-1 and Wnt signaling have since been identified as negative regulators of differentiation (Fancy et al. 2009, 2010; Huang and Franklin 2011), while accumulation of glycosaminoglycan hyaluronan in MS lesions might further contribute to an environment that is not conducive to remyelination (Back et al. 2005).

In addition to the presence of inhibitory factors, the absence of stimulatory factors may play a role in remyelination failure. MS lesions are rarely devoid of any inflammatory activity. However, chronic lesions are relatively noninflammatory compared with acute lesions and constitute a less active environment in which OPC differentiation might become quiescent. The complexity of the environment needed for remyelination, along with the multitude of cell types, growth factors, and signaling molecules, would suggest that the presence or absence of a single factor is unlikely to make a significant difference. Additionally, efficient remyelination might depend as much on the precise timing of action of these cells and molecules, as on their presence or absence within the lesion. For example, a pro-recruitment environment must be maintained for a sufficient amount of time to allow the lesion

to become sufficiently repopulated with OPCs to enable complete remyelination; only when repopulation is complete should the environment shift to one that supports differentiation (Franklin and Ffrench-Constant 2008). A process as intricate as remyelination relies on the carefully coordinated interplay of a wide spectrum of cells and signaling molecules, making it susceptible to failure at multiple stages and through numerous causes. Future studies of therapeutic strategies for promoting remyelination have an enormous playing field on which to work.

Enhancing Remyelination

At present, there are no clinical therapies that promote remyelination. Two major approaches are currently being tested in animal models of demyelination: cell replacement by transplantation (exogenous therapies) and promotion of repair by resident stem and precursor cell populations in the adult CNS (endogenous repair) (Franklin 2002).

Exogenous Therapies

The use of transplanted stem cells for treating demyelinating diseases is an exciting prospect for patients facing chronic, debilitating illness. The ideal stem cell should be capable of effective remyelination in the presence of an ongoing inflammatory insult. Additionally, it should provide positive effects on the local environment, such as neuroprotection, axonal trophic support, and immune modulation. Given the multifocal nature of many demyelinating diseases, an ability to travel within and between lesions following systemic administration would be beneficial.

Transplantation therapies entail administration of cells into the demyelinated lesion. The CNS presents a particularly inaccessible target for cell delivery, especially in diseases with diffuse lesions. Repeated local injections are technically demanding and carry the risk of intracerebral hemorrhage, advocating the development of alternative experimental approaches, including intracerebroventricular and intravenous injection (Ben-Hur 2011). Stem and precursor cells injected into the lateral ventricles have been shown to enter the CNS and generate myelin-forming oligodendrocytes in widely dispersed areas (Ben-Hur et al. 2003; Pluchino et al. 2003). This route presents a potential therapeutic approach, although the risk of cell aggregates blocking intraventricular flow of cerebrospinal fluid remains a theoretical concern. Intravenous administration is straightforward to perform, but significant subsequent stem cell recruitment into CNS lesions has yet to be demonstrated (Pluchino et al. 2005).

The effectiveness of any stem cell transplantation and remyelination therapy depends on the ability to suppress the effect of any ongoing disease process on new oligodendrocytes. In diseases such as periventricular leukomalacia (PvL), this is not an issue, because oligodendrocyte loss is due to an ischemic insult occurring in

utero or at the time of birth and is, therefore, transient. Equally, for the leukodystrophies, such as Pelizaeus–Merzbacher disease, cells delivered by transplantation will not have the pathogenic genetic mutation and will, therefore, not be subjected to the cell autonomous effects of the genetic defect. For MS, however, the issue of ongoing damage is a major concern. Furthermore, injecting precursor cells into an environment that already contains an abundance of normal precursor cells does not seem wholly rational. Exogenous therapies are likely to show the most promise in specific demyelinating diseases that lack normal OPCs and do not have an ongoing disease insult (Franklin and Ffrench-Constant 2008).

Neural Stem Cells

The CNS contains a heterogeneous population of mitotically active stem and precursor cells that are capable of generating all postmitotic cell lineages of the CNS. Grouped together, these neural stem cells (NSCs) have been explored for their potential therapeutic use in demyelination (Gupta et al. 2012; Kingwell 2012; Uchida et al. 2012). NSCs can be obtained from embryonic (Fig. 3), fetal, neonatal, or adult CNS tissues. Induced pluripotent stem cells and autologous biopsies may become viable sources in the future (Ben-Hur 2011), with recent work validating the generation of functional postmitotic neurons and oligodendrocytes (Czepiel et al. 2011; Karumbayaram et al. 2009; Swistowski et al. 2010; Tsuji et al. 2010).

Intravenous administration of NSCs led to the amelioration of symptoms in experimental autoimmune encephalomyelitis (EAE), an animal model of MS (Pluchino et al. 2003), suggesting that these cells might enter the CNS from the cerebral vasculature. Magnetic resonance tracking has since shown incorporation of NSCs into CNS lesions following transplantation (Politi et al. 2007) and human NSCs, when implanted into demyelinated rat spinal cord, were shown to initiate a remyelination program (Akiyama et al. 2001). A recent study has shown that human

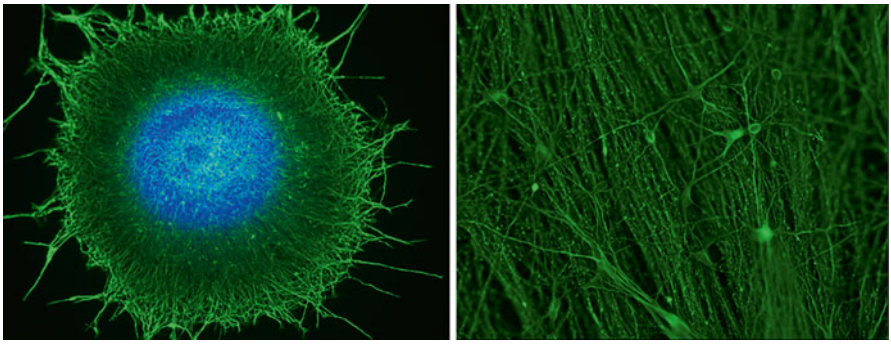

Fig. 3 Neurosphere grown from human embryonic stem cells. Stained for β3-tubulin. Images courtesy of Dr. S.R.L. Stacpoole

NSCs can differentiate into myelin-producing cells when transplanted into the brains of myelin-deficient mice, with extensive migration of the transplanted cells along white matter tracts and increasing myelination, as detected by magnetic resonance imaging (MRI) (Uchida et al. 2012). Similar findings have been reported in humans, in which a phase I, open-label trial was undertaken in four young males with global hypomyelination due to the congenital leukodystrophy, Pelizaeus–Merzbacher disease. The patients underwent NSC transplantation into the superficial subcortical white matter. Over a 12-month follow-up period, MRI revealed enhanced myelination around implantation sites, which was associated with stable or modest gains in motor and cognitive function. The findings suggested donor-derived myelination in the cell transplantation region (Gupta et al. 2012), and a phase II efficacy study of human NSCs is currently being planned (Kingwell 2012).

Conflicting studies have, however, revealed poor NSC differentiation into neuronal and glial cells, with minimal replacement of damaged cells (Martino and Pluchino 2006). Growing evidence suggests that much of the beneficial effects of NSC transplantation result from modulation of the CNS microenvironment rather than by direct cell replacement. Systemically delivered NSCs have been shown to migrate to areas of CNS damage and subsequently release immunomodulatory soluble molecules, such as cytokines and chemokines, to ultimately promote recovery in EAE mice (Einstein et al. 2003; Pluchino et al. 2005) and nonhuman primates (Pluchino et al. 2009). Furthermore, an immunosuppressive effect of NSCs on T-cell populations has been documented in the perivascular space of the CNS and in peripheral lymph nodes, with a pro-apoptotic influence, as well as reduced proliferation of antigen-specific encephalitogenic T cells (Einstein et al. 2003, 2007; Pluchino et al. 2005).

In addition to protecting the CNS from autoimmunity, NSCs have been shown to reduce astroglial scarring, enhance endogenous progenitor cell function, and increase survival of mature neural cells (Martino et al. 2010; Martino and Pluchino 2006; Pluchino et al. 2005). The induction of an environment permissive to axonal regeneration has been documented (Pluchino et al. 2005), as well as neurotrophic growth factor production, including nerve growth factor and brain-derived neurotrophic factor (Lu et al. 2003). Additionally, transplanted NSC induction of matrix metalloproteinases has been detected, potentially with the purpose of degrading the extracellular matrix, thereby facilitating axonal outgrowth and passage through the inhibitory glial scar (Zhang et al. 2007).

Enhanced remyelination efficiency has been reported following NSC administration in a spinal cord injury model, with both endogenous OPCs and the transplanted cells contributing to this enhanced repair (Keirstead et al. 2005). In aged rodent models, endogenous OPCs underwent increased proliferation and differentiation following NSC transplantation, hence improving the rate and extent of remyelination (Einstein et al. 2009). Therefore, growing evidence would suggest that NSCs promote CNS repair and that they act not through direct cell replacement but by modulation of the immune response and support of endogenous repair activities exerted by resident precursor cells. Translating these initial findings into controlled clinical trials to obtain rigorous safety and efficacy data will be the next challenge.

Oligodendrocyte Precursor Cells

When transplanted into regions of demyelination, OPCs elicit remyelination and restore neurological function (Windrem et al. 2004, 2008) and thus may represent an alternative source for stem cell therapies. One significant disadvantage, however, is the apparent limited ability for OPCs to migrate through normal intact regions of the CNS (Blakemore et al. 2000; Gensert and Goldman 1997), thereby necessitating direct targeting of individual lesions for transplantation. Sourcing sufficient numbers of autologous OPCs presents another difficulty, although the potential future use of autologous-induced pluripotent stems cells may provide a solution.

Adult OPCs exhibit a protracted cell cycle time and can be considered to exist in a somewhat dormant state, requiring prolonged growth factor stimulation before becoming proliferative, responsive cells (Psachoulia et al. 2009; Wolswijk and Noble 1992). As a result, precursors derived from embryonic (Fig. 4) or early postnatal CNS tissues may offer superior migratory, proliferative, and differentiation capabilities and may, therefore, represent a more effective remyelinating cell source than endogenous cells (Ben-Hur 2011). Results from one study demonstrated that fetal OPCs, following transplantation into the congenitally dysmyelinated brain, migrated more widely and engrafted more efficiently compared with adult OPCs. However, the adult cells generated oligodendrocytes more efficiently and were able to myelinate the recipient brains more rapidly (Windrem et al. 2004). Further work is needed to determine the potential efficacy of OPCs within the clinically diseased CNS, as well as to clarify the range of therapeutic effects. At present, there is no evidence to indicate that transplanted OPCs, whether administered systemically or locally into the CNS, have immunomodulatory properties (Archer et al. 1997; Ben-Hur 2011; Brustle et al. 1999; Jadasz et al. 2012).

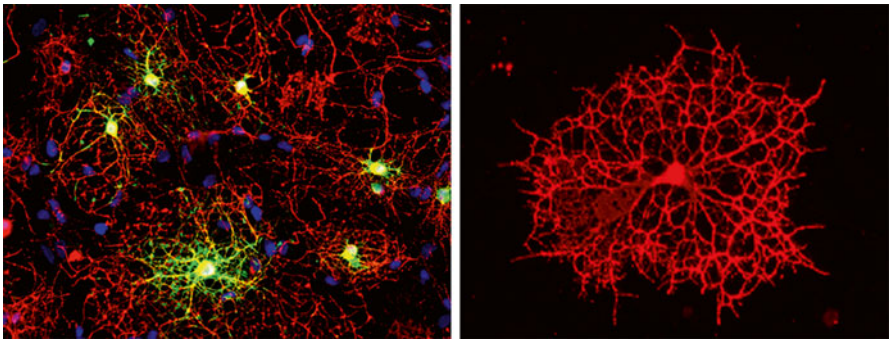

Fig. 4 Human embryonic stem cell-derived oligodendrocytes. Stained for O4 (*red*) and myelin basic protein (*green*). Images courtesy of Dr. S.R.L. Stacpoole

Olfactory Ensheathing Cells

Olfactory ensheathing cells (OECs) are differentiated glial cells that ensheath the axons of the first cranial nerve and can be isolated from nasal mucosal biopsies, thereby providing a readily accessible autologous source. OECs appear capable of remyelinating experimentally demyelinated axons (Barnett et al. 2000; Franklin et al. 1996; Imaizumi et al. 1998; Kato et al. 2000), with additional neurotrophic factor secretion (Lipson et al. 2003; Woodhall et al. 2001) and promotion of axonal growth (Chung et al. 2004; Jiao et al. 2011; Ramon-Cueto et al. 2000; Runyan and Phelps 2009). Embryonic-derived OECs were found to achieve the most efficient remyelination within the rat spinal cord, compared with fetal and adult sources (Coutts et al. 2012). Preliminary trials of autologous OEC transplantation into the injured spinal cord in both humans and dogs have demonstrated the feasibility and safety of this technique (Feron et al. 2005; Jeffery et al. 2005; Lima et al. 2006; Mackay-Sim et al. 2008), while a recent study demonstrated improved locomotor outcome in chronic, clinical spinal cord injury in the dog (Granger et al. 2012). Further work is needed to ascertain the long-term benefits of such therapies and their potential role as a component of a multifaceted, therapeutic approach to demyelinating disease.

