The role of neurogenesis in stroke recovery

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Contents

Abstract

This review investigates the role of neurogenesis after stroke. Neural stem cells reside in the SVZ of the adult mammal, including humans. In response to stroke, proliferation in the SVZ is upregulated, giving rise to neuroblasts that migrate to the ischemic area. Here, some of them differentiate into mature neuronal cell types and survive long-term. Signs of integration have been found, but the capacity of these newborn neurons to replace neurons lost after stroke is not well understood. The mechanisms that govern proliferation, migration, differentiation, integration and survival of newborn neural precursors and neurons are investigated. The role of the vasculature, specialized niches in the SVZ, astrocytes and angiogenesis during the poststroke neurogenic response receive attention. A range of factors that have been shown to influence the neurogenic response are reviewed. It becomes clear that the role of the neurogenic response after stroke in repairing the brain is not well understood. The positive effect on stroke recovery that is associated with neurogenesis may be the result of functional integration, trophic support, neuroprotection, angiogenesis, or a combination. Taking advantage of the neurogenic capacity of the brain in stroke therapy seems promising, but many difficulties remain to be overcome.

List of abbreviations

Ara-C = cytosine-β-D-arabinofuranoside
bFGF = basic fibroblast growth factor
CREB = cAMP response element-binding protein
CSF = cerebrospinal fluid
ECM = extracellular matrix
EE = environmental enrichment
EGF = epidermal growth factor
EPO = erythropoietin
EPOR = EPO receptor
FGF2 = fibroblast growth factor
GABA = gamma aminobutyric acid
GFP = green fluorescent protein
HB-EGF = heparin-binding epidermal growth factor-like growth factor
HDAC = histone deacetylase
IGF-I = insulin-like growth factor-I
MCAO = middle cerebral artery occlusion
MMP = matrix metalloproteinase
MSC = bone marrow stromal cell
NMDA = N-methyl-D-aspartic acid
NO = nitric oxide
OB = olfactory bulb
PSA-NCAM = polysialylated-neural cell adhesion molecule
RA = retinoic acid
RMS = rostral migratory stream
rtPA = recombinant tissue plasminogen activator
SB = sodium butyrate
SCF = stem cell factor
SDF-1 = stromal cell derived factor-1
SGZ = dentate subgranular zone
Shh = sonic hedgehog
SVZ = subventricular zone
TNF-α = tumor necrosis factor alpha
TNF-R1 = tumor necrosis factor receptor 1
VEGF = vascular endothelial growth factor

Introduction

Stroke is a disease with high incidence, in the USA alone approximately 795 000 people suffer a new or recurrent stroke each year, which comes down to one person every forty seconds. Even though someone dies of stroke in the USA every four minutes, the large majority of people that experience stroke survive, yielding an estimated 7 000 000 Americans ≥ 20 years of age who have had a stroke. Beside a major healthcare problem, stroke is a large economic burden on society (Roger et al 2010).

The only current pharmacological treatment of acute ischemic stroke is thrombolysis with recombinant tissue plasminogen activator (rtPA; Hacke et al 2004). The therapy has several drawbacks. It must be performed shortly after acute stroke, it is only selectively applicable to patients and complications may occur, as it increases the induction of hemorrhagic transformations (Katzan et al 2004; Molina 2011; Zhang et al 2005). Recovery from stroke is clinically approached by behavioral treatment, which largely depends on brain plasticity (Brown & Schultz 2010).

The limited result of stroke treatments has driven the search for therapies directed at restoring neurological function after stroke. A promising approach is augmenting the innate neurogenic capacity of the adult brain to repair damaged areas after stroke.

Adult neurogenesis is the formation of new neurons out of neural stem cells. Even though adult neurogenesis is now robustly established, this has not always been the case. In neurobiology there has been a longstanding dogma that no newborn neurons were generated in the adult mammalian brain (Gross 2009). Even though in 1912 it was already shown that adult rats have mitotic cells in the subventricular zone (SVZ; Allen 1912), this was not noticed or paid attention to by other neuroscientists. The highly influential neuroscientist Ramón y Cajal wrote in 1928 that "In the adult centers the nerve paths are something fixed, ended and immutable. Everything may die, nothing may be regenerated" (Ramón y Cajal, 1928). Reports in the 1960's by Altman clearly showed the SVZ retains its neurogenic capability into adulthood (Altman 1962; 1969). However, these reports were also largely ignored by contemporary scientists and the central dogma persisted. In 1970 the textbook 'Developmental Neurobiology' by Jacobson (1970) stated that "…there is no convincing evidence of neuron production in the brains of adult mammals" (Gross 2009). Even though Kaplan confirmed findings by Altman in the 1980's (Kaplan & Bell 1984), it wasn't until the course of the 1990's when new techniques for labeling mitotic cells and markers for specific cell types were developed, that the dogma was overcome (Cameron et al 1993; El Maarouf et al 2006; Gould et al 1999; Lois & Alvarez-Buylla 1994; Magavi et al 2000). The discovery of neurogenesis in the human adult dentate gyrus was very important for the research field of adult neurogenesis in animal models (Eriksson et al 1998).

The adult mammalian brain has two main neurogenic zones where neural stem cells reside: the SVZ and the dentate subgranular zone (SGZ) of the hippocampus (Doetsch et al 1999; Jin et al 2001; Kaplan & Hinds 1977). There exist studies showing adult neurogenesis in other areas of the brain, but these results remain controversial (Gould 2007).

The SVZ neurogenic system has been extensively studied in rodents, where it has been shown that thousands of newborn neurons are generated on a daily basis (Kim & Szele 2008). Neural precursors migrate to the olfactory bulb (OB), where they differentiate and mature into newborn neurons which can survive long-term and integrate in the circuitry of the OB. Understanding the basic mechanisms behind these processes is important, because similar mechanisms are likely to play a role in stroke-induced neurogenesis.

Since the establishment of adult neurogenesis, many studies have been directed at investigating the potential of adult neurogenesis for therapeutic use. The discovery of a neurogenic response after stroke (Arvidsson et al 2002; Jin et al 2001; Parent et al 2002; Zhang et al 2001) has begged the question whether this process could be improved to help the treatment of stroke patients. The normal neurogenic response after stroke is not sufficient for repair of the damaged brain in stroke models or humans. Therefore, it has been a topic of great scientific interest to understand the neurogenic response, with the prospect of manipulating it to increase brain repair after stroke.

It has been established that not only stroke damage to the brain, but also a wide range of other types of damage induce neuroblast migration to the area of damage (Kim & Szele 2008). The mechanisms that lead to neuroblast migration after several types of brain trauma might be similar to stroke-induced neurogenesis and migration. Even though other brain trauma and the subsequent neurogenic response is thus of interest, also for stroke research, this review will focus only on stroke-induced neurogenesis.

Neurogenesis in the SGZ of the hippocampus is also upregulated after stroke (Yagita et al 2001), similar to neurogenesis in the SVZ after stroke. Also, newborn neuronal precursors from the SGZ have been found to migrate to areas of ischemic damage (Jin et al 2001). Even though the role of SGZ neurogenesis after stroke is interesting, it is beyond the scope of this review, which focuses on SVZ neurogenesis after stroke.

The existence of a neurogenic system in the SVZ in humans has been controversial (Bedard & Parent 2004; Sanai et al 2004), but Curtis and colleagues (2007) have provided compelling evidence that indeed a similar system is in place in the human brain. In addition, a neurogenic response to stroke has been found in the adult human brain (Jin et al 2006; Macas et al 2006; Marti-Fabregas et al 2010; Minger et al 2007). These finding provide the necessary confidence that results obtained in animal stroke models may be useful in human approaches in stroke treatment.

Neurogenesis is a broad concept which involves the entire process of generation of neural precursors to mature neurons. Neurogenesis in the SVZ starts with proliferation of neural progenitors, giving rise to neuroblasts (Jin et al 2001; Zhang et al 2001). Neuroblasts migrate and differentiate, and some mature into neurons. Neurogenesis is measured by numbers of neuroblasts generated through neural progenitor proliferation, by numbers of neuroblasts/immature neurons migrating, and by numbers of mature neurons formed. Although technically only the number of mature neurons formed constitutes a measure of neurogenesis, earlier stages of the neurogenic process are often taken as indirect measures of neurogenesis. In this paper, ‘neurogenesis in the SVZ’ applies mainly to proliferation leading to the generation of neural precursors, whereas ‘neurogenesis’ applies to all the processes that take place between neural stem cell proliferation and mature neuronal integration in the target area.

Rodent models of stroke are essential tools for the investigation of stroke mechanisms and the subsequent neurogenic response. The most commonly used model of stroke is middle cerebral artery occlusion (MCAO), which was developed in rats (Koizumi et al., 1986; Longa et al 1989) and later adapted to mice (Clark et al 1997). In MCAO an occluding suture is placed in the internal carotid artery at the junction of the anterior and middle cerebral arteries, blocking blood flow into the middle cerebral artery causing ischemia of the area innervated by this artery. Transient MCAO is achieved by removal of the occlusion after twenty minutes to two hours, allowing for reperfusion of the ischemic area, whereas in permanent MCAO the occlusion is maintained and no reperfusion is

allowed. MCAO obstructs blood flow and causes unilateral ischemic damage in the striatum, or both the striatum and cortex. In this paper we will focus on this type of focal stroke.

In animal models, similar to the situation in human stroke brains, a focal (often large) area of the brain is affected by ischemic damage. The damaged area is irregularly shaped and depends on the innervation of the brain and the location of blood flow obstruction. The core of the stroke area is usually most severely damaged, with surrounding tissue transiently ischemic for a shorter period called the ischemic penumbra (Durukan & Tatlisumak 2007). Experimental stroke induces a neurogenic response, which involves cell proliferation in the SVZ, migration of neural precursors to the stroke area, and maturation and survival of newborn neurons in the ischemic region (Arvidsson et al 2002; Jin et al 2001; Parent et al 2002).

This review will first briefly discuss the process of neurogenesis, migration and integration in healthy adult rodents. It is important to understand the mechanisms that govern these processes in healthy animals, since after stroke many of the same mechanisms are likely involved in altered stroke-induced processes. Next, neurogenesis in response to stroke will be discussed. Attention will be given to the different types of neural precursors that give rise to mature neurons, migration of neural precursors, and their differentiation into mature neuronal cell types. A separate chapter is devoted to the important role that angiogenesis plays in relation to these processes. The role of the existing vasculature, which plays an important role in proliferation and migration, will be addressed throughout this review. Finally, the integration and survival of mature neurons in the ischemic area will be discussed.

The aim of this review is to clearly bring into focus the current state of knowledge on the role of neurogenesis after stroke. Understanding the mechanisms involved in stroke-induced neurogenesis could help us understand why they fail to regenerate the brain after stroke, and perhaps suggest strategies to manipulate these mechanisms to improve the recovery after ischemic stroke.

Neurogenesis in the adult SVZ

Two specialized niches in the adult brain, the SGZ of the hippocampus and the SVZ are known for their neurogenic capacity. SVZ neurogenesis is associated with olfaction (Doetsch & Hen 2005), while neurogenesis in the SGZ is associated with spatial learning and memory (Lee & Son 2009). The SVZ is the largest neurogenic site in the adult brain (Doetsch & Alvarez-Buylla 1996) and has been shown to be present in mammals (Doetsch et al 1999) as well as humans (Curtis et al 2007; Sanai et al 2004). The SVZ generates new neurons in the adult, which travel rostrally along the rostral migratory stream (RMS) toward the OB, where they differentiate and integrate as inhibitory interneurons (Fig. 1).