Schwann Cells

Schwann cells are neural crest-derived myelinating cells of the peripheral nervous system (PNS). However, they have been found to mediate CNS remyelination in both experimental models and human demyelinating disease (Dusart et al. 1992; Itoyama et al. 1983). It was presumed that these central Schwann cells were derived from the peripheral nervous system, from sites such as the spinal and cranial nerve roots, and that they migrated into the CNS after disruption to the astrocytic glia limitans (Duncan and Hoffman 1997; Franklin and Blakemore 1993). However, fate mapping has confirmed that the majority of Schwann cells in areas of CNS remyelination are derived from OPCs (Zawadzka et al. 2010). What could cause CNS-resident precursors to become Schwann cells, which are normally found only in the PNS? In a study using the EAE model, it was found that OPCs robustly generate oligodendrocytes, but very few Schwann cells, suggesting that the inflammatory microenvironment may exert a strong influence on the direction of OPC differentiation (Tripathi et al. 2010). In particular, BMPs, members of the transforming growth factor-β family of secreted growth factors, may stimulate Schwann cell differentiation. Pretreatment of adult OPCs with BMPs in vitro—prior to transplantation into demyelinated spinal cord—results in enhanced Schwann cell remyelination (Crang et al. 2004). Conversely, overexpression of Noggin, an inhibitor of BMP signaling, reduces Schwann cell remyelination (Talbott et al. 2005).

The implications of Schwann cell remyelination of CNS axons remain unclear. The major constituent of Schwann cell myelin is myelin protein zero (Mpz), compared with proteolipid protein (PLP) in oligodendrocyte myelin. Peripheral nerve myelin is generally not targeted in the autoimmune pathology of MS, potentially due to various myelin constituents providing a reduced antigenic target for immune cells (Duncan and Hoffman 1997). Thus, theoretically, remyelination with peripheral myelin may hinder progression of further autoimmune-mediated demyelination and so its promotion may prove beneficial in the treatment of MS. Both Schwann cell and oligodendrocyte remyelination are associated with a return of saltatory conduction (Smith et al. 1979); however, the relative ability of Schwann cells or oligodendrocytes to promote axon survival, a major function of myelin (Nave and Trapp 2008), has yet to be established. Further work is needed to ascertain whether OPC differentiation into Schwann cells has a beneficial or deleterious effect compared to oligodendrocyte remyelination.

Schwann cells isolated from a sural or sciatic nerve biopsy, expanded in culture, and transplanted into the CNS resulted in the production of compact myelin and restored normal conduction velocity (Bai et al. 2012; Baron-Van Evercooren et al. 1992; Blakemore and Crang 1985; Honmou et al. 1996; Kohama et al. 2001). Further work has assessed Schwann cell precursors isolated from the neural crest—specifically the boundary cap cells, which form the border between the developing PNS and CNS—and found these cells demonstrated enhanced migratory and myelinating properties (Aquino et al. 2006; Zujovic et al. 2010). Taken together, these studies suggest that Schwann cells may represent a promising candidate for cell replacement therapy in myelin disorders.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) reside in the bone marrow and most connective tissues and are characterized by their ability to differentiate into cells of the mesenchymal lineage, including osteoblasts, adipocytes, and chondrocytes (Minguell et al. 2001). MSCs have powerful immunomodulatory effects when administered systemically, and demonstrate "therapeutic plasticity" by adapting their fate and function to the specific environmental needs arising from different pathological conditions. Intravenously infused MSCs have been shown to reduce demyelination, increase neuroprotection, modulate inflammation, and enhance endogenous OPC differentiation and remyelination in the EAE model (Bai et al. 2009; Barhum et al. 2010; Gerdoni et al. 2007; Gordon et al. 2008; Kassis et al. 2008; Lanza et al. 2009; Lu et al. 2003; Zhang et al. 2005, 2006). The immunomodulatory effects of MSCs include promotion of T-cell anergy, stimulation of regulatory T-cell production, reduction of B-cell proliferation and differentiation, and interference with the antigen-presenting function of dendritic cells, with overall reductions in encephalitogenic T-cell proliferation, inflammatory CNS infiltrates, and, ultimately, demyelination (Beyth et al. 2005; Gordon et al. 2008; Kassis et al. 2008; Selmani et al.

2008; Uccelli et al. 2006; Zappia et al. 2005). In a phase I/II, open-safety clinical trial, MSCs were administered intravenously and intrathecally into patients with chronic MS or amyotrophic lateral sclerosis, a motor neuron disease. No major adverse effects were encountered following treatment, but minor adverse effects that included headache and transient fever were reported. An immunomodulatory effect was detected in the patients, including an increased proportion of regulatory T cells and a decreased lymphocyte proliferative response, while clinical follow-up showed an improvement in the mean expanded disability status scale (Karussis et al. 2010).

Several lines of evidence suggest that MSCs directly protect neural tissues through paracrine mechanisms (Li et al. 2002; Minguell et al. 2001; Uccelli et al. 2008) and in EAE mice that received MSCs axonal loss was significantly reduced (Zhang et al. 2006). MSCs are presumed to promote survival of damaged tissues by secreting large amounts of bioactive factors that inhibit scarring and apoptosis, as well as stimulating angiogenesis and mitosis of tissue-intrinsic stem or precursor cells. Studies have confirmed the presence of soluble factors that promote oligodendrocyte and neuronal survival, including hepatocyte growth factor, in MSC-conditioned medium (Bai et al. 2012; Steffenhagen et al. 2012), as well as having a strong antioxidant effect (Lanza et al. 2009). In a recent phase II clinical trial, autologous MSCs were safely given to patients with secondary progressive MS involving the visual pathways. Treated patients showed evidence of structural, functional, and physiological improvement in some visual endpoints, suggesting that the transplanted cells were inducing neuroprotection (Connick et al. 2012).

Intravenously administered MSCs have been visualized within demyelinated CNS lesions (Azizi et al. 1998; Gordon et al. 2010; Zhang et al. 2006), and some studies suggest that MSCs can transdifferentiate into cells of the neuroectodermal lineage (Deng et al. 2001; Li et al. 2000; Mahmood et al. 2001; Padovan et al. 2003; Sanchez-Ramos et al. 2000; Woodbury et al. 2000). These results suggest that MSCs could contribute to cell replacement in the injured CNS. However, there is a lack of clear evidence that transdifferentiation occurs with any frequency within the damaged CNS, and there is no data to suggest that MSCs differentiate into oligodendrocytes (Gerdoni et al. 2007; Gordon et al. 2010; Jadasz et al. 2012; Uccelli et al. 2011). Nevertheless, various studies have demonstrated the ability of MSCs to recruit local OPCs to the site of demyelination, thereby inducing oligodendrogenesis and promoting remyelination (Bai et al. 2009; Constantin et al 2009; Cristofanilli et al. 2011; Zhang et al. 2005). Further work has revealed that MSCs activate oligodendrogenic differentiation and the maturation program in NSCs (Rivera et al. 2006), and when NSCs are transplanted with MSCs, they acquire an oligodendrocytic phenotype (Rivera et al. 2009).

Irrespective of little or no integration into the CNS, MSCs not only act in the periphery to inhibit autoimmune attack of the CNS, but they can also support tissue repair within the CNS (Uccelli et al. 2011). Given the abundance and accessibility of MSCs, the potential for autologous transplant purposes, feasibility of systemic administration, as well as relative simplicity of in vitro cultures, MSCs represent an attractive cellular tool for remyelination therapies. However, in cases of chronic inflammation and neural damage, MSCs appear unable to ameliorate the clinical

course of disease and improve neurological parameters, with their beneficial effects seemingly limited to acute disorders involving active inflammation (Uccelli et al. 2011; Zappia et al. 2005). Thus, the timing of transplantation may prove to be crucial. Cells introduced during the non-active phase of the disease could fail to survive until a time when their actions are required, while those introduced later in the disease progression may miss the window of active inflammation for which their immunomodulatory properties are adapted (Ben-Hur 2011; Uccelli et al. 2011).

Further work is now needed to ascertain the most appropriate diseases for MSC use, as the therapeutic dose range, optimal frequency, timing, and route of administration, with future rigorous controlled clinical trials focusing on these parameters in combination with the generation of improved safety and efficacy data.

In summary, the stem cell approach to the treatment of demyelinating diseases has shifted from focal injection of cells toward a systemic delivery and from the regenerative potential of precursor cells toward their immunomodulatory properties. However, it remains to be determined whether cellular injection is a better immunomodulatory strategy than the many pharmacological approaches to immunomodulation that currently exist. Furthermore, long-term survival of transplanted cells remains to be seen, particularly in an environment as "hostile" as MS. Encouraging results have emerged from preliminary clinical trials, but markers for myelin repair and neuroprotection are still lacking (Franklin et al. 2012). Their development would facilitate the determination of clinical efficacy of stem cell therapies in demyelinating disease.

Endogenous Therapies

Because remyelination can be complete, and because the precursor cells responsible for remyelination are abundant throughout the adult CNS, a conceptually attractive approach to enhancing remyelination is to target the endogenous regenerative process. Such an approach necessitates a detailed understanding of remyelination mechanisms and why it fails to then pinpoint areas suitable for therapeutic manipulation. Identifying the key regulatory pathways involved should enable the design of regenerative medicines that enhance remyelination, thereby also protecting axons. At present, research efforts are focused on stimulating OPCs directly and creating the appropriate local environment conducive to effective remyelination.

The inhibition of OPC differentiation in chronically demyelinated lesions presents a vital therapeutic target whereby direct OPC stimulation or antagonism of inhibitory factors could encourage effective remyelination. Several negative regulators of OPC differentiation have recently been identified, including LINGO-1 and Wnt signaling. LINGO-1 is a protein made by oligodendrocytes and neurons that inhibits oligodendrocyte differentiation and myelination. Intrathecal delivery of a LINGO-1 antibody in the EAE model was shown to enhance remyelination and increase axonal integrity, suggesting that it could serve as a candidate for therapeutic remyelination enhancement (Mi et al. 2004). Wnt signaling controls temporal and spatial regulation of cell proliferation, migration, and survival and has been shown to negatively regulate OPC differentiation during remyelination (Fancy et al. 2009).

Given the growing interest to develop Wnt inhibitors as cancer therapeutics, it may prove possible in the future to utilize these therapies for OPC differentiation stimulation and remyelination enhancement (Huang and Franklin 2011).

While most studies have focused on the negative regulators of OPC differentiation, positive regulators are now emerging, including leukemia inhibitory factor (LIF) and retinoid X receptors (RXRs). LIF is produced by myelin-reactive T cells infiltrating demyelinating lesions and has been shown to enhance oligodendrocyte survival and myelin repair while additionally decreasing demyelination (Deverman and Patterson 2012; Marriott et al. 2008). RXRs are nuclear receptors that regulate cell proliferation and differentiation and are highly expressed in active and remyelinating MS lesions. RXR knockout mice show an accumulation of undifferentiated OPCs in demyelinated lesions, while RXR agonists, such as 9-*cis*-retinoic acid, stimulate OPCs to differentiate in culture and enhance remyelination following focal demyelination in the rat CNS. RXR agonists can also attenuate inflammation by regulating macrophage activity. These agonists could provide dual benefits of immunomodulation and acceleration of OPC differentiation (Huang et al. 2011).