The SVZ neurogenic niche is located adjacent to the lateral ventricle, directly below the ependymal layer, a single layer of ependymal cells that lines the lateral ventricle (Doetsch & Alvarez-Buylla 1996). The SVZ lies largely on the lateral side of the lateral ventricle, but it also extends to parts of the dorsal and medial walls of the lateral ventricle and even the RMS (Alvarez-Buylla et al 2008).

Normal adult neurogenesis involves proliferation, migration, differentiation and integration. These processes are controlled in a spatial and temporal manner by cues from the cellular environment and diffusible factors in the brain (Ohab & Carmichael 2008). Here, we look at these basic mechanisms and their controls in normal neurogenesis in the healthy animal, before we turn to the factors and mechanisms involved in stroke-induced neurogenesis. It is important to understand the controls over normal neurogenesis, since some of the same mechanisms will likely play a role in the neurogenic response after stroke. These may be used to facilitate the recovery after stroke, and could lead to directed therapies of stroke.

Astrocytes are the most abundant cells in the mammalian brain and are extremely important in normal brain functioning (Freeman 2010). Astrocytes have many functions in relation with neurons, they regulate synapse environment and play an active role in synapse signaling (Freeman 2010). Astrocytes can also regulate blood flow and signal between each other through gap junctions, forming networks of astrocytes (Allen & Barres 2009). Astrocytes influence neuronal synapse formation, thus playing a role in synaptic plasticity (Ullian et al 2001). The role of astrocytes as neural stem cells (Doetsch et al 1999) was an unexpected discovery, since it conflicted with the notion of stem cells as undifferentiated cells. However, the neural stem cell that gives rise to a range of other cells has convincingly been shown to be a glial fibrillary acidic protein (GFAP)-positive astrocyte-like cell type that resides in the SVZ (Doetsch et al 1999). These neural stem cells (also called type B cells) are nestin- and glial fibrillary acidic protein GFAP-positive (Merkle et al 2004). They first give rise to rapidly dividing nestin- and distalles homeobox (Dlx2)-positive, GFAP- and polysialylated-neural cell adhesion molecule (PSA-NCAM)-negative transitory amplifying progenitor cells (also called type C cells), which in turn give rise to Dcx-, TUJ1- and PSA-NCAM-positive neuroblasts (also called type A cells; Doetsch et al 1999; Doetsch et al 1997; Doetsch et al 2002; Garcia-Verdugo et al 1998; Garcia et al 2004).

The SVZ is a specialized cellular and extracellular matrix (ECM) niche (Mercier et al 2002). This neurogenic niche is closely associated with the vasculature. Particularly transitory amplifying progenitor cells and active neural stem cells are found in close proximity to blood vessels. Almost 50% of these cell types were found within 5 μm of the vasculature. Neuroblasts migrating in chains were not as close, but they do follow along the vasculature in the SVZ (Shen et al 2008; Tavazoie et al 2008). A subset of neural stem cells lies within the ependymal layer and is in contact with both the lateral ventricle and the vasculature. Underneath this lies a layer of neural stem cells with tangential

processes that run in the direction of neuroblast migration (Shen et al 2008). In the neurogenic niche the cell-cell interaction between astrocytic neural stem cells and other neural precursors seems to be important for neurogenesis (Lim & Alvarez-Buylla 1999; Song et al 2002). A population of astrocytes possibly distinct from neural stem cells plays an important role in the SVZ neurogenic niche. In the SVZ, astrocytes are in close contact with neural progenitor cells, with each other through gap junctions and the vasculature through astrocytic end feet. Astrocytes play an active role in the maintenance, self renewal and proliferation of neural progenitors. It is not clear whether astrocytic neural stem cells play a dual role in the SVZ, or that distinct astrocytic populations exist simultaneously (Ma et al 2005). Thus, the SVZ neurogenic niche is a specialized niche where neural progenitors can be influenced by factors from neighboring neural precursors, ependymal cells, astrocytes, axon terminals, cerebrospinal fluid (CSF) from the lateral ventricle, and the vasculature.

Proliferation in the SVZ is controlled by a wide range of factors. Molecular cues from neighboring cells such as ephrin, sonic hedgehog (Shh), and other signaling systems play a role in the cell fate decisions of neural stem cells in the SVZ. Ephrin signaling promotes neurogenesis in the SVZ and ectopic ephrin administration disrupts neuroblast migration (Conover et al 2000). Shh has been suggested to affect neurogenesis in the SVZ by regulating proliferation of neural stem cells and transitory amplifying progenitor cells (Ahn & Joyner 2005). Other factors that stimulate neurogenesis in the healthy rodent brain include Notch1 and erythropoietin (EPO), as well as several trophic factors.

The Notch1 signaling pathway is known to play an important role in brain development. Its function in controlling neurogenesis was recently found to be preserved in the adult brain. Expression of Notch1 ligands Jagged1 and Delta1 is found in the adult SVZ and neurogenesis in the rat brain is upregulated following intracerebral Notch1 administration and downregulated after Notch1 inhibition (Wang et al 2009a). Thus, Notch1 promotes neurogenesis in the SVZ, likely through its receptors Jagged1 and Delta1.

EPO promotes neural stem cell proliferation *in vitro* (Tsai et al 2006). Recombinant EPO administration can induce Shh expression in rats (Wu et al 2010). Shh combined with epidermal growth factor (EGF) promotes neurogenesis in the SVZ. Also, inhibition of Shh signaling by cyclopamine leads to decreased proliferation in the SVZ (Palma et al 2005) and inhibits carbamylated EPO-induced neurogenesis. Carbamylated EPO is an EPO derivative that leads to increased SVZ neural progenitor cell proliferation *in vitro*, the promotion of neural progenitor cells into neurons, as well as the upregulation of Shh (Wang et al 2007a). In the SVZ neurogenic niche, neural stem cells and other neural progenitor cells are in close contact with astrocytes. Astrocytes in this region secrete Shh and can induce neurogenesis (Jiao & Chen 2008). These data suggest that Shh plays an important role in SVZ neurogenesis, and that astrocytes may influence proliferation through Shh secretion. Shh mediates carbamylated EPO-induced neurogenesis and may also be involved in EPO-induced neurogenesis. It thus seems that the effect of EPO on neurogenesis is mediated through Shh.

Several trophic factors play a role in regulating proliferation in the SVZ. Administration of the trophic factor stem cell factor (SCF; Jin et al 2002a) or heparin-binding epidermal growth factor-like growth factor (HB-EGF; Jin et al 2002b) intraventricularly in rats leads to an increase in proliferation of neural precursors in the SVZ. Vascular endothelial growth factor (VEGF) infusion leads to increased proliferation of neural precursors in the SVZ, generating neuroblasts (Jin et al 2002c).

After neural progenitor proliferation, the newly generated neuroblasts in the SVZ enter the RMS through which they migrate in chains of tangential neuroblasts to the OB. Time-lapse videomicrography has shown that individual neuroblasts travel at an average speed of ~120 μm/hr

along the chains in SVZ explants (Wichterle et al 1997). In acute slices they were found to migrate at an average speed of ~50 µm/hr (Bolteus & Bordey 2004) and ~89 µm/hr (Hirota et al 2007). Possibly, migrating neuroblasts leaving explants encounter less physical hindrance and are therefore able to move faster than in acute slices. Reports of *in vivo* migration speed are scarce, one report states that neuroblasts migrate to the SVZ at an average speed of ~25 µm/hr (Lois & Alvarez-Buylla 1994). This slower migration *in vivo* compared to *in vitro* could be due to inhibitory signals in the intact brain. It also has to be taken into account that *in vivo* observations are never direct, rather they depend on tracers combined with immunohistochemical techniques, making it harder to precisely measure individual neuroblast chain migration speed.

The RMS can be seen as a 'glial tube', since it has boundaries formed by astrocytes and blood vessels, which interact with the migrating chains of neuroblasts (Doetsch & Alvarez-Buylla 1996; Doetsch et al 1997). Neuroblasts migrate rostrally until they reach the OB, where they dissociate from each other and turn to migrate radially into the cell layers of the OB. Differentiation of neural precursors to specialized neurons takes place in the RMS and OB. Almost all neuroblasts differentiate into granule inhibitory interneurons, while only a small portion (less than 3%) mature into periglomerular inhibitory interneurons (Doetsch & Hen 2005). The mechanisms that direct differentiation are not fully understood, but the neurotransmitter gamma aminobutyric acid (GABA) is known to regulate early dendritic growth in new neurons in the OB (Gascon et al 2006). Interestingly, a small portion of newborn neurons migrate into the neostriatum where they form calretinin-positive neurons (Dayer et al 2005). Even though this amount is in no comparison to the number of neuroblasts that follow the SVZ-RMS-OB pathway (Yang et al 2008), this does indicate that the intact brain already has the capacity, and thus perhaps some of the machinery prepared for migration of neuroblasts from the SVZ to the striatum.

It has been suggested that progenitors in the SVZ have a certain specificity in terms of what types of neurons they can give rise to. Regional subtypes of neural stem cells may give rise to different types of mature interneurons. The timing and location of neurogenesis may be crucial, or it may be the specific characteristics of individual neural stem cells that dictate the type of interneurons and its chances of survival (Lledo et al 2008). Different theories on this subject still remain to be investigated, which may also have implications for neurogenesis after stroke.

Neuroblasts express cAMP response element-binding protein (CREB) from when they are formed, but interestingly it is only transiently phosphorylated during neuroblast differentiation into a neuronal cell type. Some unidentified signal causes neuroblast CREB phosphorylation during late rostral migration and initial radial migration in the OB. CREB phosphorylation diminishes as the cells acquire adult neuronal morphology, as judged by elongated dendrites and spinal formation. Experiments interfering with CREB show impaired neuronal differentiation and survival in the OB (Giachino et al 2005). These results suggest an important role for the CREB pathway in neuronal maturation.

Migration along the SVZ-RMS-OB pathway is controlled by a variety of proteins from the ECM, neighboring cells, blood vessels and other guiding molecules. The exact mechanisms are not fully understood, but many factors that play a role in these processes will be discussed shortly here. To investigate the altered migration of neuroblasts after stroke, it is important to understand the mechanisms that govern migration from the SVZ through the RMS to the OB.

The process of migration is tightly regulated to prevent SVZ neuroblasts from entering the striatum and to allow them to travel the long distance forward through the RMS into the OB. Neuroblasts start their migration in the SVZ, where they are born (Doetsch & Alvarez-Buylla 1996).

GABA tightly regulates neuroblast migration speed in the anterior part of the SVZ and in the RMS through neuroblast $GABA_A$ receptor activation. Astrocytes forming the glial tube express the high-affinity GABA transporter subtype GAT4 on their processes ensheathing migrating neuroblasts. Both inhibition of GABA uptake by these astrocytes and increased GABA release by neuroblasts reduce the migration rate of neuroblasts (Bolteus & Bordey 2004). These results show that astrocytes of the glial tube help create a microenvironment that controls neuroblast migration. Taken together, these data show that GABA negatively influences speed of neuroblast migration in the RMS.

The chain-like manner of migration, which starts in the SVZ and continues throughout the RMS, allows the neuroblasts to migrate along one another (Wichterle et al 1997). This process is influenced by cell-surface molecules such as PSA-NCAM (Cremer et al 1994; Doetsch & Alvarez-Buylla 1996; Ono et al 1994) and α6β1 integrin (Jacques et al 1998). Integrin adhesive interactions, particularly in the β1/laminin system play a role in directing neuroblast chain migration (Belvindrah et al 2007; Ohab & Carmichael 2008). Ectopic laminin as well as disruption of integrin binding to laminin results in redirection of neuroblasts from the RMS (Emsley & Hagg 2003). The β1/laminin system regulates the adhesion of neural progenitors to the vasculature not only in the RMS, but also in the dorsal SVZ (Shen et al 2008). Thus, the ECM, and specifically laminin is very important for neuroblast migration.

The growth factor insulin-like growth factor-I (IGF-I) is expressed in the SVZ and OB in the adult brain. IGF-I promotes neuronal migration and positioning in the OB as well as the exit of neuroblasts from the SVZ. (Hurtado-Chong et al 2009). IGF-I is thus an important factor at vital turning points in the migratory route of neuroblasts from the SVZ to their final destination in the OB.

Astrocytes in the SVZ niche, ependymal cells lining the SVZ, as well as neuroblasts in both the SVZ and RMS express the receptor tyrosine kinase ErbB4 (Anton et al 2004). Neuregulins, a collection of polypeptides with an EGF-like domain, bind to and activate ErbB receptors. Neuregulin1 and -2 are specifically expressed by astrocytes and neuroblasts in the SVZ and RMS and regulate neural progenitor proliferation and migration by activating its receptor ErbB4. Neuregulin2 promotes proliferation of neural stem cells, generating more neuroblasts. Neuregulin1 is involved in the initiation of migration of newborn neuroblasts in the SVZ (Ghashghaei et al 2006).

The chains of neuroblasts migrating through the RMS are closely associated with a scaffold of blood vessels. Migrating neuroblast and endothelial cells are in very close contact with each other and are often separated by only a very thin layer formed by astrocytic processes, as shown with electron microscopy (Whitman et al 2009). This blood vessel scaffold for neuroblast migration could guide migration by secreting substances that control the process.

Chemorepulsants guide migrating cells by repressing their migration in the direction of the chemorepulsant. A gradient of the chemorepulsant Slit guides neuroblast migration in the SVZ-RMS-OB pathway via activation of its receptors Robo-2 and Robo-3 (Wu et al 1999). Slit is expressed in the septum (Hu & Rutishauser 1996; Wu et al 1999) and the choroid plexus (Hu 1999; Sawamoto et al 2006), which is separated from the SVZ by the lateral ventricle and the ependymal layer lining the lateral side of the lateral ventricle. Despite this physical boundary Slit is able to enter the SVZ through tissue diffusion. The Slit gradient is maintained by the beating of ependymal cells lining the ventricles creating CSF flow and thus establishing a gradient of Slit in the SVZ. Slit concentration is highest in the caudal region and progressively lower in more rostral regions of the SVZ guiding neuroblasts rostrally toward the OB (Sawamoto et al 2006). In another function of Slit, it acts on Robo-1 and -2, which is expressed by astrocytes that form the glial tube. In this way, Slit affects the morphology and arrangement of astrocytes that form the RMS glial tube. Neuroblasts thus facilitate their own

migration by expressing Slit, which through neuron-astrocyte communication influences astrocytes and helps to maintain astrocytic tunnels in the RMS (Kaneko et al 2010).

In traveling through the brain parenchyma neuroblasts must overcome the barrier of the ECM. Matrix metalloproteinases (MMPs) are enzymes that degrade the ECM by breaking down ECM molecules. This way, they regulate the physical constraints that the ECM poses for cells and substances. It has been suggested that migrating neuroblasts interact with and modify the ECM during migration through expression of matrix metalloproteinases (Bovetti et al 2007a; Yang et al 2005). However, it is unclear whether this applies to neuroblasts migrating in chains as well (Bovetti et al 2007a).

Since the migrating neuroblasts have the OB as their destination, it would not be surprising if this structure was the source of chemoattractants that guide migration to the OB. In fact, there is some evidence pointing in this direction (Liu & Rao 2003). However, the issue is subject to debate because of conflicting reports. For example, in mice that have undergone bulbectomy the RMS persists and newly generated cells continue to migrate rostrally for up to three weeks after bulbectomy (Kirschenbaum et al 1999). Also, disconnecting the OB from the RMS does not prevent migration rostrally through the RMS either (Jankovski et al 1998). On the other hand, others found an unknown 'attractive activity' emanating from the OB as well as a reduction of OB-directed migration in the RMS after bulbectomy (Liu & Rao 2003). These results urge researchers to continue to take the OB serious as a possible source of attractive substances for migrating neuroblasts.

The neurotransmitter N-methyl-D-aspartic acid (NMDA) plays a role in neuroblast development along the migratory pathway. The NMDA receptor is activated by the astrocytes of the glial tube ensheathing the chains of migrating neuroblasts. The activation of the NMDA receptor, expressed by neuroblasts during migration through the RMS, appears crucial for their survival (Platel et al 2010).

When migrating neuroblasts reach the OB, they have to stop their rostral migration and start migrating radially, into the different cell layers of the OB. What are the cues that tell the migrating neuroblasts that they have reached the OB and it is time to change direction? First, the neuroblasts have to detach from the neuroblast chain in which they migrate. In the OB the neuroblasts dissociate from the RMS neuroblast chain under the influence of the extracellular protein reelin (Hack et al 2002) and the ECM glycoprotein tenascin-R. Tenascin-R is expressed in the deep layers of the OB, surrounding the RMS, but not in the RMS (Saghatelyan et al 2004). This fits well with the role tenascin-R plays in directing migration at the OB level. Radial migration then takes place for a short distance through close contact with vascular endothelial cell and astrocyte contact, until the maturing neurons reach their final destination and integrate into the OB circuitry. The radial migration of neuroblasts in the OB is, similar to the situation in the RMS, closely associated with blood vessels. According to some, neuroblasts connect to the blood vessels through astrocyte end feet and the ECM (Bovetti et al 2007b). Others suggest that the interaction between blood vessels in the OB and radially migrating neuroblasts is mediated by BDNF synthesized by endothelial cells, acting on p75NTR expressed on neuroblasts (Snapyan et al 2009).

BDNF itself has also been investigated as a regulator of neuroblast migration. *In vitro* experiments have shown that BDNF can act as a chemoattractant for postnatal neuroblasts from the RMS (Chiaramello et al 2007). A recent study in acute brain slices has shown that altering the levels of BDNF has complex effects on neuroblast direction and motility during migration through the RMS. For example, blocking BDNF results in increased motility and a directional shift toward the OB. This indicates that yet unknown factors may be present that in the absence of BDNF increase migration

toward the OB. Furthermore, they showed that the effects of BDNF were likely mediated through its receptor TrkB (Bagley & Belluscio 2010).

Even though many signals have been identified as regulators of SVZ neurogenesis, neuroblast migration to the OB and integration as mature neurons, the precise controls over these processes are not yet fully elucidated. The origin of the signals involved, their place in signaling pathways and the way the different factors relate to each other are still poorly understood. The complexity and multitude of controls adds to the difficulty of elucidating the exact mechanisms involved. Understanding the controls of these processes in healthy animals may shed light on similar processes that occur after stroke. The controls of stroke-induced neurogenesis may be similar, but likely significant differences will also be found, since the situation after stroke is quite different from normal. In the next section we investigate neurogenesis in response to stroke.

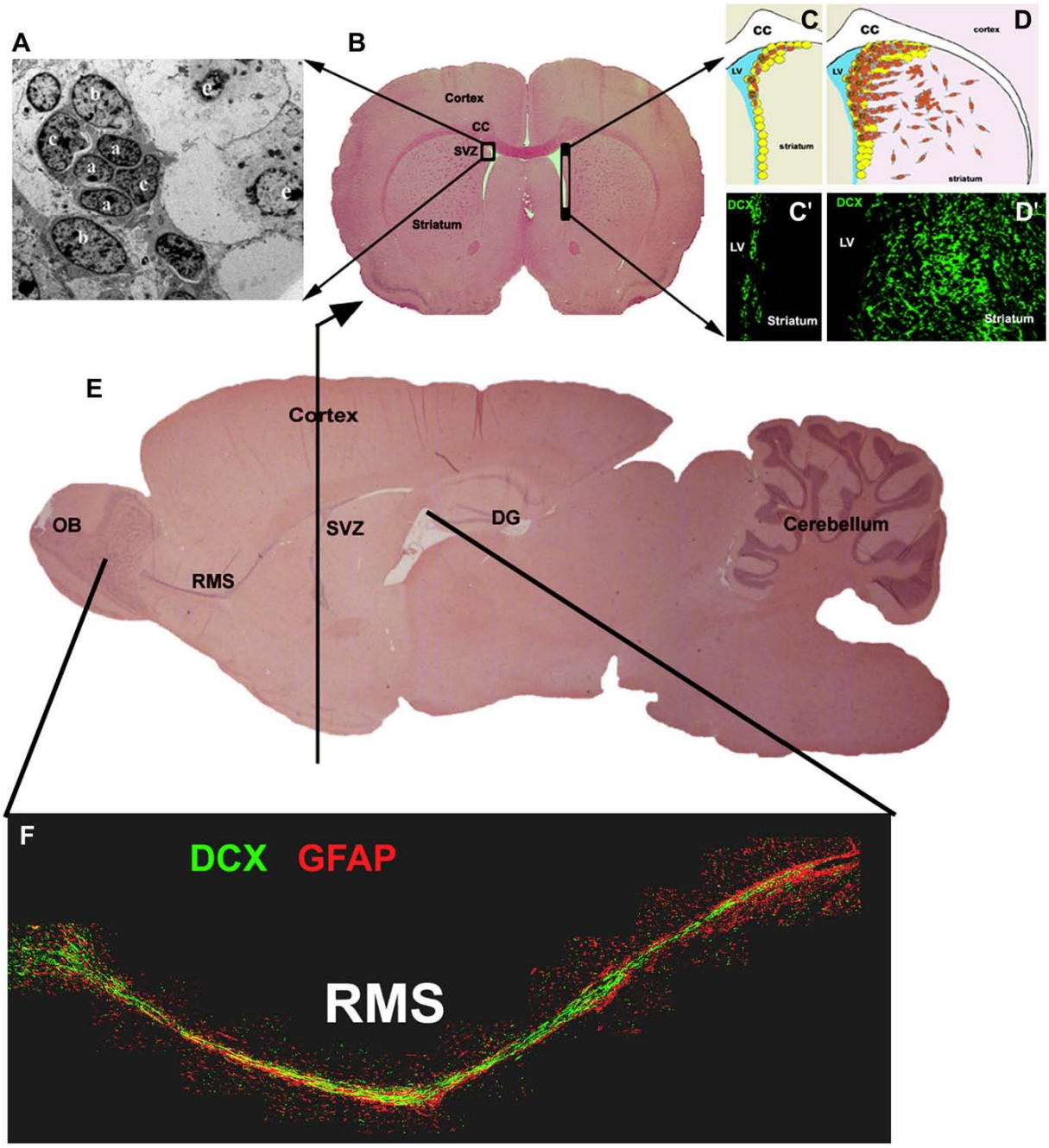

Fig. 1. Overview of adult neurogenesis in the intact brain and after stroke. (A) SVZ neurogenic niche, showing neural stem cells (b), transitory amplifying progenitor cells (c), neuroblasts (a) and ependymal cells (e). (B) schematic coronal view of the adult mouse brain taken at the vertical line in E. (C, C') Coronal schematic (C) and immunohistochemical (C') representation of the normal mouse brain, showing neural stem/progenitor cells (yellow) and neuroblasts (red), adjacent to the lateral ventricle (LV, blue) and showing neuroblasts in C' in green. (D, D') Coronal schematic (D) and immunohistochemical (D') representation of the poststroke mouse brain, showing neuroblasts migrating into the striatum on the stroke side. Colors are the same as in C and C'. (E) Schematic sagittal view of adult mouse brain showing the SVZ, RMS and OB. (F) Immunohistochemical representation of the SVZ-RMS-OB pathway in the mouse (from right to left). In green are Dcx-positive migrating neuroblasts, in red are GFAP-positive astrocytes that form the glial tube. CC = corpus callosum and DG = dentate gyrus. Picture taken from Zhang and colleagues (2008).