Pharmacological manipulation to generate a favorable environment for endogenous stem/precursor cells of the CNS is likely to generate new and valuable treatment options for demyelinating diseases. Furthermore, "environment-enhancing" therapeutics could be combined with stem cell transplantation therapies to improve survival and function of transplanted cells. In particular, manipulation of the inflammatory response to recreate the active environment needed for remyelination has been under considerable investigation. Much success has already been achieved with treatments such as interferon-β, which acts to shift a detrimental Th1 or Th17 immune response into a protective Th2 response, as well as alemtuzumab, an anti-CD52 humanized monoclonal antibody that causes long-term T-cell depletion (Cohen et al. 2012; Coles et al. 2012). The true complexity of the signaling environment needed for successful remyelination is being uncovered, as well as the enormous lesion diversity between diseases and between individuals with the same disease (Franklin et al. 2012). As this understanding expands, new and exciting treatment options are likely to come to light.

Conclusions

Since the discovery that OPCs are widely dispersed throughout the adult CNS, there has been a growing interest in regenerative therapies for demyelinating diseases. Much of the research has, and continues to, focus on unraveling the complex biology of remyelination to identify new means of therapeutic manipulation. Pharmacological approaches to enhance endogenous OPC differentiation and to create a pro-remyelination environment could ultimately be combined with exogenous cell transplantation therapies to provide an array of treatments to tackle these heterogeneous diseases and provide the best possible outcome for affected patients.

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Stem Cell-Based Therapies for Spinal Cord Regeneration

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Introduction

Approximately 12,000 new spinal cord injury (SCI) cases are reported in the USA each year (https://www.nscisc.uab.edu/PublicDocuments/fact_figures_docs/Facts%202012%20Feb%20Final.pdf). The sudden impairment/loss of motor, sensory, and autonomic functions turns the life of mostly young patients instantly upside down. To date, effective restorative therapies that target molecular and cellular mechanisms known to prevent structural and functional recovery are not available.

Most spinal cord injuries are induced by blunt trauma with subsequent compression of the spinal cord, leading to the disruption of axonal tracts. The loss of spinal cord parenchyma, including astroglia, oligodendroglia, and neurons, as well as microglial activation, macrophage and leukocyte infiltration, and demyelination of preserved axons, represents key factors contributing to the poor intrinsic regenerative capacity of the adult spinal cord. Moreover, growth-inhibitory factors, such as myelin-based inhibitors [i.e., Nogo, MAG (myelin-associated protein), and OMgp (oligodendrocyte myelin glycoprotein)], inhibitory extracellular matrix components [i.e., CSPG (chondroitin sulfate proteoglycans)], diffusible chemorepellents [i.e., semaphorins, ephrins, and netrin (Busch and Silver 2007; Giger et al. 2010)], and

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the lack of growth-promoting molecules [i.e., neurotrophic factors], represent additional components preventing recovery from SCI (Lu and Tuszynski 2008; Thuret et al. 2006).

To achieve improvement in the most severe complete SCI cases, cell replacement strategies are likely needed to compensate for the loss of spinal cord parenchyma at the site of injury, as well as remote regions, with the ultimate goal to induce functional recovery in spinal cord-injured individuals (Barnabe-Heider and Frisen 2008; Hulsebosch 2002; Collins 1983). Solely enhancing the intrinsic capacity for cell replacement seems insufficient to promote beneficial structural repair after a devastating trauma with substantial tissue damage.

Ideally, grafted cells should serve as a substrate for axonal growth, axonal remyelination, and replacement of lost neurons or as neuronal relays to reconnect damaged axons and secrete growth factors to promote axonal outgrowth/remyelination. Studies have identified numerous cell types that meet some or all of these objectives. The most attractive cell types can differentiate into neural phenotypes of the adult spinal cord in a phenotypically appropriate fashion. Olfactory ensheathing cells and Schwann cells derived from the peripheral nervous system (PNS) and the transition zone between the PNS and central nervous system (CNS), respectively, cannot replace lost cells in a phenotypically appropriate fashion. These cells do not reside in the CNS (Field et al. 2003; Ramon-Cueto and Avila 1998), yet they can elicit substantial axonal regrowth and myelination in the injured adult CNS (Akiyama et al. 2004; Blakemore and Crang 1985; Honmou et al. 1996; Imaizumi et al. 2000; Kohama et al. 2001; Weidner et al. 1999; Li et al. 1997; Lu et al. 2002; Ramon-Cueto et al. 2000; Sasaki et al. 2004; Steward et al. 2006; Takami et al. 2002; Lu et al. 2006). Other cell types, such as fibroblasts, which are not cellular components of the CNS, cannot mimic glial or neuronal function and can only serve as cellular substrates for axonal growth and/or vehicles for the local delivery of growth-promoting factors after genetic modification (Grill et al. 1997; Tuszynski et al. 2003; Jin et al. 2002). The present review will focus on neural stem/progenitor cells, which have the potential to differentiate into astroglia, oligodendroglia, and neurons and are thus theoretically able to reconstitute the original CNS tissue composition as close as possible.

Stem Cell Nomenclature

The developmental stage, at which cells are isolated, defines stem cells as embryonic, fetal, or adult stem cells.

Embryonic Stem Cells

Embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst and remain pluripotent, meaning that they retain the potential to give rise to all cell types of the three germ layers (ectoderm, endoderm, and mesoderm), with exception

to extraembryonic tissues (Marcus and Woodbury 2008). The first ES cells were isolated and cultivated from mice, and a few years later, human-derived ES cells followed (Reubinoff et al. 2000; Thomson et al. 1998). ES cells have the capacity for unlimited proliferation in vitro (Evans and Kaufman 1981; Martin 1981; McDonald et al. 1999), representing a promising cell type for transplantation paradigms. However, major concerns regarding ethical issues, the necessity for immunosuppression, the risk for tumor formation, and possible chromosomal instability after long-term cultivation challenge the widespread application of this particular cell type (McDonald et al. 1999; Vats et al. 2005; Nussbaum et al. 2007).

Fetal Stem Cells

Fetal stem cells are postembryonic cells, which are multipotent. Thus, they can only differentiate into cell types within a specific lineage, e.g., neural stem cells or hematopoietic stem cells. Fetal stem cells are isolated from specific regions, similar to adult stem cells, but they lack the differentiation potential of ES cells. The proliferative capacity of fetal stem cells is still superior in comparison to adult stem cells (Rao 1999; Pojda et al. 2005; Johe et al. 1996; Wu et al. 2002a; O'Donoghue and Fisk 2004). However, the need for immunosuppression following transplantation, as well as ethical concerns, presents major drawbacks for this method.

Glial- or neuronal-restricted precursor cells (GRPs or NRPs) can be isolated from the fetal spinal cord (rat E12 through E14) and are capable of self-renewal (Rao et al. 1998; Lee et al. 2000). GRPs can be maintained in culture for a prolonged time without loosing their differentiation potential (Wu et al. 2002b; Davies et al. 2006; Rao and Mayer-Proschel 1997; Rao et al. 1998; Gregori et al. 2002). In addition, they can differentiate into two distinct astrocyte populations (type 1 and type 2 astrocytes), as well as oligodendrocytes, but not into neurons (Wu et al. 2002b; Han et al. 2004). NRPs, on the other hand, can differentiate into several neuronal phenotypes, but not into oligodendrocytes or astrocytes (Kalyani and Rao 1998; Mujtaba et al. 1999; Rao 1999).

Adult Neural Stem/Progenitor Cells

Adult neural stem/progenitor cells (NSCs) are multipotent and can be isolated from two neurogenic regions: 1) the subventricular zone of the lateral ventricles, which normally gives rise to neurons of the olfactory bulb, and 2) the subgranular zone of the hippocampal dentate gyrus. In addition, adult NSCs also exist in non-neurogenic regions of the CNS, such as the striatum, neocortex, and spinal cord (Palmer et al. 1999; Palmer et al. 1995; Yamamoto et al. 2001; Weiss et al. 1996). Besides their potential for self-renewal, adult NSCs proliferate and can be expanded in vitro in the presence of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) (Roy et al. 2000; Wachs et al. 2003; Gritti et al. 1996). After withdrawal of

growth factors and/or exposure to serum, adult NSCs differentiate into neurons, astrocytes, and oligodendrocytes in vitro (Arsenijevic et al. 2001; Gage et al. 1995; Johansson et al. 1999; Reynolds and Weiss 1992; Weiss et al. 1996; Shihabuddin et al. 1997; Cao et al. 2002).

Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) are obtained by reprogramming differentiated somatic cells to induce a pluripotent, embryonic-like state. By introducing four genes (Oct3/4, Sox2, c-Myc, and Klf4) to mouse and human somatic cells, such as fibroblasts, these cells have been reprogrammed to become iPSCs (Takahashi and Yamanaka 2006; Park et al. 2008; Takahashi et al. 2007; Yu et al. 2007; Nakagawa et al. 2008). Another method to generate iPSCs is to replace the oncogenes c-Myc and Klf4 with NANOG and LIN28 (Yu et al. 2007). Similar to ES cells, iPSCs give rise to cells of all three germ layers (Takahashi and Yamanaka 2006). In the context of potential clinical applications, iPSC isolation from patient skin allows for autologous transplantation, thus avoiding ethical concerns and immunological problems (Salewski et al. 2010; Fujimoto et al. 2012; Tsuji et al. 2011). However, the yield of iPSCs obtainable from adult skin biopsies remains rather low. Furthermore, uncontrolled proliferation of iPSC grafts is a major concern and has to be closely monitored (Yamanaka 2009; Takahashi and Yamanaka 2006; Maherali et al. 2007; Maherali and Hochedlinger 2008; Wernig et al. 2007). Due to potential epigenetic alterations, inappropriate reprogramming, and the possibility of chromosomal aberrations, iPSCs are thought to be more tumorigenic than ES cells (Ben-David and Benvenisty 2011; Wernig et al. 2008; Yamanaka 2007; Miura et al. 2009). Therefore, each iPSC line needs to be pre-evaluated to assess the risk of tumor formation, as well as to confirm their safety following cell grafting, before their use can be translated into clinical applications (Tsuji et al. 2010).

Therapeutic Targets of Neural Stem Cell Transplantation

Remyelination

Axonal demyelination and loss of nerve conduction resulting from oligodendrocyte loss contributes to functional impairment after SCI (Gledhill et al. 1973; Hulsebosch 2002; Basso et al. 1996). Post-mortem studies in spinal cord-injured individuals indicate a preserved rim of axons in motor complete SCI (Kakulas 1999; Sherwood et al. 1992). However, these spared axons are frequently demyelinated (Kakulas 1999). The intrinsic capacity for oligodendroglial replacement, and consecutive remyelination of demyelinated axons in the adult spinal cord, remains limited. The poor remyelination capacity is at least partially due to inadequate proliferation and terminal differentiation of oligodendroglial precursor cells into mature myelinating

oligodendrocytes (Levine and Reynolds 1999). Therefore, approaches to enhance oligodendrocyte myelination represent a viable option for repairing the injured spinal cord.

In vitro, adult NSCs differentiate into neurons, astroglia, and oligodendroglia (Cao et al. 2002; Shihabuddin et al. 1997; Weiss et al. 1996). However, following transplantation into the injured spinal cord, adult NSCs differentiate predominantly into astroglia, with only few cells becoming oligodendroglia, while neuronal differentiation is essentially never found (Akiyama et al. 2001; Vroemen et al. 2003; Hofstetter et al. 2005; Cao et al. 2001; Pfeifer et al. 2006; Pfeifer et al. 2004; Mothe and Tator 2008; Vroemen et al. 2007; Karimi-Abdolrezaee et al. 2006).