Neurogenesis in the adult SVZ in response to stroke

Different types of brain injury including stroke have been shown to lead to increased SVZ neurogenesis (Kim & Szele 2008). Several studies have shown that in response to stroke, neurogenesis in the SVZ is significantly upregulated (Arvidsson et al 2002; Jin et al 2001; Parent et al 2002; Zhang et al 2001) and that neuroblasts leave the SVZ and migrate toward the area of ischemic damage in the striatum (Fig 1; Arvidsson et al 2002; Parent et al 2002) and cortex (Jin et al 2003; Ohab et al 2006).

Stroke leads to dynamic changes in the ischemic area over time. In the acute phase after stroke, many cells are dying and the environment is characterized by excitotoxic neurotransmitters, free radicals and proinflammatory mediators. The inflammatory response leads to microglial activation, general cell apoptosis and can limit endogenous neurogenesis and the growth and survival of transplanted cells (Durukan & Tatlisumak 2007). At the same time, during the acute phase cytokines and neurotrophic factors are starting to be released that could promote cell survival and growth (Locatelli et al 2009). The changes in neurogenesis after stroke have been investigated acutely as well as for longer periods after stroke.

In the SVZ, the cell cycle length is shortened and the proportion of proliferating SVZ cells is increased after stroke (Zhang et al 2006b). First reports seemed to indicate that increased cell proliferation in the SVZ is only transiently upregulated after stroke (Bernal & Peterson 2004; Zhang et al 2004b). Indeed, four days after stroke increased overall cell proliferation in the SVZ is found, an effect which six weeks after stroke has faded. Rapid initiation of migration to the stroke site (within three to four days) is also consistent with early neurogenesis. However, it was also shown that migration of newborn neuroblasts to the stroke site can be sustained for four months (Thored et al 2006). This result clearly demonstrates that neurogenesis is in fact a long-lasting effect after stroke.

BrdU injections the first two weeks after stroke revealed that directly after this period, 46% of the neuroblasts in the damaged striatum were newly generated after stroke. After sixteen weeks similar numbers of neuroblasts were found in the damaged striatum, but these were not BrdU-positive, indicating they were generated at a later time point than the first two weeks. Indeed, when BrdU injections were given in weeks seven and eight after stroke, directly thereafter the neuroblasts in the damaged striatum were BrdU-positive, but after twelve weeks they were not labeled. This suggests that neuroblasts found in the striatum are continuously generated (Thored et al 2006). By injecting self-inactivating lentiviruses expressing green fluorescent protein (GFP) into the SVZ one day before stroke, Liu and colleagues (2009) have shown that GFP- and GFAP-positive neural stem cells can be found in the SVZ six weeks after stroke. At that time neural stem cells still give rise to GFP- and Dcx-positive neuroblasts (Liu et al 2009).

The long-lasting migration of newborn neurons to the stroke area is not the result of continued high levels of proliferation in the SVZ. Cell proliferation in the SVZ in response to stroke is most pronounced in the early stages after stroke (Thored et al 2006), with a peak around one (Zhang et al 2001) to two weeks after stroke (Jin et al 2001). The transient increase of overall cell proliferation in the SVZ after stroke indicates that the source of later neurogenesis lies in the activation of a limited number of neural stem or transitory amplifying progenitor cells. The found neurogenesis that was persistent for long periods after stroke seems to be caused by an increase in the number of neural stem and transitory amplifying progenitor cells in the ipsilateral SVZ (Thored et al 2006).

It was convincingly shown that all neurogenesis induced by stroke is derived from a GFAP-positive precursor in the SVZ. In a transgenic mouse line in which GFP is expressed in any cell that has expressed GFAP (Ohab et al 2006) all newborn (BrdU- and Dcx-positive) neuroblasts after stroke colocalized with GFP. This indicates that after stroke, as in normal neurogenesis, the population of GFAP-positive astrocytic neural stem cells in the SVZ gives rise to new neurons.

Using BrdU birthdating in combination with cell type-specific markers Jin and colleagues (2001) showed that after stroke neurogenesis was upregulated in Dcx- and PCNA (marker for proliferation)-positive cells in the SVZ with a peak after two weeks. In another study using the cell cycle antigen cdc2, expressed by proliferating cells in the SVZ, increased SVZ cell proliferation was found on the ipsilateral stroke side at fourteen days after stroke. BrdU-labeled cells were found in the peri-infarct striatum two to three weeks after stroke. The majority of these newborn cells were migrating neuroblasts, as such identified by markers and morphological features, such as elongated cell bodies and bipolar processes (Parent et al 2002).

The SVZ niche is tightly regulated to maintain its proliferative environment. It is clear that stroke influences neurogenesis in the SVZ. During normal ongoing neurogenesis in the healthy animal, many factors from the cellular environment, ECM, and soluble factors induce neurogenesis and direct migration to the OB. What is the effect of stroke on this environment? It would seem logical that the same mechanisms that regulate normal SVZ neurogenesis and proliferation also play a role after stroke. However, the changes in SVZ neurogenesis and proliferation indicate that these mechanisms, if they are indeed employed, are altered after stroke. What are the changes in signaling that prompt the SVZ stem cells to produce higher numbers of neuroblasts? Where do these signals come from? How do these changes provide the newborn cells with cues to leave the SVZ-RMS-OB migration pathway and start moving into the direction of ischemic damage? These are the questions that arise. Here, we investigate the stroke-induces mechanisms behind increased neurogenesis after stroke.

The changes in the SVZ niche are illustrated by changes in gene expression. Stroke upregulates the expression of genes in the SVZ that are involved in embryonic development, such as TGF-β and BMP superfamily, Wnt family and Notch signals. This suggests recruitment of some of the same signaling pathways after stroke as employed during development. Stroke upregulates genes that are involved in neurogenesis and migration, such as Notch signals, FGFs, integrin α6, MMPs and Hif1α, as well as genes associated with angiogenesis, such as angiopoietins (Liu et al 2007). Poststroke control of gene expression may be mediated by epigenetic adjustments. In response to stroke the brain upregulates growth factors that promote neurogenesis and neuronal survival (Li et al 2010). Growth factors have been found in the CSF after stroke both in animal models (Leker et al 2009; Marti et al 2000) and humans (Krupinski et al 1998). Also, intraventricular administration of growth factors has been shown to increase SVZ neurogenesis (Leker et al 2009).

Regulation of epigenetic controls over gene expression by histone deacetylase (HDAC) inhibitors can be involved in numerous different processes ranging from cell proliferation and differentiation to apoptosis and cell cycle arrest (Marks et al 2000; Vigushin & Coombes 2004). Reports on the effect of HDAC inhibitors after stroke have shown that they decrease brain damage and improve functional outcome (Kim et al 2007; Ren et al 2004). In a later study, Kim and colleagues (2009) have shown that poststroke cell proliferation in the SVZ is promoted by treatment with the HDAC inhibitor sodium butyrate (SB). They also found that BDNF-levels in the ischemic hemisphere were reduced after stroke, but that this effect could be stopped by administering SB. Since BDNF is known to play a role in neurogenesis (Benraiss et al 2001), it was considered that the effect of SB on

neurogenesis was mediated through the BDNF/TrkB signaling pathway. Indeed, when TrkB activity was blocked by its antagonist K252a prior to MCAO, the SB-induced neurogenesis in the SVZ was almost completely inhibited (Kim et al 2009). Others have also shown that inactivation of TrkB, which is expressed by astrocytes and ependymal cells in the SVZ, leads to reduced proliferation in the SVZ and less surviving newborn neurons in the OB (Galvao et al 2008). These findings hold therapeutic value, since they may point to a mechanism for long-term beneficial effects of HDAC inhibitors after stroke.

BDNF upregulation promotes neurogenesis (Benraiss et al 2001) and influences neuroblast migration (Bagley & Belluscio 2010). This mechanism also seems to function in the ischemic brain, BDNF released by stroke-induced neural stem or neural progenitor cells may have a neuroprotective effect and promotes stroke-induced proliferation (Li et al 2010).

BDNF is one of many growth factors that are involved in proliferation and other neurogenic processes after stroke. Growth factors that are upregulated after stroke and stimulate neurogenesis in the SVZ also include HB-EGF, VEGF, fibroblast growth factor (FGF2), EGF, BMP7 and EPO. These and a few other controls over neurogenesis will be discussed here. Administration of HB-EGF, a member of the EGF-family, to the lateral ventricle three days after stroke increases proliferation in the SVZ, generating nestin-positive neural precursors and Dcx-positive neuroblasts (Jin et al 2002b). Treatment with adenovirus expressing HB-EGF also leads to increased migration to the ischemic striatum where the newborn cells mature into a neuronal cell type, shown by the expression of mature neuronal markers such as NeuN and Map2 (Sugiura et al 2005).

In the search for stroke therapies, approaches to promote neurogenesis have included cellular (Chen et al 2001) and pharmacological approaches (Leker et al 2009). To investigate the benefits of a combined approach, Chen and colleagues (2004) administered the nitric oxide (NO) donor NONOate, and bone marrow stromal cells (MSCs) to the poststroke brain. They found this combined treatment leads to increased neurogenesis in the ipsilateral SVZ and striatum. It also increased expression of VEGF and basic fibroblast growth factor (bFGF) in the ischemic boundary region (Chen et al 2004). This study clearly shows that that NONOate and MSC together have a strong effect on poststroke neurogenesis, likely through multiple pathways. Thus, VEGF was shown to be involved in poststroke neurogenesis. It had already been shown that VEGF infusion leads to increased proliferation of neural precursors in the SVZ (Jin et al 2002c). Not surprisingly, overexpression of VEGF after stroke also leads to increased neurogenesis in the striatum of rats (Wang et al 2007b). In another study, the same group shows that overexpression of VEGF after stroke leads to reduced infarct volume and increased proliferation in the SVZ, striatum and cortex of the ischemic hemisphere, compared with controls (Wang et al 2009b). However, in rats with focal cerebral ischemia treatment with VEGF intraventricularly leads to no further increase of proliferation in the SVZ, when compared to the normally observed increase in proliferation in the SVZ in response to stroke (Sun et al 2003). The neuroproliferative effect of VEGF previously found in rat SVZ (Jin et al 2002c) does not add to the neuroproliferative effect observed after focal cerebral stroke. This suggests that focal cerebral stroke- and VEGF-induced neuroproliferation may involve the same, VEGF-mediated mechanism. VEGF treatment after focal cerebral stroke leads to higher numbers of newborn cells in the SVZ at 28 days after stroke, indicating a neuroprotective effect of VEGF rather than an increase in proliferation (Sun et al 2003).

Overexpression of FGF2 increases proliferation in the SVZ, leading to higher numbers of migrating newborn cells found in the corpus callosum and the ischemic cortical area after focal ischemia. Inhibition of proliferation in the SVZ with Ara-C led to a sharp, but not complete reduction

of newborn cells in the peri-infarct cortex. The persisting newborn cells were identified as microglia, supporting the notion that all newborn neurons after stroke originate in the SVZ (or SGZ). Interestingly, an improvement in functional recovery was also found, indicating that the neurogenic effect augmented brain repair (Leker et al 2007).

Intraventricular application of EGF after stroke increases proliferation in the SVZ resulting in increased numbers of newborn neuroblasts. Low doses of EGF (40 ng/day) from day two to day nine after stroke increases the number of proliferating cells eleven-fold in the medial, but not lateral, striatum. High doses (400 ng/day) of EGF even lead to a seventeen-fold increase, showing a significant dose-dependent effect of EGF administration. The specific time period of EGF administration was chosen because it coincides with EGFR expression on nestin-positive neural precursors in the SVZ after stroke. Later administration of EGF (400 ng/day starting at day 21 poststroke) was less effective, indicating the importance of early EGF administration to improve proliferation of transitory amplifying progenitor cells and neural stem cells in the SVZ, likely through EGF/EGFR signaling (Teramoto et al 2003).

In a stroke model relying on devascularization of the motor cortex, intraventricular infusions of EGF and EPO together lead to increased neurogenesis in the damaged cortical area, and contribute to functional motor recovery (Kolb et al 2007). EPO administration by itself was also found to increase neurogenesis; rats treated with EPO after stroke have increased numbers of newborn neural precursors and neuroblasts in the SVZ. EPO treatment increased VEGF levels in the ischemic boundary region and BDNF levels (Wang et al 2004). These neurotrophic factors may be responsible for the effects after EPO treatment. The observed increase in newborn cells 28 days after stroke might be the result of increased survival of newborn cells, since VEGF has a positive effect on the survival of newborn cells in the SVZ after stroke (Sun et al 2003). In EPO receptor (EPOR) and EPO conditional knockout models, the neurogenic response after stroke is decreased (Tsai et al 2006). These data provide strong evidence for an important role of EPO in poststroke neurogenesis.

Another growth factor involved in stroke-induced neurogenesis is bone morphogenetic protein 7 (BMP7). Administration of BMP7 intraventricularly after stroke leads to increased proliferation in the SVZ and in the ischemic cortex. The newly generated cells were mainly NeuN- and Nestin-positive, indicating a neuronal identity (Chou et al 2006). These findings suggest a mechanism for the previously observed functional recovery of stroke after BMP7 treatment (Chang et al 2003). Several growth factors thus stimulate neurogenesis after stroke either directly, or through induction of other (growth) factors.

Notch1 is a transmembrane receptor which plays an important role in stroke-induced neurogenesis in the SVZ. The Notch receptor is activated by binding of its ligands Delta-like 4 and Jagged1. On activation, the transmembrane receptor Notch is cleaved and releases the Notch intracellular domain (NICD). The NICD is directly involved in the transcriptional control of Notch downstream targets, which includes hairy and enhancer of split (Hes) family transcription factors and Shh. Following cerebrocortical focal ischemia the Notch ligand Delta-like 4 and FGF2 were delivered to the brain by osmotic pump, and both were found to increase proliferation in the SVZ. Only the combined administration of Delta-like 4 and FGF2 improved recovery after stroke, leading to an improvement in motor skills (Androutsellis-Theotokis et al 2006). In another study, stroke-induced neurogenesis, measured by the number of BrdU-positive cells in the SVZ, was downregulated after Notch1 inhibition (Wang et al 2009a). Notch1 was further shown to be expressed by neuroblasts in the SVZ, and its ligand Jagged1 by astrocytes in the SVZ. Following stroke, Notch1 is activated. This is shown by expression of the activated form of Notch1, Notch intracellular domain (NICD) which is

increased at four and 24 hours after MCAO. Furthermore, the activity of the enzyme γ-secretase, responsible for cleavage and activation of Notch1 was increased, and downstream targets of Notch1, Hes1 and Shh, were both upregulated after MCAO (Wang et al 2009a). Shh is, given its role in normal neurogenesis in the SVZ together with EGF, a potential regulatory factor in poststroke neurogenesis, but more research is needed to confirm this. Together, these data suggest an important role for the Notch1 signaling pathway, possibly involving Shh, in regulating stroke-induced neurogenesis.

Tumor necrosis factor alpha (TNF-α) is a cytokine that functions through its receptor tumor necrosis factor receptor 1 (TNF-R1). TNF-α has been implicated in neuronal cell death after stroke (Marchetti et al 2004). Stroke leads to TNF-R1 and TNF-α upregulation in the SVZ and striatum of the affected hemisphere (Iosif et al 2008; Lambertsen et al 2007). Cell proliferation in the ipsilateral SVZ is increased in TNF-R1$^{-/-}$ mice one week after stroke, leading to increased numbers of (Dcx-positive) neuroblasts. TNF-R1 is expressed by progenitor cells in the SVZ, suggesting that the decrease in proliferation observed after TNF-R1 deletion may be mediated directly on the progenitor cells themselves (Iosif et al 2008). Astrocytes also express TNF-R1 (Dopp et al 1997), so mechanisms involving astrocytes in the SVZ could also be in play. These results show that TNF-R1 plays an important negative role in neurogenesis after stroke. It is interesting that TNF-R1 deletion, but not TNF-α deletion leads to increased proliferation after stroke (Iosif et al 2008). This suggests that the TNF-R1 signaling pathway is not dependent on TNF-α and can be activated by another TNF-α ligand, such as lymphotoxin α. Remarkably, whereas several growth and other factors that are upregulated after stroke are positive regulators of neurogenesis, TNF-α and TNF-R1 are upregulated, but are negative regulators of SVZ neurogenesis. Blocking TNF-R1 signaling could become a clinical approach to improve neurogenesis in the SVZ after stroke.

Adding a factor to the poststroke animal is a much-applied method of investigating methods that influence neurogenesis. In one study, this was combined with behavioral treatment, in the form of environmental enrichment (EE). Poststroke treatment with retinoic acid (RA) combined with EE increases neurogenesis in the SVZ and the striatum (Plane et al 2008). This effect was found to be present for as late as 61 days poststroke. A less often used, but very effective method is depletion of a signal from the animal model. Such an approach was taken for the complement component (C3)-derived peptide C3a. As the name implies, C3a is derived from C3. C3a has been implied in a range of functions including stimulation of inflammation and neuroprotection. Mice that are deficient in C3 (and thus also C3a) or that have undergone treatment to block the C3a receptor after stroke have impaired stroke-induced neurogenesis (Rahpeymai et al 2006).

It has become clear that many signaling pathways, many involving growth factors and cytokines, influence poststroke neurogenesis in the SVZ neurogenic niche. The discussed factors are by no means complete, but we have discussed some of the most important and best studied controls over poststroke SVZ neurogenesis. The diversity and myriad of mechanisms that play a role shows how complex the process is. Researchers are on their way of understanding how the brain regulates poststroke neurogenesis in the SVZ, but the available knowledge is still incomplete. More studies are needed to better understand what goes on at what time point, in which brain region and which cells are involved. However, as we have shown here, several signals such as TNF-R1, EGF, VEGF and EPO are candidates for use in treatments aimed at enhancing poststroke neurogenesis in the SVZ.

Many of these studies have found a strong correlation between increased neurogenesis in the SVZ and improvement of functional recovery (Leker et al 2007; Plane et al 2008; Tsai et al 2006; Wang et al 2004). The logical expectancy is that the newborn neural precursors migrate to the

ischemic area and integrate to replace neurons lost after stroke. In the next section we investigate the process that follows proliferation in the SVZ, migration to the ischemic area.

Stroke-induced migration of neuroblasts from the SVZ to the ischemic area

The migration of neuroblasts toward the ischemic area observed in response to stroke is very interesting to investigate because understanding the mechanisms involved may lead to better treatments of stroke. New neuroblasts generated in the SVZ are influenced after stroke in such a way that instead of following their natural pathway toward the OB, they divert and travel toward the ischemic area (Arvidsson et al 2002). Mechanisms have to be activated that disrupt the tight regulation in the normal SVZ, where neuroblasts are prevented from entering the striatum. Therapies directed at improving recovery after stroke may benefit greatly from more knowledge on the factors that guide these newborn neurons. If the migratory response can be enhanced, perhaps more young neurons can contribute to functional neuronal replacement in the damaged area or play other beneficial roles in the recovery after stroke.

Both the ischemic striatum and the ischemic cortex are target areas for directed migration of neuroblasts after stroke. Neuroblasts have repeatedly been found to migrate into the ischemic striatum (Jin et al 2003; Parent et al 2002; Zhang et al 2004a). Seven days after focal cortical stroke, neuroblasts are found in the peri-infarct region in the cortex (Ohab et al 2006). Migration to the ischemic striatum differs from migration to the ischemic cortex. The striatum is reached directly from the SVZ, adjacent to the striatum. Neuroblasts that travel to the ischemic cortex do so via the corpus callosum (Ohab & Carmichael 2008). Neuroblasts must traverse tissue boundaries of the striatum and sometimes cortex and white matter of the corpus callosum and can travel a distance of four mm in the murine brain (Tsai et al 2006).

The migrating neuroblasts stem from astrocytic neural stem cells in the SVZ (Yamashita et al 2006). Migration is initiated within three to four days and can be sustained for at least four months after stroke (Thored et al 2006). Migrating neuroblasts were frequently observed in chains extending from the SVZ to the ischemic striatum. This appeared very similar to chains found in the RMS. Both were found to have astrocytes in close contact with the neuroblast chains (Parent et al 2002; Teramoto et al 2003). The neuroblasts migrating in chains have elongated cell bodies and are closely associated with thin astrocytic processes and blood vessels (Yamashita et al 2006).