Similar results have been reported for fetal-derived NSCs, which generate only a small proportion of cells (<10 %) that differentiate into oligodendrocytes up to 8 months post-transplantation in the injured spinal cord (Cao et al. 2001; Iwanami et al. 2005; Wu et al. 2002a). Other studies have shown that both ES cell and fetal NSC grafts contain significantly higher proportions of cells that express markers for mature oligodendrocytes (Salazar et al. 2010; Yasuda et al. 2011; Cummings et al. 2005; McDonald et al. 1999). The grafting of human brain-derived fetal NSCs into spinal cord-injured immunodeficient and myelin-deficient mice yielded up to 64 % adenomatous polyposis coli (APC)-positive oligodendrocytes, which were also associated with remyelination of axons 4 months after transplantation (Cummings et al. 2005).

Increased cell survival and oligodendroglial differentiation in vivo has been shown after infusion of growth factors (PDGF-AA, bFGF, and EGF), in combination with adult NSC grafts (Karimi-Abdolrezaee et al. 2006), grafting of ciliary neurotrophic factor (CNTF) expressing oligodendrocyte progenitor cells (OPCs) (Cao et al. 2010) or multi-neurotrophin-expressing GRPs (with BDNF and NT-3 activity) (Cao et al. 2005; Cao et al. 2010), and after grafting of neurogenin-2-expressing adult NSCs (Hofstetter et al. 2005). Survival/differentiation of graft-derived oligodendrocytes varied between 50 and 400 % in these studies. In contrast, the number of transplanted oligodendrocytes decreased, and neuronal differentiation increased following grafting of fetal NSCs in combination with valproic acid administration, a histone deacetylase inhibitor (Abematsu et al. 2010).

The pre-differentiation of NSCs into OPCs, which are already restricted toward an oligodendroglial lineage, is another method that has been utilized to increase absolute numbers of ES cell- and NSC-derived oligodendrocytes (Cao et al. 2010; Keirstead et al. 2005; Nistor et al. 2005). Depending on the maturation stage, OPC differentiation, survival, and migration differ significantly. Immature OPCs survive better after transplantation, migrate more extensively from the implantation site, and remyelinate more efficiently (Archer et al. 1997; Groves et al. 1993; Warrington et al. 1993; Zhang et al. 1999; Franklin 2002). In addition, OPCs derived from fetal or adult tissue, which have been purified by fluorescence-activated cell sorting of $A2B5^{+}$/PSA-$NCAM^{-}$ cells, result in different characteristics. Both OPC phenotypes mediate robust myelination; however, adult-derived OPCs myelinate more rapidly, generate a higher number of oligodendrocytes, and ensheath more host axons per donor cell. In addition, adult cells migrate shorter distances compared to fetal-derived OPCs (Windrem et al. 2004).

Graft-induced remyelination is mostly visualized by toluidine-blue- or Luxol fast blue-stained semi-thin sections and electron microscopy (Abematsu et al. 2010; Cao et al. 2010; Cummings et al. 2005; Keirstead et al. 2005; Yasuda et al. 2011; Zhang et al. 2007). The ratio between myelin sheath thickness and axon diameter can be used to discriminate remyelination by Schwann cells or oligodendrocytes from preserved myelin. Remyelinated axons typically exhibit thin myelin sheaths relative to axon diameter (Keirstead et al. 2005). These measurements indicate that oligodendrocyte-mediated remyelination, rather than prevention of oligodendroglial cell death, accounts for the observed behavioral recovery. Remyelination by grafted human fetal NSC-derived oligodendroglia has also been verified in shiverer mice, where engrafted human cells were selectively ablated with diphtheria toxin, resulting in secondary demyelination (Cummings et al. 2005).

In terms of locomotor recovery, minimal improvement of around 2 points on the Basso, Beattie, & Bresnahan (BBB) motor recovery scale (range from 0 to 21; 0 no observable movement vs. 21 normal locomotion) (Basso et al. 1996) was the most common finding in studies examining remyelination (Cao et al. 2005; Hofstetter et al. 2005; Karimi-Abdolrezaee et al. 2006; Keirstead et al. 2005; McDonald et al. 1999; Mitsui et al. 2005; Zhang et al. 2007). Two studies grafting human ES cell-derived OPCs and CNTF-overexpressing rat OPCs, respectively, reported an average improvement of around 4 points on the BBB locomotor rating scale (Cao et al. 2010; Keirstead et al. 2005), which was paralleled by restoration of neurophysiological parameters (transcranial magnetic motor-evoked potentials, magnetic interenlargement reflex responses) (Cao et al. 2010). In addition to oligodendroglial differentiation/remyelination, axonal sprouting around the injury site was reported as a structural correlate of transplanting CNTF-overexpressing OPCs. Transplantation of fetal NSCs into the contused spinal cord of non-obese diabetic (NOD)/severe combined immunodeficiency (SCID) mice elicited restoration of motor-evoked potentials and improved locomotor function (2 points in the Basso Mouse Scale; BMS). In control animals receiving grafts of fetal shiverer mice-derived NSCs, only thin, poorly formed, myelin sheaths were observed as the correlate of impaired functional outcome (Yasuda et al. 2011).

Axonal Regrowth

Axonal regrowth can contribute to structural repair via two different mechanisms: (1) transected axonal endings regrow across or around the lesion and reinnervate previous target neurons distal to the lesion; (2) remaining intact axons that bypass the lesion site extend collateral sprouts, which may partially compensate for transected axons. To accomplish true axonal regeneration across the lesion, a growth-permissive substrate that fills the cystic lesion cavity needs to be introduced. Cellular grafts not only provide a growth-permissive scaffold but also further recruit endogenous growth-promoting cells, such as Schwann cells, into the lesion site. Neurotrophic factors or factors that neutralize inhibitory molecules can also

promote collateral sprouting of axons. Local delivery of such growth-promoting molecules can be achieved via cell grafts, which produce relevant growth factors either naturally or following genetic modification.

Overall, few studies have reported neural stem/progenitor cell graft-mediated axonal regeneration/sprouting. Enhanced regrowth of anterogradely labeled corticospinal axons for a short distance into the graft has been shown following grafting of adult spinal cord-derived NSCs into rat cervical dorsal column transections (Pfeifer et al. 2004). Of note, grafted adult NSCs alone are not able to fill the cystic lesion site. Co-grafting with fibroblasts or bone-marrow stromal cells (Sandner et al. 2012) represents an effective strategy to maintain NSCs within the lesion site. Regenerating axons were found to be co-localized with graft-derived glial-fibrillary-acidic-protein GFAP-expressing astroglia, suggesting that NSC-derived astrocytes provide a cellular scaffold for injured axons (Pfeifer et al. 2004). Similar mechanisms have been described following transplantation of GRP-derived astroglia into the injured rat spinal cord (Davies et al. 2006). Fetal spinal cord-derived GRPs were purified through A2B5 sorting, and consecutive exposure to bone morphogenetic protein 4 (BMP4) in vitro resulted in differentiation of A2B5-negative astroglia. These GFAP-expressing astrocytes formed a permissive substrate for regenerating the ascending sensory and descending rubrospinal axons through and across the graft after unilateral lesions of the dorsal or dorsolateral funiculi. This observed axonal regeneration was paralleled by improved locomotor recovery, as assessed by grid-walk analysis. Interestingly, fetal grafts without astroglial differentiation failed to promote functional improvement.

Unmodified spinal cord-derived fetal NSCs, grafted as cell suspensions into rat spinal cord dorsal column crush lesions, were not able to fill the cystic lesion site and failed to promote corticospinal and sensory axonal regeneration (Webber et al. 2007). Infusion of CNTF antibody, in combination with fetal NSC-soaked Gelfoam grafted into a thoracic over-hemisection lesion in adult rats, resulted in regrowth of anterogradely labeled corticospinal axons at and caudal to the lesion site (Ishii et al. 2006). This effect was attributed to CNTF deprivation with consecutive reduction of astroglial scar formation rather than direct or indirect graft-mediated mechanisms. Limited axonal sprouting has also been reported in studies using co-grafts of fetal/neonatal cortical or hippocampal NSCs, together with olfactory ensheathing or Schwann cells (Wang et al. 2010; Wang et al. 2011), although the underlying mechanisms remain unknown. Neuroprotection, rather than graft-mediated enhancement of axonal growth, might be one mechanism resulting in increased axonal density in animals with grafts of stem/progenitor cells. Increased density of serotonergic, noradrenergic, and corticotrophin-releasing factor-positive axons caudal to the lesion site has been demonstrated following transplantation of NRPs/GRPs in rat thoracic contusion SCI. However, grafted cells were not spatially associated with these axons. Functionally, locomotor and bladder functions (reduced detrusor hyperreflexia) were improved (Mitsui et al. 2005).

Several studies reporting axonal sprouting/regeneration also described partial functional recovery (Davies et al. 2006; Mitsui et al. 2005; Wang et al. 2010; Wang

et al. 2011). An impressive improvement of locomotor function (BBB scores of 17 vs. 9.1 in the control group) within 12 weeks post-injury was demonstrated after co-grafting of fetal NSCs and olfactory ensheathing cells (Wang et al. 2010). Neonatal NSCs overexpressing TrkC, co-grafted with NT-3-overexpressing Schwann cells, also induced superior locomotor function (BBB score improvement from 0.5 in control animals to 7.6 in grafted animals). Moreover, motor- and sensory-evoked potentials recovered almost to the level of uninjured animals (Wang et al. 2011). Independent investigators need to replicate these findings before firm conclusions can be drawn. Taken together, at present there is only limited evidence for robust functional recovery by neural stem cell-mediated axonal regeneration.

Neuronal Replacement

Significant degeneration of lower motor neurons is observed in cervical and lumbar spinal cord injuries, which contributes to severe and irreversible paresis in upper and lower extremities. In these cases, therapeutic efforts should focus on neuronal replacement strategies, rather than aiming for long-distance axonal regeneration to promote functional recovery. In addition, transplanted neurons might serve as "relays" across the lesion in the injured spinal cord. Transected axons may form synapses with graft-derived neurons that can extend into the distal spinal cord to connect with appropriate target neurons.

A key prerequisite for neuronal replacement is that grafted stem/progenitor cells differentiate into appropriate mature neurons. However, unmodified adult NSCs do not differentiate into neurons after grafting to the injured spinal cord (Pfeifer et al. 2004; Pfeifer et al. 2006; Vroemen et al. 2003; Mothe and Tator 2008). In contrast, in the majority of studies, fetal NSCs have been shown to differentiate into neurons after spinal cord grafting (Yan et al. 2007; Okada et al. 2005; Cummings et al. 2005; Cummings et al. 2006; Ogawa et al. 2002), as demonstrated by immunohistochemistry (beta-III-tubulin, NeuN, and defined neurotransmitter phenotypes). Some of the fetal NSC graft-derived neurons also exhibited electron microscopic evidence of synapse formation with the host neurons (Cummings et al. 2005). Only limited evidence exists that relevant integration of graft-derived neurons into the spinal or supraspinal circuitry occurs. Robust neuronal differentiation into GABAergic and cholinergic neurons was demonstrated after grafting NSCs from human fetal spinal cord into the lumbar spinal cord of nude rats. Graft-derived neurons were innervated by host axons and subsequently established synapses with host neurons (Yan et al. 2007). However, these effects were investigated in a peripheral nerve lesion model (lumbar rhizotomy and excitotoxic root lesion), not in a SCI model. E13.5 rat spinal cord-derived NRPs/GRPs differentiated into GABAergic and glutamatergic neurons after grafting into a rat spinal cord lesion model (dorsal column transection) (Bonner et al. 2010). Axonal outgrowth over a limited distance into the host spinal cord from these neurons was promoted by injection of a lentiviral vector expressing the neurotrophic factor BDNF. In a subsequent study, the same cells

grafted into a dorsal column lesion at cervical level C1, followed by BDNF-expressing lentiviral injections, elicited sensory axonal regeneration into the graft, as well as graft-derived axonal outgrowth into the dorsal column nuclei along the neurotrophin gradient. Synaptic connections between sensory axons and graft-derived neurons on one side, and graft-derived neurons and dorsal column nuclei on the other side, were confirmed by electron microscopy and electrophysiological analysis. These findings provide convincing evidence for a "relay" function of stem cell grafts (Bonner et al. 2011).