The number of astrocytes in the SVZ remains unchanged after stroke. This indicates that astrocytic neural stem cells that give rise to transitory amplifying progenitor cells and neuroblasts in the SVZ do not multiply; rather, they only generate cells further down the neural lineage. In the ischemic striatum however, the number of astrocytes, measured with the astrocytic markers vimentin and GFAP, was higher after stroke. These astrocytes may play a role in directing neuroblast migration. Neuroblasts migrating into the ischemic striatum have been found moving in chains, closely connected with astrocytes (Parent et al 2002). This is very similar to the chain migration of neuroblasts in the RMS through a glial tube (Doetsch & Alvarez-Buylla 1996). In addition, EGF infusion leads to an increase in astrocytes (GFAP-positive) with long processes and the formation of a network of astrocytic processes linking the SVZ and the ischemic striatum (Teramoto et al 2003). The known role of astrocytes in guiding normal neuroblast migration suggests they may perform a similar function here. These findings support a role for astrocytes as guiding cells providing trophic support to neuroblasts migrating to the infarcted brain area.

Mechanisms involving different cell types, blood vessels, and signaling pathways have been described to play a role in directing neuroblasts from the SVZ to the ischemic boundary in the striatum and cortex. On the origin of migrating neuroblasts, the question can be asked whether it is

only stroke-induced neuroblasts that migrate to the ischemic area. It could also be that neural precursors that are continuously generated in the SVZ/RMS and designated for integration in the OB are also available for migration to the ischemic area. Evidence points in the direction of the latter being the case. When the mitosis inhibitor cytosine-β-D-arabinofuranoside (Ara-C) was used to stop proliferation after stroke, neuroblasts were still found in the ischemic striatum. A sharp reduction in the number of neuroblasts was observed, but still 25% of normal numbers remained to be found in the ischemic striatum (Arvidsson et al 2002). This shows that neuroblasts generated normally, destined to travel to the OB can also migrate to the ischemic striatum. Also, it indicates that stroke-induced migration can occur at the expense of normal neuroblast migration in the SVZ-RMS-OB pathway (Ohab et al 2006). Still, the majority of neuroblasts found in the ischemic striatum originate from stroke-induced proliferation in the SVZ.

Stroke in humans varies largely in severity (Jorgensen et al 1995). The response to stroke may correlate with stroke severity, and to investigate this Thored and colleagues (2006) looked whether the increase of neuroblasts in the striatum was stroke severity-dependent in mice. They compared the number of Dcx-positive neuroblasts after two hour MCAO (leading to severe damage in the striatum as well as damage in the parietal cortex) with thirty minute MCAO (resulting in a less severe lesion restricted to the striatum). Both two and six weeks after stroke the number of neuroblasts in the ischemic striatum after two hour MCAO was higher compared with thirty minute MCAO. This indicates that stroke severity affects recruitment of new neurons to the damaged area (Thored et al 2006). Stroke severity must therefore somehow influence the mechanisms regulating the recruitment of neuroblasts after stroke. This might be achieved by the inflammatory response to stroke (Durukan & Tatlisumak 2007). Since different types of injuries have been found to invoke neuroblast migration to the damaged area, it was hypothesized that an inflammatory response may play a role in directing neuroblasts to the site of injury (Imitola et al 2004).

Indeed the chemokine stromal cell derived factor-1 (SDF-1) and its receptor CXCR4 are involved in neuroblast migration after stroke as well as neural progenitor cell proliferation. SDF-1 was previously shown to play an important role in adult neuroblast migration (Peng et al 2004). SDF-1 is upregulated in post-stroke brains (Robin et al 2006; Thored et al 2006) and is expressed by reactive astrocytes and endothelial cells in the ischemic boundary for up to sixteen weeks after stroke. Activated microglia in the damaged area also express SDF-1. SDF-1 guides CXCR4-expressing implanted neural stem cells to the damaged area (Imitola et al 2004; Thored et al 2006). Furthermore, secreted SDF-1 can form a gradient to attract adult SVZ neural progenitor cells in a CXCR4-dependent manner (Kokovay et al 2010). The possible SDF-1 gradient is all the more interesting, since it seems that different concentrations of SDF-1 exert different effects. In a recent study it was shown that migration of neural progenitor cells migrating toward a low concentration of SDF-1 (4-20 ng/ml) was increased by treatment with C3a. Conversely, when a high concentration of SDF-1 (500 ng/ml) was used in the migration assay, C3a inhibited migration. C3a only has an effect on migration in the presence of SDF-1 and this effect of C3a is probably mediated through SDF-1-dependent ERK phosphorylation in neural progenitor cells (Shinjyo et al 2009). These results provide a possible mechanism for neuroblasts migrating toward sources of SDF-1 in the ischemic area, and stop once they reach their target area.

Several experiments, both *in vitro* and *in vivo* show that the SDF-1/CXCR4 pathway plays an important role in neuroblast migration. *In vitro* exposure of CXCR4-expressing neural stem cells to SDF-1 triggered intracellular processes associated with survival and migration through the SDF-1/CXCR4 pathway (Imitola et al 2004). Exposure of SDF-1 to neurospheres increases neural

progenitor cell migration. This effect was attenuated by treating the neurospheres with a neutralizing antibody against CXCR4 (Robin et al 2006). Furthermore, overexpression of CXCR4 promotes migration, and migration is further enhanced when SDF-1 is added (Liu et al 2008). Similarly, inhibition of CXCR4 expression decreased migration in a neurosphere assay. This effect was shown to be dose-dependent (Kokovay et al 2010). Interestingly, overexpression of the migration-promoting receptor CXCR4 seems to have a negative effect on proliferation (Liu et al 2008). Finally, NO donor induced-upregulation of SDF-1 after MCAO also enhances SVZ explant cell migration (Cui et al 2009). The SDF-1/CXCR4 pathway does not seem to have an effect on differentiation (Liu et al 2008). Also the *in vivo* regulation of stroke-induces neuroblast migration by SDF-1/CXCR4 signaling has been confirmed. Blockage of CXCR4 does not lead to change in the number of neuroblasts, but it does suppress their migration to the ischemic striatal area (Thored et al 2006). Using a NO donor to upregulate SDF-1 in the ischemic border and CXCR4 in the SVZ after stroke promotes neuroblast migration toward the ischemic area (Cui et al 2009). Furthermore, CXCR4 is expressed by neuroblasts, transitory amplifying cells as well as neural stem cells (Kelly et al 2004; Kokovay et al 2010). Neural stem cells are least attracted by SDF-1, although activated (highly EGFR-positive) neural stem cells are more strongly attracted than non-activated neural stem cells and astrocytes. Neuroblasts and transitory amplifying progenitor cells both show a significant chemotaxis toward an SDF-1 signal. Blocking CXCR4 with AMD3100 leads to an almost complete attenuation of migration of neural stem and transitory amplifying progenitor cells, but of only about half of the neuroblasts (Kokovay et al 2010). The explanation for this could lie in the differential expression of the laminin receptor α6β1. Neural stem cells and transitory amplifying progenitor cells both express α6 integrin and β1 integrin, forming the α6β1 laminin receptor. Neuroblasts also express this receptor, but to a much lesser extend. Laminin is expressed by vascular endothelial cells and is abundant in the surrounding ECM. Adhesion of neural stem cells and neural progenitors to the vasculature is mediated through laminin/α6β1 interaction (Shen et al 2008). SDF-1 induces upregulation of α6 expression in neural stem cells and transitory amplifying progenitor cells. This effect of SDF-1 is much lower in neuroblasts (Kokovay et al 2010). These differential effects of SDF-1 on neuroblasts compared with neural stem and transitory amplifying progenitor cells may be related to the increased migration mainly of neuroblasts after stroke. SDF-1 promotes the adhesion of neural stem cells and transitory amplifying progenitor cells to the vasculature, but not as much in neuroblasts, giving the neuroblasts more 'freedom' to migrate. Clearly, SDF-1 and CXCR4 work closely together in controlling neuroblast migration. Much has been discovered about the complex functioning of this pathway. For example the selective expression by certain cell types, the effects on other processes such as proliferation and differentiation, the dosage-dependent functioning and its relation to other factors such as NO and C3a. These results are all useful for the design of therapeutic agents that could manipulate neuroblast migration after stroke.

Many other factors that influence cell migration from the SVZ to the ischemic boundary after stroke include have still gone unmentioned. They include growth factors, hormonal factors, signaling from the target area and interactions with the ECM, and will be discussed here.

EPO, which plays a role in poststroke neurogenesis, was also found to enhance migration after stroke (Wang et al 2004). Expression of EPO and its receptor EPOR are increased in the damaged area and the ischemic boundary after stroke (Bernaudin et al 1999). This makes EPO and EPOR possible attractive signals for migrating neuroblasts. However, it cannot be excluded that the observed increase in migration is the result of increased neurogenesis (Wang et al 2004). Further

research will be needed to elucidate the mechanisms involved in the positive effect of EPO and EPOR on migration after stroke.

Infusion of EGF promotes migration from the SVZ toward the ischemic cortex. In a study using a stroke model of pial blood vessel devascularization, which resulted in the death of cortical cells in the motor cortex, EGF infusion for seven days after stroke (days four to eleven poststroke) leads to increased directed migration to the stroke area. Migrating cells were also found in the corpus callosum and the ischemic area in the motor cortex. Strikingly, in this stroke model, when EGF infusion was followed by EPO infusion from day eleven poststroke, cortical regeneration of the lesion area by SVZ-derived neural precursors was found. Furthermore, this regeneration leads to marked functional recovery (Kolb et al 2007). Early EGF administration (starting on day two poststroke) also gives rise to high numbers of migrating neuroblasts expressing the neuronal marker TUJ1 (Doetsch et al 1997) in the medial ischemic striatum, extending deeper into the ischemic striatum in a gradient (Teramoto et al 2003). It was speculated that the observed effect on migration of EGF may be in part have been the result of increased transitory amplifying progenitor cell proliferation caused by EGF (Doetsch et al 2002). However, it does seem that EGF influences migration itself, as it has also been shown to promote migration in the non-ischemic brain (Craig et al 1996). EGF infusion can induce the formation of a network of astrocytic processes between the SVZ and ischemic striatum, providing a possible mechanism for EGF-induced migration after stroke (Teramoto et al 2003). These data suggest that migrating neuroblasts derived from SVZ precursors migrate toward the ischemic striatum and other infarcted brain areas, and that this process is promoted by EGF signaling.

BDNF has a positive effect on poststroke migration; rats treated with intravenous BDNF after stroke have more neuroblasts migrating into the striatum on the affected side (Schabitz et al 2007). In another study, treatment of poststroke animals with atorvastatin, which increases levels of VEGF, VEGFR2 and BDNF in the ischemic border, led to increased migration of neuroblasts *in vivo*. *In vitro*, using SVZ explant cultures, it was shown that atorvastatin promotes migration and this effect is inhibited by blocking of BDNF (Chen et al 2005). These results demonstrate the important role of growth factors in regulating stroke-induced neuroblast migration.

Activated astrocytes may play an important role in directing stroke-induced migration to the ischemic boundary (Parent et al 2002). Activated microglia and reactive astrocytes in the ischemic area secrete the neuroblast attracting chemokine SDF-1 after MCAO and hypoxic-ischemic injury (Imitola et al 2004; Thored et al 2006). On the non-ischemic side astrocytes were present, but no reactive astrocytes expressing SDF-1 were found (Imitola et al 2004). The inflammatory response after ischemic injury may well have been the cause of SDF-1 upregulation in reactive astrocytes, since the inflammatory agent interleukin-1β (IL-1β) has been associated with SDF-1 upregulation in astrocytes (Zhou et al 2002).