Transplanted fetal spinal cord tissue into the neonatal injured spinal cord supports survival of axotomized neurons and axonal regrowth, resulting in locomotor improvement (Bregman and Reier 1986; Bregman 1987; Diener and Bregman 1998; Bernstein-Goral and Bregman 1993, 1997; Houle and Reier 1988). Fetal spinal cord grafts within the adult uninjured spinal cord were shown to promote host-derived axonal growth into the transplant. However, axons failed to grow across the graft and back into the host spinal cord (Kunkel-Bagden et al. 1992; Bregman et al. 1997; Bernstein-Goral and Bregman 1993). Based on a more recent study, which demonstrated remarkable axonal growth after grafting green-fluorescent-protein GFP-expressing rat cortical E14 NSCs into the lesioned rat motor cortex (Gaillard et al. 2007), NSCs derived from E14 GFP-transgenic rat spinal cord were transplanted subacutely into the completely transected adult rat spinal cord (Lu et al. 2012). The NSC grafts differentiated into astrocytes, oligodendrocytes, and neurons. Graft-derived axons elongated robustly from the thoracic graft site both into the white matter of the lumbar spinal cord and cervical spinal cord in a highly oriented fashion. Frequently, axon collaterals terminated in the spinal cord gray matter, and GFP-expressing axon terminals formed synapses with host dendrites, which suggested that graft-derived neurons form functional relays in the injured spinal cord. Importantly, axonal growth occurred despite the presence of numerous growth-inhibitory molecules in the injured spinal cord, underlining the importance of a proper neuron-intrinsic axonal regrowth capacity. A remarkable locomotor improvement of 5 points in the BBB locomotor scale was paralleled by partial restoration of nerve conduction across the transection site. Both locomotor and electrophysiological recoveries were abolished by spinal cord re-transection. In subsequent experiments, grafts of human fetal-derived stem cell lines achieved comparable results after thoracic spinal cord transection (Lu et al. 2012), which highlights their relevance for a potential clinical translation.

Neuronal replacement was also proposed as an underlying mechanism of functional recovery after grafting of iPSCs (Fujimoto et al. 2012; Nori et al. 2011; Tsuji et al. 2010). In these studies, murine- or human-derived neural progenitors generated from iPSCs were grafted into NOD/SCID mice at 7 to 9 days following SCI. The grafted cells survived, integrated into the host tissue, and differentiated into all three CNS lineages (neurons, astrocytes, and oligodendrocytes). The majority of these cells differentiated into neurons (50–75 %) (Fujimoto et al. 2012; Nori et al. 2011), which formed synaptic connections with host neurons. Roughly one third of the grafted iPSCs differentiated into astroglia and oligodendroglia. The exact mechanisms underlying the observed functional recovery remain to be determined.

Stem Cell Transplantation: Clinical Application

Clinical trials are mandatory to assess the risks and benefits of a cell-based therapy before such an approach can be translated into a routine clinical application. The point in time when a promising experimental therapy should be investigated in a clinical trial is frequently the subject of major controversy. On one side, expectations and hopes of patients asking for effective therapies to cure severe disabilities have to be addressed. On the other side, careful scientists ask for caution in conducting premature clinical trials. To ensure that clinical trials in SCI are justified and conducted properly, a consensus for standards for the appropriate conduct of clinical trials has been established (Tuszynski et al. 2007; Steeves et al. 2007; Lammertse et al. 2007; Fawcett et al. 2007). A number of case series/clinical trials using cell-based therapies in SCI have been conducted, which indicated different degrees of compliance with these guidelines (Hug and Weidner 2012). The majority of studies have investigated bone-marrow-derived cell grafts (Pal et al. 2009; Callera and do Nascimento 2006; Chernykh et al. 2007; Deda et al. 2008; Geffner et al. 2008; Kumar et al. 2009; Park et al. 2005; Sykova et al. 2006; Yoon et al. 2007; Saito et al. 2008). In this chapter, only clinical trials investigating NSC-based therapies will be reviewed.

In December 2010, StemCells, Inc. initiated a phase I/II clinical trial based on preclinical evidence that fetal human brain-derived stem cells can differentiate into myelinating oligodendrocytes and synapse-forming neurons mediating functional recovery in rodent models of SCI (Cummings et al. 2006; Cummings et al. 2005; Hooshmand et al. 2009; Salazar et al. 2010; Uchida et al. 2000). The trial received final approval in July 2011 and is currently recruiting patients. The aim of the trial is to assess the safety and preliminary efficacy in 12 patients with AIS (American Spinal Injury Association impairment scale) grade A–C thoracic SCI at three to 12 months post-injury. Patients receive cell suspension grafts injected directly into the SCI lesion site, followed by transient immunosuppression. Subsequently, patients will be assessed over a 1-year period to monitor safety and to measure recovery of sensation, motor, and bowel/bladder function. An additional 4-year follow-up observational study will conclude the study. As of January 2013, four patients have been enrolled. No side effects have been reported to date (Dr. Armin Curt, Zürich, personal communication).

Based on preclinical evidence (Sharp et al. 2010; Keirstead et al. 2005) that human ES cell-derived oligodendrocyte progenitor cells enhance remyelination and neuroprotection after SCI, Geron Corp. initiated a multicenter trial (Mayor 2010). In 2009, the first clinical trial to evaluate safety and efficacy (recovery of neurological function) following human ES cell transplantation in acute SCI was approved. Patients with a complete thoracic SCI (AIS A) were included. Two million human ES cell-derived oligodendrocyte progenitor cells were injected into the spinal cord lesion site within 14 days after injury, accompanied by immunosuppression with tacrolimus. Surprisingly, in November 2011, the company discontinued the clinical trial for financial reasons after five patients underwent the cell transplantation procedure. Apparently, the establishment of proper cell purification protocols, problems with cyst formation after transplantation, and slow patient recruitment caused

an unexpected rise in costs. Over the course of 12 years from bench to bedside, developmental costs for this therapeutic approach were estimated at $170 million (http://www.bbc.co.uk/news/health-11517680). According to the company, the therapy did not promote any improvement, but was well tolerated with no serious adverse events. (Kaiser 2011).

Summary and Conclusions

Preclinical studies investigating neural stem cell grafting in SCI reported partial structural and functional improvement. Based on this evidence, two clinical trials investigating neural stem cell grafts in SCI subjects were initiated. Unfortunately, the first phase I/II trial investigating ES cell-derived grafting in acute SCI was terminated. Despite the disappointment related to the premature termination of this clinical trial, the experience gathered from this pioneer study will significantly facilitate planning and execution of future clinical studies of this kind.

Current concepts employing neural stem cell grafts, as glial scaffolds for regenerating axons (Pfeifer et al. 2004; Davies et al. 2006) or as relays after neuronal differentiation (Bonner et al. 2011; Lu et al. 2012), hold great promise for promoting substantial structural restoration as a prerequisite for a successful regenerative therapy in SCI. Future research efforts need to focus on accessible stem cell sources, which do not raise ethical concerns. iPSCs – once issues of tumor formation and efficient cell propagation and differentiation have been addressed – may serve as a reliable and effective stem cell-based regenerative therapy in SCI. Moreover, relevant glial or neuronal phenotypes need to be enriched and further modified in their capacity to promote structural repair. Robust restoration of spinal neural circuits in animal models of chronic severe spinal cord contusion injury will be the most valuable predictor for successful clinical trials in SCI.

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Direct Reprogramming of Somatic Cells into Induced Neuronal Cells: Where We Are and Where We Want to Go

Giacomo Masserdotti and Benedikt Berninger

A Brief History of Reprogramming

The majority of development of a multicellular organism follows a seemingly straightforward and unidirectional path: from the zygote, a totipotent cell is generated following fusion of the male and female gametes, and the produced cells acquire ever more specialized functions. This differentiation process is accompanied by a gradual loss of stemness and cellular plasticity. In other words, cells progressively restrict their differentiation potential by shaping their gene expression profile (or transcriptome) via epigenetic mechanisms that (a) maintain a certain pool of genes that are active or poised for subsequent activation and (b) stably repress other genes that control or constitute alternate cellular fates. This (meta)stable state of gene expression patterns is commonly referred to as genetic programming of a given cell type.

Despite this largely unidirectional flow of differentiation, the possibility that a specialized somatic cell can revert back to a pluripotent state, or even re-specify towards a different specialized cellular fate, has fascinated scientists since the early

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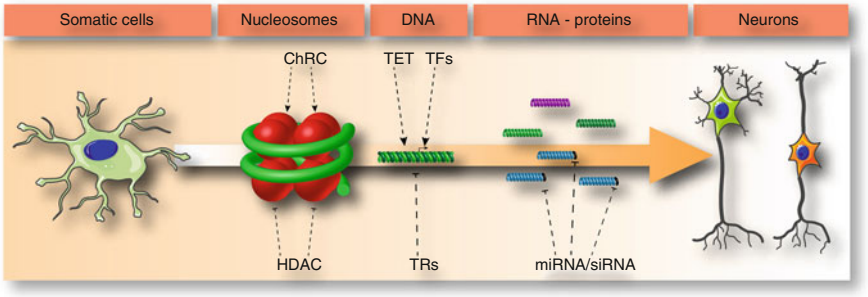

Fig. 1 Summary of different molecular mechanisms employed for somatic cell-to-neuronal reprogramming. Synergies between transcriptional and posttranscriptional/epigenetic mechanisms can be employed for successful conversion of cells that exhibit a higher barrier to reprogramming, such as cells of adult or human origin. *ChRC* chromatin remodeling complex, *HDAC* histone deacetylase, *TET* ten-eleven translocation protein, *TFs* transcription factors, *TRs* transcription repressors, *miRNA* microRNA, *siRNA* small interfering RNA

twentieth century, when DNA was still yet to be discovered. Hans Spemann, awarded the Nobel Prize in 1935 for the discovery of the "Organizer Center," performed the first experiment of somatic cell nuclear transfer in 1928. By transferring a nucleus isolated from a single cell of a 16-cell salamander embryo to an enucleated cell of another salamander embryo, Spemann obtained viable embryos that developed into normal salamanders. This simple, yet groundbreaking, experiment was a major milestone for subsequent cloning attempts, ultimately leading to the successful cloning of mammals. His technique employed two fundamental concepts: firstly, a nucleus from a terminally differentiated cell retains all the information required to generate a fully viable organism, and, secondly, this information can be reactivated upon proper conditions (transfer into a totipotent enucleated cell).

It took about 40 years to definitively prove that the nucleus from a fully differentiated adult cell can be re-instructed, or reprogrammed, to generate an entire new organism. In 1962, John Gurdon showed for the first time that an enucleated oocyte from the African frog *Xenopus laevis* could generate an adult frog following transfer of a nucleus isolated from a mature intestinal cell (Gurdon 1962).

Although these experiments demonstrated the possibility to reprogram the nucleus of a differentiated cell towards a pluripotent state, virtually nothing was known about the underlying mechanisms of pluripotency reactivation. For instance, how many gene products are required to successfully reprogram a somatic nucleus? And would it be possible to reprogram one type of differentiated cell into another without passing through a pluripotent intermediate state by a process coined direct lineage reprogramming?

In 1973, Peter Jones reported the ability of an anticancer drug, 5-azacytidine, to convert cultured fibroblasts into muscle cells, and 14 years later, Lassar and Weintraub cloned the master gene responsible for this conversion—*MyoD* (Taylor and Jones 1979; Lassar et al. 1986; Davis et al. 1987). Subsequent experiments

showed that *MyoD* induces expression of muscle cell markers in different cell lines derived from pigment, nerve, fat, and liver cell types. This finding was initial proof that a single gene is sufficient to elicit a full differentiation program and inspired further research aimed at identification of similar master fate regulators. From an historical point of view, the remarkable discovery of Shinya Yamanaka, i.e., successful reprogramming of differentiated cells into pluripotent stem cells by four defined transcription factors, was the final logical step along a research line aimed at understanding the mechanisms that govern pluripotency and differentiation (Takahashi and Yamanaka 2006).