Poststroke treatment with RA alone and in combination with EE, leads to increased numbers of neuroblasts migrating to the ischemic boundary (Plane et al 2008). Since RA increases proliferation in the SVZ leading to higher numbers of neuroblasts, the observation of higher numbers of migrating neuroblasts could be due to increased neuroblasts available for migration. Therefore, even though increased migration is observed, RA may not directly influence mechanisms involved in migration.

Since normal migration is influenced by the cell adhesion molecule PSA-NCAM (Cremer et al 1994; Doetsch & Alvarez-Buylla 1996; Ono et al 1994), this mechanism may also be involved in stroke-induced neuroblast migration to the stroke site. Presence of PSA on the cell surface decreases cell-cell interactions thus promoting cell motility and migration. A link between natural PSA level adjustments and migration in has not been found in the natural poststroke migratory response.

However, artificially increasing PSA levels leads to increased migration of neuroblasts from the SVZ to the site of injury in the cerebral cortex (El Maarouf et al 2006).

Since neuroblasts travel through the brain tissue, they have to overcome the boundary of the ECM. MMPs can degrade ECM proteins and aid in directing migrating cells by selective degradation. In response to stroke, SVZ neuroblasts expression of MMP-3 and MMP-9 is upregulated and broad spectrum inhibition of MMPs with GM6001 decreases neuroblast migration into the striatum (Lee et al 2006).

The role reelin plays in neuroblast detachment from the neuroblast chain and the initiation of radial migration raises the question whether reelin may be involved in stroke-induced migration and in the decision of neuroblasts to leave the SVZ-RMS-OB pathway. The latter issue has not yet been resolved. It has been shown that migration after stroke was decreased in reelin-deficient mice (Won et al 2006).

Post-stroke migration is regulated by a number of attractive and trophic factors. Restrictive factors, that inhibit neuroblast migration to specific areas after stroke may also be an important mechanism after stroke. It has been suggested that migration-inhibiting signaling found *in vitro* and during development, such as chondroitin sulfate proteoglycans (CSPGs) and semaphorins may play a role in creating tissue boundaries for post-stroke migration (Ohab & Carmichael 2008). Such a mechanism seems likely, but this suggestion is based solely on upregulated expression of these factors after stroke and requires further investigation.

Since neuroblasts differentiate into calretinin-expressing interneurons in the damaged striatum after stroke, the effect of stroke on the numbers of calretinin-expressing neuroblasts in the SVZ-RMS-OB pathway was measured. In both the ipsilateral as well as the contralateral RMS and OB high numbers of calretinin-positive neuroblasts were found. Newborn calretinin-positive neurons in granular and glomerular layers of the OB also did not differ between contralateral and ipsilateral sides (Liu et al 2009). Thus, stroke does not effect the number of interneurons in the OB. Even tough the number of migrating cells in the RMS seems to diminish after stroke (Ohab et al 2006), this may not affect the number of surviving calretinin-positive neurons in the OB, since more than 50% of new cells are lost after they migrate into the OB (Petreanu & Alvarez-Buylla 2002).

Many factors that influence stroke-induced migration of neuroblasts from the SVZ to the ischemic area have been discussed, such as SDF-1, EPO, EGF, RA, PSA-NCAM and MMPs. These may hold therapeutic value for stroke treatment. Angiogenesis and the existing vasculature are closely associated with poststroke neuroblast migration and stroke-induced neurogenesis in general, which will be discussed in the following section.

Angiogenesis and the role of the vasculature after stroke

Over the past decade an important relationship between angiogenesis and neuroblast migration has been uncovered. Neuroblasts localize around the damaged area, attracted by factors not present in healthy tissue, or at least upregulated in the affected tissue. One feature that sets the peri-infarct area apart from healthy tissue is the angiogenic response. Stroke induces angiogenesis in the infarcted region and the ischemic penumbra (Beck et al 2000; Kovacs et al 1996; Marti et al 2000). Angiogenesis increases blood supply to the damaged area, providing trophic support and oxygen, reducing further ischemic damage in the period following stroke (Chopp et al 2007). The newly formed blood vessels aid in recruiting new neurons to the damaged area (Thored et al 2007). Vascular endothelial cells have been shown to express factors that attract migrating neuroblasts to the infarcted area. The migratory path of neuroblasts through the striatum to the stroke site is associated with blood vessel guidance and contact (Yamashita et al 2006). Also in the neurogenic SVZ a close association between neural progenitors and the vasculature exists. Dividing transitory amplifying progenitor cells and neural stem cells are in close contact with vascular endothelial cells (Kokovay et al 2010). This neurogenic environment in the SVZ has been called a 'neurovascular niche' because of its close association with the vasculature (Ohab et al 2006).

In the vascular niche neuroblasts are not located as closely to the vasculature compared with neural stem cells and transitory amplifying progenitor cells. However, in the dorsal SVZ chains of migrating neuroblasts do appear to migrate along the vasculature stroke (Shen et al 2008; Tavazoie et al 2008). This is similar to the situation in the healthy RMS, where chains of migrating neuroblasts are also closely associated with blood vessels (Bovetti et al 2007b; Whitman et al 2009). Furthermore, several studies show a close physical relation between neuroblasts and blood vessels in the poststroke striatum (Thored et al 2007; Yamashita et al 2006). Neuroblasts are associated with both newly formed and pre-existing blood vessels. Imaging studies have revealed that neuroblasts follow a vascular network from the SVZ to the ischemic striatum and that they extend a leading process in close physical association with blood vessels (Kojima et al 2010). These studies show that neural precursors during early and late stages of development, in the SVZ as well as during migration to the ischemic site are found in close association with the vasculature. The vasculature acts as a guidance mechanism for neuroblasts migrating to the ischemic stratum.

Angiogenesis in the ischemic region can be seen as a response mechanism of the brain to restore oxygen and nutrient supply to the affected area. In the tissue surrounding the infarcted area the angiogenic response is initiated rapidly. Using PECAM-1 as a marker for endothelial cells and Ki67 as a proliferation marker, angiogenesis was found from two days after cerebral stroke in the ischemic border and core (Marti et al 2000). Later it was shown that proliferation of endothelial cells in this area starts as soon as one day after stroke. This leads to an increase in bloodvessels in the area at three days poststroke (Hayashi et al 2003). Angiogenesis continues for at least three weeks after stroke (Beck & Plate 2009). Studies over longer time periods will be needed to investigate whether angiogenesis continues after three weeks.

Directly after stroke, the angiogenic response is preceded by a reaction that is the opposite of angiogenesis. Endothelial cells of the vasculature undergo apoptosis at twelve hourse after stroke. This is followed by an angiogenic response marked by proliferation of endothelial cells in the peri-infarct region. It is speculated that Ang-2 is responsible for this transient apoptotic effect. A dual role for Ang-2 that is dependent on VEGF levels is hypothesized. At first, with low levels of VEGF, Ang-2

has an apoptotic effect on endothelial cells, after VEGF levels have risen it counteracts the apoptotic effect of Ang-2 (Beck et al 2000).

Areas of vascular sprouting have been found in the ischemic penumbra. Within these areas newly born, immature neurons were found. Blocking of angiogenesis results in 90% less neuroblasts in the area surrounding ischemic damage than normally present after stroke (Ohab et al 2006). This strongly indicates a close connection between neurogenesis and angiogenesis exists after stroke.

The exact mechanisms by which angiogenesis affects neuronal migration are not clear, but some factors that play important roles in this relationship have been discovered. Factors such as NO, SDF-1, MMPs, VEGF, HB-EGF, EPO and angiopoietins have all been shown to be connected with angiogenesis and neurogenesis in response to stroke.

First, we look at the effects mediated by the pathway involving angiopoietin-1 (Ang1) and its receptor, the tyrosine kinase Tie2, on angiogenesis, neurogenesis and neuroblast migration. Treatment with a NO donor after stroke was shown to increase expression of the endothelial growth factor Ang1 in the ischemic boundary area (Cui et al 2009). Interestingly, blood vessels that connect the ischemic area and SVZ along the complete migratory route of neuroblasts are immunoreactive for Ang1 (Ohab et al 2006). NO treatment also increased expression of Tie2 in the SVZ. NO treatment also promotes neuroblast migration to the ischemic area. Finally, it was shown that NO treatment after stroke enhances SVZ explant cell migration, likely through the Ang1/Tie2 pathway (Cui et al 2009). Interestingly, the Ang1/Tie2 pathway is also known to enhance angiogenesis in the ischemic brain (Zacharek et al 2006). Thus, NO promotes neuroblast migration after stroke through the Ang1/Tie2 signaling pathway. The effect of NO on neuroblast migration could be through NO-induced angiogenesis, but more research is needed to confirm this. The Ang1/Tie2 signaling pathway is involved in both neurogenesis and angiogenesis.

In addition to its positive effect on neurogenesis, administration of MSCs to poststroke animals can induce angiogenesis in the area surrounding the infarct (Chen et al 2003). When investigators looked at the combinatorial treatment of stroke with a NO donor and MSCs, they found it promotes angiogenesis as well as neurogenesis in the SVZ. In this study, no upregulation of angiopoietin was reported, but no immunochemistry for angiopoietin was performed, therefore it may have been upregulated still. Instead, they found upregulation of the growth factors bFGF and VEGF in the ischemic boundary (Chen et al 2004).

VEGF is one of the first known and best studied vascular-endothelial-specific growth factors and is crucial for vasculogenesis and angiogenesis during rodent development as well as in adulthood (Risau 1997; Yancopoulos et al 2000). VEGF is expressed in the ischemic penumbra after stroke and is induced by hypoxia. It is transiently upregulated after stroke in the ischemic border, from 6 to 24 hours after stroke, but expression is not found in the ischemic core. VEGF receptor (VEGFR) expression is induced 48 hours after stroke in the ischemic border and in vessel-like structures invading the ischemic core, later VEGFR expression is also found in the infarct area. The area of VEGF expression corresponds largely with the area where angiogenesis occurs in response to stroke. Furthermore, the transcription factors hypoxia-inducable factor-1 and -2 (HIF-1 and -2), which are involved in the regulation of VEGF and VEGFR expression, are increased in the area surrounding ischemic damage after 72 hours (Marti et al 2000). This very specific pattern of upregulation of the VEGF signaling system in the area where angiogenesis is induced together with VEGF's known role in promoting angiogenesis suggest this signaling pathway plays an important role in regulating angiogenesis after stroke.

Logically, it was investigated whether VEGF administration could promote angiogenesis after stroke. It was found that treating ischemic mice with VEGF can have a positive or a negative effect on recovery. Intravenous administration of recombinant human VEGF (rhVEGF) directly (one hour) after stroke increases blood brain barrier leakage and may lead to hemorrhagic transformation and accelerated ischemic damage (Zhang et al 2000). Also, blockage of VEGF in a stroke model reduces edema formation and tissue damage (van Bruggen et al 1999). On the other hand, rats treated with intravenous VEGF two days poststroke have more small- and microvessels in the ischemic border in the cortex and show increased functional recovery during the early stages after stroke (up to four weeks; Zhang et al 2000). Others have also found that VEGF, when overexpressed, promotes angiogenesis in the frontal cortex after stroke (Sun et al 2003; Wang et al 2009b). It seems that VEGF has a positive effect on stroke recovery by promoting angiogenesis, but in the acute phase of stroke it causes exacerbation of brain damage, due to increased blood vessel permeability.