Following *MyoD* cloning, seminal works identified other genes capable of reprogramming specific cell types into other lineage-related cells. For instance, Thomas Graf's lab showed that C/EBPα or C/EBPβ converts B cells into macrophages at very high efficiency (Xie et al. 2004). Similarly, Douglas Melton's group demonstrated the feasibility of in vivo conversion of exocrine cells into β-islet cells by the expression of three transcription factors—Pdx1, Ngn3, and Mafa (Zhou et al. 2008) [for a comprehensive review, see Graf (2011)].

From Somatic Cells to Neurons

The abovementioned studies demonstrated that the fate of a differentiated cell can be altered: in all of these cases, however, direct reprogramming was observed between cells of a common germ layer origin (e.g., fibroblasts and muscle cells share a mesodermal origin, as do B cells and macrophages, while exocrine cells and β-islet cells are derived from the endoderm). This fact raised the question as to whether direct lineage reprogramming was achievable across distinct germ layers.

Vierbuchen et al. (Vierbuchen et al. 2010) investigated the possibility of reprogramming fibroblasts (cells of mesodermal origin) into neuronal cells (cells derived from the neuroectoderm). To this end, they introduced several transcription factors (Vierbuchen et al. 2010) into cultured murine embryonic fibroblasts (MEFs) or perinatal tail-tip fibroblasts (TTF) via lentiviral vectors and assessed the potential conversion of the transduced cells into neuronal cells. Among the genes examined, a combination of three transcription factors—*B*rn2 (Pou3f2), *A*scl1, and *M*yt1l, termed "BAM"—was the most efficient in generating synaptically active neurons (Vierbuchen et al. 2010). It is worth mentioning that some transcription factors (Pax6, Neurog2, Sox2, etc.) included in their screening, which were found to exert little, if any, effect in the assays, have been shown to reprogram other cell types into induced neurons. These results suggested the potential relevance of the cellular context. Wernig's laboratory further investigated the ability of the BAM combination to obtain neuronal cells from other cell types and showed that this was the case even for postmitotic hepatocytes. However, the cells left a trace of their origin, which may point to an incomplete erasure of an epigenetic memory (Marro et al. 2011). Finally, reprogramming of human fetal and perinatal fibroblasts required an additional fourth factor, NeuroD1, for efficient conversion (Pang et al. 2011), suggesting that cells of human origin exhibit more barriers to reprogramming.

However, as discussed below, direct lineage reprogramming can be markedly enhanced by interfering with signaling pathways (Ladewig et al. 2012).

Soon after the first successful conversion of fibroblasts into neurons, other labs reported combinations of transcription factors capable of specifically generating dopaminergic neurons (Caiazzo et al. 2011; Pfisterer et al. 2011; Kim et al. 2011) or spinal motor neurons (Son et al. 2011). Moreover, this approach could be used to generate neurons from skin fibroblasts isolated from Alzheimer's patients, revealing interesting differences in the processing of Aβ peptides between patient- and control-derived neurons, but not fibroblasts (Qiang et al. 2011). More recently, our group demonstrated that human pericytes are efficiently converted into induced neuronal cells upon co-expression of Ascl1 and Sox2 (Karow et al. 2012) (for a summary of combinations employed to date, see Table 1). Together, these results demonstrate high reproducibility and robustness of direct conversion by defined transcription factors.

Reprogramming Astroglial Cells into Neurons

The interpretations of Ramón y Cajal led to the thought that the mammalian brain was an immutable organ: in the adult, it [the brain] may undergo degenerative processes, or traumatic injury, but lacks any significant regenerative ability (Cajal 1914). However, two major discoveries of the last two decades suggest a rationale for aiming to repair the damaged brain from within: the fact that most, if not all, neurons generated during development are of glial origin and the discovery of the lifelong maintenance of neurogenic niches in the adult brain (Malatesta and Gotz 2013). The identification of the two neurogenic niches in the adult brain (i.e., the subependymal zone lining the lateral ventricles and the subgranular zone of the dentate gyrus) demonstrated that the brain retains a certain degree of plasticity, because newly generated neurons not only survive but successfully integrate into the already established neuronal circuitry, thereby making unique contributions to neuronal information processing (Marin-Burgin and Schinder 2012; Nakashiba et al. 2012; Alonso et al. 2012). Yet, it came as a surprise that the neural stem cells residing in both neurogenic niches exhibited astroglial/radial glial hallmarks (Kriegstein and Alvarez-Buylla 2009). This observation, together with the fact that neurons and classical parenchymal astroglia share a common radial glial origin (Malatesta et al. 2000; Noctor et al. 2001), raised the possibility of converting apparently non-neurogenic astroglia into neurons, if provided with an appropriate transcriptional program. These fascinating findings may eventually lead to healing the diseased brain by recruiting endogenous cells for self-repair. Such notions are even further reinforced by the observation that in diverse brain insults—stroke, stab wound injury, and epilepsy—parenchymal astroglia not only become reactive, dedifferentiate, and resume proliferation but also acquire the capacity to give rise to neurosphere-initiating cells with characteristics of neural stem cells (Wetherington et al. 2008; Ridet et al. 1997; Robel et al. 2011; Buffo et al. 2008; Sirko et al. 2013).

Table 1

Factors	Vehicle	Expression	Species	Cell of origin	Age	Neurons	References
Ascl1	Retrovirus	Constitutive	Mouse	Astrocytes	Postnatal	GABAergic	Berninger et al. (2007)
Ascl1 + Brn2 + Myt1l	Lentivirus	Inducible	Mouse	Fibroblast	Embryo	Glutamatergic	Vierbuchen et al. (2010)
Ascl1 + Brn2 + Myt1l + NeuroD1	Lentivirus	Inducible	Human	Fibroblasts	Adult	Glutamatergic	Pang et al. (2011)
Ascl1 + Brn2 + Zic1	Lentivirus	Constitutive	Human	Fibroblasts	Adult	Glutamatergic	Qian et al. (2012)
Ascl1 + Dlx2	Retrovirus	Constitutive	Mouse	Astrocytes	Postnatal	GABAergic	Heinrich et al. (2010)
Ascl1 + Lmx1a + Nurr1	Lentivirus	Inducible	Mouse	Fibroblasts	Embryo	Dopaminergic	Caiazzo et al. (2011)
Ascl1 + Lmx1b + Nurr1	Lentivirus	Inducible	Mouse	Astrocyte cell line	Postnatal	Dopaminergic	Addis et al. (2011)
Ascl1 + Sox2	Retrovirus	Constitutive	Human	Pericytes	Adult	Glutamatergic	Karow et al. (2012)
Brn2 + Ascl1 + Myt1l + Lhx3, Hb9, Isl1, Neurog2	Retrovirus	Constitutive	Mouse	Fibroblast	Embryo	Motor neuron	Son et al. (2011)
Brn2 + Ascl1 + Myt1l + Lmx1a + Foxa2	Lentivirus	Inducible	Human	Fibroblast	Embryo	Dopaminergic	Pfisterer et al. (2011)
miR124 + miR9* + NeuroD2 + Ascl1 + Myt1l	Lentivirus	Constitutive	Human	Fibroblast	Adult	GABAergic, glutamatergic	Yoo et al. (2011)
Neurog2	Retrovirus	Constitutive	Mouse	Astrocytes	Postnatal	Glutamatergic	Berninger et al. (2007)
sh-PTB	Lentivirus	Constitutive	Human, mouse	Fibroblast, cell lines	Embryo	GABAergic, glutamatergic	Xue et al. (2013)

With such vision in mind, a first attempt to convert astroglia into neurons was performed in a simplified context: the petri dish. Primary cultures of astrocytes from the mouse cerebral cortex, isolated at a postnatal stage when astroglia undergo massive expansion in vivo (Ge et al. 2012), were infected with a retrovirus encoding a neuronal fate determinant in radial glia, *Pax6* (Jordan et al. 1992; Gotz et al. 1998): as hypothesized, some *Pax6*-transduced cells acquired a neuronal morphology and expressed the neuronal marker βIII-tubulin (Heins et al. 2002), providing proof-of-principle evidence that astroglia can indeed be converted into neuronal cells.

To impose specific neuronal phenotypes, Berninger et al. (Berninger et al. 2007) transduced astroglial cultures with viruses encoding two key players of embryonic forebrain neuronal specification, *Neurogenin2* (*Neurog2*) and *Ascl1* (Berninger et al. 2007). Interestingly, both transcription factors induced a very efficient neuronal conversion of postnatal astrocytes—up to 70 % for Neurog2 and 40 % for Ascl1—and provided the first evidence that Neurog2 not only elicits generic neurogenesis but also promotes a specific neuronal fate. Indeed, similarly to in vivo development (see below), *Neurog2*-transduced cells expressed T-box brain 1 (Tbr1), a transcription factor characterizing the glutamatergic lineage (Englund et al. 2005; Hevner et al. 2001), already 4 days after reprogramming, while *Ascl1*-transduced cells were devoid of this protein. Both *Neurog2*- and *Ascl1*-reprogrammed neuronal cells showed a progressive acquisition of neuronal electrophysiological properties but failed to establish a functional presynaptic output. Thus, to further promote functional maturation of the induced neuronal cells, retroviral vectors engineered for resistance towards silencing and overall stronger expression were adopted in a following study to secure high and stable expression of the transgenes. Under these conditions, *Neurog2*-induced neuronal cells could form fully functional glutamatergic synapses (Heinrich et al. 2010; Heinrich et al. 2011). Conversely, *Ascl1*- and/or *Dlx2*-transduced astrocytes gave rise to neuronal cells exhibiting features of GABAergic neurons, such as action potential discharge patterns characteristic of distinct types of interneurons, GABAergic synaptic transmission, and GABA neuronal marker expression (Heinrich et al. 2010).

While sequential expression of Eomes/Tbr2 and Tbr1 during Neurog2-induced direct astroglial-to-neuronal conversion may be pivotal for acquisition of a glutamatergic phenotype, SoxC transcription factors appear to play a crucial role in reprogramming astroglia into induced neuronal cells; astroglia deficient for both Sox4 and Sox11 exhibited impaired neurogenesis upon forced expression of Neurog2 (Mu et al. 2012). Intriguingly, both SoxC factors play important roles in adult neurogenesis in the dentate gyrus, indicating that astroglial-to-neuronal conversion and physiological neurogenesis from adult neural stem cells share similar paths.

More recently, a successful attempt of converting astrocytes into dopaminergic neurons was reported (Addis et al. 2011). Postnatal astrocytes were transduced via a lentiviral system allowing for expression of the three transcription factors *Ascl1*, *Lmx1B*, and *Nurr1*. Reprogrammed cells were positive for the dopaminergic marker tyrosine hydroxylase (TH) and expressed other midbrain dopaminergic markers, such as Pitx3, Lmx1A, FoxA2, vMat2, and the potassium channel Girk2. Notably, induced dopaminergic neuronal cells fired action potentials and released dopamine, indicating neuronal functionality.

Another cell population endogenous to the brain that may lend itself for direct conversion into neurons comprises the so-called pericytes, a cell type tightly associated with microvessels in the healthy brain. Similar to astrocytes, pericytes become activated following CNS injury. For instance, following spinal cord injury, a subtype of pericytes becomes highly proliferative, thereby contributing to scar formation (Goritz et al. 2011). Intriguingly, a study from our own lab revealed that cells of pericytic origin proliferate in culture following isolation from the adult human cerebral cortex (Karow et al. 2012). Moreover, these cells proved amenable to direct reprogramming into induced neuronal cells when forced to express Sox2 and Ascl1. Interestingly, Sox2 alone has also been shown to instruct some degree of neurogenesis from cord blood-derived cells (Giorgetti et al. 2012), as well as to allow for the direct conversion of MEFs into neural stem cell-like cells (Ring et al. 2012).

The Role of Pro-neural Genes in Reprogramming

Looking at the combinations of factors employed for reprogramming and summarized in Table 1, one main conclusion can be drawn: the most effective combinations all contain at least one pro-neural gene (i.e., Ascl1 or Neurog2), as well as other factors that have been implicated in neurogenesis and fall into other transcription factor classes, like POU domain (Brn2), homeobox (Dlx2, Isl1), LIM domain (Lmx1a and b), basic helix-loop-helix (NeuroDs), forkhead (Fox2a), and SRY-box high-mobility group (Sox2) transcription factors. This points to a prominent role of pro-neural genes for neuronal reprogramming, similar to during development. Indeed genetic studies in *Drosophila melanogaster*, as well as vertebrate models, have provided evidence that a small number of genes that encode transcription factors of the basic helix-loop-helix (bHLH) class are both necessary and sufficient to initiate development of the neuronal lineages in cells of ectodermal origin. Therefore, these genes have been called "pro-neural genes."

In *D. melanogaster*, five genes have been found to regulate the early steps of neural development: achaete (ac), scute (sc), lethal of scute (lsc), and asense (ase), which form the achaete-scute complex (asc) and atonal (ato) (Garcia-Bellido 1979; Gonzalez et al. 1989; Villares and Cabrera 1987). Several genes homologous to asc and ato have been identified in vertebrates. The vertebrate asc family includes ash1 (Mash1/Ascl1 in mouse, Cash1 in chick, Zash1 in zebrafish, and Xash1 in *Xenopus laevis*). The number of vertebrate genes related to ato is larger, but only two of them (Math1/Atoh1 and Math5/Atoh5 in mouse) can be considered orthologous to the founding member. Other vertebrate ato-related genes can be grouped into distinct families (i.e., the neurogenin [Neurog], the NeuroD, and the Olig families), characterized by the presence of family-specific residues in their bHLH domain. Like other bHLH factors, pro-neural proteins are known to bind DNA as heterodimeric complexes formed with the ubiquitously expressed E proteins, namely, E12 and E47, encoded by the gene E2A, or with the proteins HEB and E2-2 (Cabrera and Alonso 1991; Massari and Murre 2000). These heterodimeric complexes bind DNA sequences that contain a core motif, CANNTG, known as E-box. Each pro-neural gene appears to have a preferential consensus site [for a review, see Bertrand et al. (2002)].

The Dual Role of Ascl1 in CNS Development

Identified in several vertebrate species (Guillemot and Joyner 1993; Johnson et al. 1990; Zimmerman et al. 1993), *Ascl1* is expressed in specific domains of the neuroepithelium already at 8.5 days post-coitus (dpc) and later becomes broadly expressed within the ventricular zones (VZ) of the CNS (Guillemot and Joyner 1993; Lo et al. 1991). The generation of *Ascl1*-mutant mice revealed the pivotal role for *Ascl1* in specifying progenitor cells towards neuronal fates (Guillemot and Joyner 1993; Casarosa et al. 1999; Ma et al. 1997). Consistent with particularly high *Ascl1* expression in the medial ganglionic eminences (MGE) (Casarosa et al. 1999), the development of this structure is most affected among telencephalic regions in *Ascl1-mutant* embryos. Defects, such as the loss of *Nkx2.1* (in the rostral MGE), *GAD67*, and *SCG10* (Casarosa et al. 1999) expression observed in *Ascl1*-mutants, are consistent with its role in neuronal specification and differentiation. However, pronounced MGE size reduction appears to be due to reduced proliferation of VZ precursor cells, as confirmed by bromodeoxyuridine (BrdU) incorporation analysis (Castro et al. 2011). In an attempt to gain insights into the molecular mechanisms underlying the apparently contradictory roles of *Ascl1* in regulating precursor proliferation, as well as exit from cell cycle and differentiation, Castro et al. (Castro et al. 2011) combined a genome-wide transcriptome analysis with loss-of-function and gain-of-function studies (Castro et al. 2011). Surprisingly, besides regulating genes involved in cell cycle exit, specification, and differentiation, Ascl1 was also found to increase expression of positive cell cycle regulators (e.g., *E2f1*, *Cdca7*, *Tead1* and *Tead2*, and *FoxM1*).

How does Ascl1 select the right targets at a given time? Although a comprehensive and definitive answer still remains to be shown, Castro et al. (Castro et al. 2011) showed that by analyzing promoters of several Ascl1 targets, that those upstream of genes stimulating cell proliferation contained a consensus binding site for CBP/RBP-Jk, the DNA-binding nuclear adaptor protein for NICD (i.e., the intracellular transducer of the canonical Notch signaling pathway) (Kao et al. 1998). Thus, cooperation between Notch signaling and Ascl1 may account for the positive effect on progenitor proliferation, while repression of the Notch signaling cascade could lead to Ascl1 activating the differentiation program.

Likewise, selection of the appropriate Ascl1 targets can be regulated by cooperation with the POU domain transcription factor Brn2 (Castro et al. 2006). For instance, the Ascl1 target gene Delta1 contains an enhancer in which a consensus binding site for POU domain-containing proteins overlaps with the E-box. Point mutations within this consensus site abrogate Ascl1-mediated activation of Delta1, indicating that expression of the gene requires binding of both Ascl1 and POU domain-containing proteins. Bioinformatics analysis identified additional Ascl1 targets that contain POU domain consensus sites, suggesting that cooperation between these transcription factors may be a more general mechanism to regulating genes in a context-dependent manner (Castro et al. 2006).

Reprogramming by Noncoding RNAs

Transcription factors are very powerful tools to manipulate cell fate because they can regulate the expression of many genes at once and in a concerted fashion. However, their effect may depend on the presence of specific cofactors, both defining and defined by the cellular context. Another similarly powerful class of regulators comprises the various kinds of fairly recently discovered noncoding RNAs, including long noncoding RNAs (lncRNAs), short interfering RNAs (siRNAs), microRNAs (miRNAs), and piwi-interacting RNAs (piRNAs) [for review, see Carthew and Sontheimer (2009); McNeill and Van Vactor (2012); Ponting et al. (2009)].

Indeed, many of these noncoding RNAs have been implicated as regulators of neurogenesis (Fineberg et al. 2009; Li and Jin 2010; Cochella and Hobert 2012). One particularly interesting feature of these noncoding RNAs is that they may not regulate a single target gene but can control the expression of several genes at one time, not unlike transcription factors. Moreover, by targeting key players in transcriptional regulation, they can also indirectly exert a global effect on gene expression. For instance, the microRNAs miR124 and miR9/9* interfere with expression of BAF53a, a component of the brahma-associated factor (BAF) complex expressed in neuronal progenitors, but not in neurons, where it is instead replaced by BAF53b (Yoo et al. 2009). Thus, given the fact that miR124 and miR9/9* are involved in neuronal differentiation, perhaps in part due to the regulation of the BAF complex, the Crabtree laboratory investigated the effect of forced expression of these microRNAs on human fibroblasts. To their surprise, they noted that some of the cells containing these microRNAs started to also express the neuronal marker MAP2 (Yoo et al. 2011). Moreover, consistent with the role of these microRNAs in regulating BAF complex composition, the authors observed BAF53a downregulation and conversely BAF53b upregulation. Conversion efficiency of the fibroblasts into neuronal cells was very low and strictly dependent upon joint expression of both miRNAs. To increase efficiency and maturation of the induced neuronal cells, they induced coexpression of several of the abovementioned transcription factors and found the combination of miRNAs and NeuroD2/Ascl1/Myt1l (DAM) to be most effective. Notably, the induced neuronal cells were either excitatory (VGLUT1 positive) or inhibitory (Dlx1, GAD67 positive); thus the combination does not seem to confer a defined neuronal subtype specification.

Another interesting target for miR124 is the polypyrimidine tract-binding (PTB) protein gene (Makeyev et al. 2007). This gene encodes a protein involved in RNA splicing and is downregulated upon neuronal differentiation, where is substituted by nPTB (Boutz et al. 2007; Zheng et al. 2012). However, it was unclear whether the PTB-to-nPTB switch would be sufficient to induce neuronal differentiation from nonneuronal cells. To this end, Xue et al. knocked down PTB via shRNAs in several cell types, including MEFs, and, surprisingly, found that these underwent a conversion into induced neuronal cells (Xue et al. 2013). This effect was at least in part attributed to the downregulation of SCP1, a RNA polymerase II serine phosphatase associated with the REST complex. Indeed knockdown of either REST or SCP1 induced MEF conversion into neuronal cells, albeit to a low degree (Xue et al. 2013).

The Impact of the Epigenetic Landscape on Reprogramming

As mentioned earlier, the first example of direct lineage conversion was provided when fibroblasts were treated with 5-azacytidine, an anticancer drug that induces DNA hypomethylation (Taylor and Jones 1979). This finding revealed that the epigenetic landscape exerts a crucial influence on the stability of a given cell fate and that removal of epigenetic markers reinstates plasticity to acquire alternative cell fates. Given that reprogramming factors must evoke an entire new transcriptional program, it remains unclear whether these factors can overcome the barriers imposed by epigenetic modifications established during cellular differentiation. Full reprogramming would be expected to ultimately result in the eradication of these modifications if they are incongruent with the new acquired cell fate. These questions remain unclear (Vierbuchen and Wernig 2012), but some studies have provided initial insight.

For instance, a recent study comparing the ability of two bHLH transcription factors, MyoD and NeuroD2, to drive target gene expression and ultimately activate a myogenic or neurogenic program, respectively, was found to depend not only on the presence of consensus binding sites and respective flanking regions but also on the epigenetic state and, thus, local accessibility for the transcriptional machinery (Fong et al. 2012).

Transcription factors recruit and stabilize a pre-initiation complex at the promoter of their target genes (Tsai and Nussinov 2011), but transcription will not occur while the DNA remains enwrapped around the nucleosomes (Richmond and Davey 2003). Thus, chromatin remodeling complexes are required to slide these nucleosomes away from the transcriptional start site, making DNA available for transcription (for a comprehensive review, see Lessard and Crabtree (2010); Li et al. (2007); Narlikar et al. (2002)). Of note, Brg1, the ATPase subunit of the BAF chromatin remodeling complex (also called SWI-SNF), has been shown to interact with several of the abovementioned transcription factors, including MyoD (de la Serna et al. 2001), *Xenopus* Neurog1, and NeuroD (Seo et al. 2005). Intriguingly, overexpression of Brg1 and BAF155, another subunit of the BAF complex, greatly increased reprogramming of fibroblasts into induced pluripotent stem cells by the classical Yamanaka cocktail of transcription factors (Oct4, Sox2, Klf4, and c-Myc) (Singhal et al. 2010), suggesting that chromatin remodeling, and consequently enhanced chromatin access, indeed renders reprogramming more efficient.

Other mechanisms that regulate the open or closed state of DNA include histone acetylation or methylation, as well as DNA methylation (Jaenisch and Bird 2003). Histone modifications appear to be relatively dynamic and serve to poise a gene towards transcription or repression, while DNA methylation confers a more stable repression state to a gene locus. DNA methylation is mediated by specific DNA methyltransferases (DNMTs) (for a comprehensive review, see Goll and Bestor (2005)), while the mechanism of active DNA methylation removal has been revealed only recently to be a part of the DNA repair machinery (Tahiliani et al. 2009; Ito et al. 2010). Ten-eleven translocation (TET) proteins can convert 5-methylcytosine

(5-mC) into 5-hydroxymethylcytosine (5-hmC), which, in turn, can be further modified by the AID/APOBEC family of deaminases and, ultimately, repaired into cytosine (Guo et al. 2011). Intriguingly, while 5-mC is associated with transcriptional repression, 5-hmC is linked to transcriptional activation. Consistent with a potential role of TET proteins during reprogramming, loss-of-function experiments have shown that TET proteins are required for the induction of Nanog expression during the early phase of reprogramming towards pluripotency (Doege et al. 2012). Likewise, Tet2 deletion impaired or delayed C/EBPα-mediated conversion of B cell into macrophages, together with activation failure of several myeloid genes 24 h after induction of C/EBPα activity (Kallin et al. 2012). The role of TET proteins in direct conversion of somatic cells into neurons still needs to be investigated.

To date, the most remarkable example of the importance of epigenetic mechanisms in regulating cell fate decision and reprogramming originates from in vivo experiments in *Caenorhabditis elegans* (Tursun et al. 2011; Patel et al. 2012). In *C. elegans*, the transcription factor CHE-1 is required to convey the identity of a specific class of gustatory neurons, the so-called ASE neurons. When overexpressed at early embryonic development, CHE-1 induced broad activation of a reporter of CHE-1 activity, indicating that, at this stage, CHE-1 functioned with little constraint. However, when CHE-1 was expressed postembryonically, only a small number of sensory neurons expressed markers for terminal ASE fate, suggesting that CHE-1 activity was context dependent, possibly due to inhibitory mechanisms in most of the other cell types. To identify such inhibitory factors, Tursun et al. (Tursun et al. 2011) screened genes in *C. elegans* with predicted roles in chromatin regulation, showing that knockdown of *lin-53*, the ortholog of WD40 domain-containing retinoblastoma-binding protein RbAp46/48, allowed expression of ASE neuronal fate markers upon CHE-1 ectopic expression in germ cells (Tursun et al. 2011). Interestingly, *lin-53* knockdown enabled acquisition of neuronal, but not other cellular, fates. Expression of *unc-30* or *unc-3*, two transcription factors important for the specification of GABAergic and cholinergic motor neurons (Jin et al. 1994), resulted in conversion of germ cells into the corresponding neuronal subtype. In contrast, *lin-53* knockdown was not sufficient to allow *hlh-1* (the MyoD homolog in *C. elegans*) to convert germ into muscle cells. Since *lin-53* is part of several complexes containing histone deacetylase (HDAC) activity, such as the NURD nucleosome remodeling complex, histone acetylation might play a role in inhibiting CHE-1-mediated conversion. To test this hypothesis, CHE-1-induced worms were treated with two different HDAC inhibitors, and, in both cases, germ cells converted into ASE neurons, thus corroborating histone acetylation as an important inhibitory mechanism that prevents neuronal differentiation.

Given that *lin-53* also forms part of the polycomb repressor complex 2 (PRC2), the Hobert lab investigated whether the effect of *lin-53* knockdown could be attributed to PRC2 abrogation in germ cells (Patel et al. 2012). To this end, they knocked down several components of the PRC2 complex and observed a phenotype very similar to cells following *lin-53* knockdown, suggesting that PCR2 inhibited neuronal differentiation in germ cells. Taken together, these studies provide evidence that chromatin modifiers exert an important influence on susceptibility of differentiated cells to reprogram, either by impeding or facilitating reprogramming.

Improving Reprogramming Efficiency

The abovementioned examples represent a proof of principle for direct conversion of soma to neuron. However, in most cases, the conversion efficiency is rather low, thereby limiting applicability of this approach, which requires high yield of homogeneous identity. Moreover, the degree or quality of reprogramming varies dramatically with many, if not the majority, of cells undergoing only partial reprogramming.

To overcome these limitations, a protocol was recently developed utilizing small molecules to enhance reprogramming yield (Ladewig et al. 2012). Inspired by previous studies aimed at enhancing neural differentiation from human iPSCs (Chambers et al. 2009; Li et al. 2010), Ascl1- and Neurog2-cotransduced human postnatal fibroblasts were treated with the SMAD signaling inhibitor SB-431542, the GSK-3β inhibitor CHIR99021, and noggin, a functional inhibitor of BMP signaling (Groppe et al. 2002), resulting in a dramatic increase in both efficiency and final yield of reprogrammed cells. Importantly, application of the same protocol to adult human fibroblasts increased reprogramming efficiency from 1 % to 13 %. Interestingly, small molecule treatment did not affect neuronal subtype specification, as induced neurons became either glutamatergic (35 %), GABAergic (20 %), or serotoninergic (approx. 5 %). Further studies are required to determine whether inhibition of well-studied signaling pathways facilitates conversion of somatic cells towards a neuronal identity, as well as improves survival of reprogrammed cells.

Future Perspectives and Challenges

With very rapid development of the direct reprogramming approach, many potential applications can be imagined, ranging from studying disease mechanisms (Qiang et al. 2011) to cell-based therapies of neurodegenerative diseases. To fully assess and eventually employ the potential of induced neuronal cells, a more comprehensive understanding of the molecular mechanisms underlying direct reprogramming is required, as this will allow for improved rationale of the reprogramming strategies. Moreover, issues of safety need to be addressed before translating the experimental work into clinical applications.

Deciphering Molecular Mechanisms of Direct Reprogramming

To date, most efforts have focused on determining combinations of genes capable of generating neurons from various somatic cells, either of murine or human origin. However, little is known about the molecular mechanisms underlying lineage conversion. Single-cell transcriptome analysis performed during MEF conversion into iPSCs suggests that this process is biphasic. An initial phase of stochastic or probabilistic gene activation is followed by a deterministic or hierarchical phase during

which the subsequent sequence of gene activation events becomes predictable (Buganim et al. 2012). Single-cell analysis revealed that activation of pluripotency-related genes, such as *Essrb*, *Lin28*, *Utf1*, and *Dppa2*, is a good predictor of successful iPSC generation. Based on these observations, a hierarchical model was generated, allowing identification of novel combinations of reprogramming factors capable of inducing pluripotency that did not include the four factors originally employed by Yamanaka and colleagues (Takahashi and Yamanaka 2006). Does direct soma-to-neuron conversion follow a similar biphasic model of gene activation, or does conversion proceed directly after onset following a hierarchical sequence of events? These questions can only be answered once a better knowledge of the gene regulatory networks that define the distinct phases of neurogenesis is gained, starting from the neural stem cell state and leading through intermediates to a fully differentiated neuron. Nonetheless, studies on astroglial-to-neuronal conversion have shown that forced Neurog2 expression leads to sequential activation of downstream transcription factors, such as Eomes/Tbr2 and Tbr1 (Heinrich et al. 2011), not unlike what happens during specification of glutamatergic neurons during corticogenesis (Englund et al. 2005; Hevner et al. 2006). Thus, it is tempting to speculate that the onset of Tbr2 expression may represent entry into the hierarchical phase of astroglial-to-glutamatergic neuronal conversion. However, not all astroglia successfully undergo reprogramming, which may be indicative of an early probabilistic phase.

As stated above, current strategies to directly reprogram somatic cells into induced neurons employ forced expression of a pro-neural gene. Thus, from a mechanistic point of view, these are likely to involve activation of a developmental program that leads to neurogenesis through intermediates rather than directly superimposing the genetic program of a mature neuron. In fact, pro-neural genes are typically expressed at the neural stem or progenitor state but no longer when a neuron becomes postmitotic. In contrast, the so-called terminal selector genes (Hobert 2008) become activated as part of a developmental program, whose expression must be maintained throughout the life of a cell to maintain the cellular identity. Interestingly, the aforementioned examples of direct reprogramming of germ cells into specific types of neurons in *C. elegans* were based on forced expression of such terminal selector genes (Patel et al. 2012; Tursun et al. 2011). Currently, it is not known whether equivalents to these terminal selector genes, as described in *C. elegans* neurogenesis, also exist in mammals. If so, it will be interesting to determine whether terminal selector genes permit generation of specific neuronal subtypes, such as layer V corticofugal neurons or cerebellar Purkinje neurons.

Modeling Neurodegenerative Diseases

Direct reprogramming of somatic cells into induced neurons is likely to help in studying the patho-mechanisms underlying human neurodegenerative diseases that are difficult to recapitulate in animal models. For instance, human fibroblasts obtained from familial Alzheimer's disease patients (FAD) have been successfully converted into glutamatergic neurons via direct reprogramming (Qiang et al. 2011).

Compared to induced neurons from control fibroblasts, immunochemical analyses revealed altered processing and localization of amyloid precursor protein (APP) and increased production of Aβ fragments, two well-known cellular marks of Alzheimer's disease. Interestingly, these differences were not notable when comparing the parental fibroblasts. Similar approaches could be utilized to generate neurons from sporadic Alzheimer's disease patients, as well as from sufferers of other neurodegenerative diseases, to study the molecular basis of pathologies, as well as investigate the effect of therapeutic drugs. Obviously, an efficient and high-quality conversion protocol is required to make direct reprogramming equal or superior to the derivation of clinically relevant neuronal types via generation of iPSCs and subsequent neuronal differentiation for the same purpose.

Safety Issues Regarding the Use of Induced Neuronal Cells

Most protocols for direct reprogramming, if not all, are based on the delivery of the transgenes through retro- or lentiviruses (see Table 1), which integrate randomly into the host genome, potentially causing adverse effects including tumorigenesis. Other strategies should also be explored, such as the use of non-integrative viruses (for instance, AAV) (Bartel et al. 2012), modified mRNAs (Warren et al. 2010), direct protein delivery (Thier et al. 2012), or combinations of small molecules, which could trigger the activation of an endogenous silenced neuronal program. It would also be interesting to evaluate the use of viruses with an integration capacity restricted to specific defined loci within the host genome (Turan and Bode 2011).

One of the most significant advantages of direct reprogramming over iPSC-induced neuronal differentiation is that the former does not involve a pluripotent state. This makes directly induced neurons safer for transplantation, as they are less likely to give rise to a tumor. Nevertheless, only a fraction of induced neurons exhibit mature electrophysiological properties, while others fail to undergo complete reprogramming. This can be attributed at least in part to the original epigenetic state, which may not be completely remodeled by the removal of nonneuronal traits that characterize the original cell type (Marro et al. 2011) and hence interfere with successful acquisition of repressive or activating marks on nonneuronal and neuronal genes, respectively. Transplantation studies of midbrain precursors into Parkinson's patients have revealed that the beneficial effects of newly added dopaminergic neurons can be counterweighted by side effects caused by the presence of non-desired cell types such as serotonergic neurons (Pfisterer et al. 2011). Clearly, only partially reprogrammed cells are likely sources for similar unwanted side effects. Nevertheless, rapid progress is made to generate clinically relevant neurons, such as dopaminergic neurons. Results from transplantation into preclinical animal models suggest that these ameliorated functional motor deficits (Caiazzo et al. 2011; Kim et al. 2011), encouraging further improvement of reprogramming efficiency and quality.

The Ultimate Goal: In Vivo Reprogramming for Brain Repair

The ultimate goal of direct reprogramming of somatic cells into induced neuronal cells lies in the promise of recruiting endogenous cells to repair the diseased brain directly from "within." Proof-of-principle evidence for successful in vivo reprogramming has been provided for conversion of exocrine cells into B cells within the pancreas via forced expression of Neurog3, Pdx1, and Mafa (Zhou et al. 2008). Likewise, forced expression of GATA4, Tbx5, and Mef2c allowed for direct conversion of scar-forming cardiofibroblasts into cardiomyocytes following heart injury (Qian et al. 2012; Song et al. 2012). In vitro work has demonstrated that CNS-resident cells, such as astrocytes and pericytes, are indeed amenable to reprogramming into induced neuronal cells (Heins et al. 2002; Karow et al. 2012; Heinrich et al. 2010), while cell populations such as adult NG2 progenitor cells, as well as non-neuroectodermal cells such as microglia, still await assessment. Each cell type has its own merits, especially in the context of CNS injuries, to which all of these cells respond by proliferation (Buffo et al. 2008; Simon et al. 2011; Goritz et al. 2011). Early proof-of-principle studies have hinted that the possibility of direct conversion within a highly non-neurogenic environment, such as the injured cerebral cortex, may not be impossible, although precise origin of cells that gave rise to doublecortin-positive neuroblasts following retrovirus-mediated Pax6 expression has not been determined (Buffo et al. 2008). However, even if in vivo reprogramming becomes feasible, further challenges must be faced, such as successful integration of newly generated neurons into a diseased neural circuitry. In this context, important lessons can be learned from examples provided by integration of adult-generated neurons into brain regions where neurogenesis continues throughout life (Bergami and Berninger 2012).

Concluding Remarks

Direct reprogramming of somatic cells into neuronal cells is a young field of investigation, yet it stands on the shoulders of a century of exciting discoveries in the area of developmental biology and profits immensely on parallel advances in the field of induced pluripotent stem cells. The most recent technological innovations that allow for observation, manipulation, and analysis of the transcriptome and proteome at single-cell level, as well at population levels, will undoubtedly lead to an improved understanding of the underlying molecular mechanisms and as a consequence allow for optimization of employed reprogramming strategies, perhaps ultimately leading to the sole employment of small molecules. The age of reprogramming has just started, promising to reveal new principles of cellular specification during development, to uncover the etiology of many devastating diseases, and, hopefully, to develop novel cures for still incurable diseases.

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