Rats treated with EPO after stroke show increased brain levels of VEGF and BDNF. EPO treatment has also been found to enhance capillary tube formation *in vitro*. This is likely mediated through VEGF signaling, since inhibition of the VEGF receptor VEGFR-2 by an antagonist abolished this effect (Wang et al 2004).

VEGF is upregulated and mainly expressed by macrophages and GFAP-positive cells during the early phases after stroke in the ischemic area and ischemic penumbra. These GFAP-positive cells are probably reactive astrocytes, judging by their small, dark, either elongated or triangular appearance. Following this early phase after stroke, neurons start to express VEGF in the ischemic core and surrounding area, and VEGF in astrocytes and macrophages declines. This suggests that ischemic neurons take over expression of VEGF from its initial sources, macrophages and glial cells. Vascular endothelial cells of developing capillaries in the ischemic area express the VEGF receptor VEGFR directly after stroke and this expression increases over time, but ceases completely by week three poststroke. Moreover, VEGF also localizes to these endothelial cells and seems to be bound to its receptor, indicating that VEGF signals through this receptor (Kovacs et al 1996).

These studies clearly show an important role for VEGF signaling in poststroke angiogenesis. VEGF has the ability to upregulate angiogenesis and promote neurogenesis. VEGF signaling in the ischemic area involves multiple cell types and VEGF receptors. VEGF has potential as a treatment for stroke, but it is important to keep in mind the possible negative effects of VEGF on stroke outcome. Timing is of the essence here, since early VEGF administration has adverse effects, while later VEGF administration can promote recovery after stroke through angiogenesis. Well-timed VEGF administration could be used to enhance the brains repair mechanisms after stroke.

VEGF signaling thus plays an important role in the ischemic area after stroke, but what is the role of VEGF signaling in the SVZ? The existing vasculature in the SVZ is closely associated with neuroproliferation (Kokovay et al 2010). It was hypothesized that the neuroproliferative effect of VEGF was mediated by new blood vessels in the SVZ. However, no signs of increased angiogenesis in the SVZ neuroproliferative zone in response to VEGF treatment after stroke was found (Sun et al 2003). This suggests that the neuroproliferative effect caused by VEGF is not due to neovascularization in neuroproliferative zones. However, coupling of neurogenesis and angiogenesis does seem to rely, at least in part, on VEGF signaling. In vitro coculture experiments have shown that neuroblasts can stimulate angiogenesis through VEGF secretion. It was also shown that endothelial cells from poststroke brains stimulate SVZ neurogenesis in vitro. The signaling pathway involved in both cases was shown to include VEGFR2 (Teng et al 2008). Migrating neuroblasts follow the vasculature (Thored et al 2007; Yamashita et al 2006) and vascular endothelial cells express VEGFR2

(Marti et al 2000). Taken together, these results strongly suggest VEGF links poststroke neurogenesis and angiogenesis. It also seems that the pathway involving VEGF/VEGFR2 signaling is responsible for a reciprocal relationship between SVZ neurogenesis and angiogenesis in the ischemic area.

The EPO-induced upregulation of BDNF expression in combination with the observed increased angiogenesis suggests a role for BDNF in mediating this effect (Wang et al 2004). It has been shown that BDNF is expressed by astrocytes and endothelial cells, and that the supportive role of the vasculature in neuroblast migration is mediated by BDNF signaling (Leventhal et al 1999). Furthermore, stroke treatment with atorvastatin increases BDNF expression in the ischemic border by neurons and endothelial cells, likely through VEGFR2 signaling, since inhibition of this receptor attenuated atorvastatin-mediated BDNF expression in vitro. Atorvastatin treatment also enhances angiogenesis after stroke, an effect which may be mediated by BDNF (Chen et al 2005).

Another important signaling pathway related to the vasculature and involved in neurogenesis is the SDF-1/CXCR4 pathway. This signaling pathway has been found to play an important role in guiding neuroblasts to the ischemic area in a manner dependent on the vasculature (Imitola et al 2004; Kokovay et al 2010; Robin et al 2006; Thored et al 2006). SDF-1, which is known to attract migrating neuroblasts, is secreted by vascular endothelial cells. It was shown in vitro that migration of activated neural progenitor cells toward the vasculature is mediated by SDF-1/CXCR4 signaling. The role of SDF-1/CXCR4 signaling was further confirmed in vivo. SVZ neural progenitor cells transplanted into a host SVZ integrate and migrate toward blood vessels in a SDF-1/CXCR4-dependent manner (Kokovay et al 2010).

In the vascular niche, the process of neural progenitors homing to the vasculature is dependent on SDF-1/CXCR4 signaling (Kokovay et al 2010). Interestingly, endothelial SDF-1 signaling has differential effects on different stages of neural progenitor cells. When neural stem cells become activated, they upregulate EGFR expression and give rise to EGFR-positive transitory amplifying progenitor cells (Pastrana et al 2009). In the SVZ, these activated neural precursors are attracted to the vasculature, whereas no significant effect on quiescent neural stem cells was found (Kokovay et al 2010). This shows that the neurogenic capacities of neural progenitors are correlated with contact with the vasculature. This contact could enable activated neural progenitors to receive signals and/or trophic factors from the blood. After stroke, activated endothelial cells in the ischemic region secrete SDF-1, thus aiding in the recruitment of new neurons to the damaged area (Ohab et al 2006; Zhang et al 2008). It has been suggested that SDF-1 is only secreted by endothelial cells in the cortical ischemic area, and not in the striatum, where SDF-1 is secreted by astrocytes and macrophages (Ohab & Carmichael 2008). These results show that the SDF-1/CXCR4 plays many roles in poststroke neurogenesis and migration, with a strong association with the vasculature. A thorough understanding of how this pathway is involved in restorative processes after stroke, and the important role for the vasculature in these processes may provide targets for therapeutic approaches.

Several factors have been found that promote both angiogenesis and neurogenesis in the ischemic area after stroke. It is not always clear what mechanisms are involved. Also, angiogenesis could lead to the observed neurogenic effect, or vice versa.

A factor that has been implicated in neural progenitor migration after stroke and also influences the vasculature is HB-EGF. In addition to its effect on neurogenesis and migration, treatment with this neurotrophic factor after stroke leads to increased vascular density in the ischemic boundary (Sugiura et al 2005). HB-EGF is known to induce the secretion of VEGF by smooth

muscle cells (Abramovitch et al 1998). Thus, VEGF signaling is a likely mechanism of HB-EGF-induced angiogenesis.

Similar to normal neuroblast migration, MMPs play an important role in stroke-induced migration. In healthy mice the metalloproteinase 21 (ADAM21) and likely other MMPs are present around chains of migrating neuroblasts. Blocking MMP function slows down neuroblast migration in normal migration (Bovetti et al 2007a; Yang et al 2005). Neuroblasts migrating to the ischemic striatum in response to stroke express MMP9, and MMP inhibition decreases migration into the striatum (Lee et al 2006). Interestingly, the vasculature itself may also secrete MMPs to enhance neuroblast migration. EPO, known to promote neuroblast migration, seems to do this in part by activating endothelial cells. EPO-activated endothelial cells upregulate their expression of MMP2 and MMP9, which promotes migration of neural progenitor cells *in vitro* (Wang et al 2006). These data suggest the (newly generated) vasculature and neuroblasts work together to digest the ECM, facilitating neuroblast migration through white matter or striatal tissue after stroke.

The vasculature, angiogenic and overall neurogenic response work together in restoring damaged tissue after stroke. Several factors, such as VEGF, angiopoietin and EPO promote the neurogenic and migratory response after stroke, with a close association with the vasculature and/or angiogenesis. Newly formed blood vessels in the ischemic area, secrete factors such as SDF-1 that attract migrating neuroblasts, which express the corresponding receptor CXCR4. During migration, the vasculature may help migrating neuroblasts to digest the normally non-migratory ECM. It is clear that the vasculature and angiogenesis are not only closely associated physically, but also mechanistically, with neurogenesis and the migration of neuroblasts after stroke.

Fate of newborn cells migrating to ischemic areas: differentiation, survival and integration

A successful recovery after stroke not only depends on neurogenesis in the SVZ and neuroblast migration to the infarct area. It also requires differentiation of these newly formed neural progenitors into mature neuronal cell types and the subsequent integration in a functional network. Integration of new neurons could replace neurons lost after stroke, thus restoring normal function of the infarcted brain area to some degree.

The brain has the ability to upregulate neurogenesis in response to stroke (Arvidsson et al 2002; Jin et al 2001; Zhang et al 2001). After stroke, neuroblasts migrate to the ischemic area through directed migration (Jin et al 2003; Parent et al 2002; Zhang et al 2004a). Maturation of migrating neuroblasts is crucial for a meaningful integration in the target area. It has been found that part of the migrating neuroblasts express the mature neuronal marker NeuN (Arvidsson et al 2002), indicating that neuroblasts mature as they migrate to the infarcted area. Having established a mature neuronal phenotype of newly generated cells after stroke, an important additional issue is what specific types of neurons stroke-induced neurogenesis can give rise to.

In principle, neuroblasts originating in the SVZ can give rise to several mature neuronal subtypes, as is known from the neurons integrating in the OB of healthy animals. The majority of newly generated neurons that integrate in the OB circuitry are GABAergic or calretinin-positive interneurons, but some express TH, suggesting a dopaminergic cell type (Winner et al 2002). In healthy animals SVZ neural stem cells thus give rise to multiple, although limited, types of neurons. In addition to the neuronal cell types generated in the adult OB, it has been found that after injecting purified SVZ neural progenitors directly into the striatum they can give rise to oligodendritic and astrocytic cell types (Seidenfaden et al 2006). This suggests that in principle neural progenitors have the capacity to generate a wide range of neuronal and other cell types.

After the dogma of ‘no neurogenesis in the adult brain’ was overcome it was still believed that neurogenesis only took place in the few neurogenic zones of the brain (Wiltrout et al 2007). At the turning of the millennium the first study was published showing that regenerative neurogenesis can take place in normally non-neurogenic areas of the brain (Magavi et al 2000). Previously it had been shown that specific apoptotic cell death in the cortex directed transplanted endogenous multipotent precursors to integrate into the neural circuitry (Sheen et al 1999; Snyder et al 1997). Magavi and colleagues (2000) provided the first ‘proof of principle’ for adult mammalian neurogenesis induced by targeted cellular apoptosis in the cortex, without transplantation. Even though they did not show the origin of these newly generated neurons, they suspected the SVZ might provide them. Importantly, in these studies the environment of the apoptotic cells in the cortex was maintained, allowing for the surrounding cells to provide directing and trophic signals for newly generated and integrating neurons (Sohur et al 2006; Wang et al 1998). This does not match the situation in the ischemic stroke core, where tissue structure has largely been disrupted, but it may be similar to the ischemic penumbra, where neurons undergo apoptosis (Durukan & Tatlisumak 2007).

The infarct area provides a very unique niche for neuronal integration. The infarct area after MCAO is generally large, comprising substantive parts of the striatum and cortex. In this area, neuronal injury and DNA damage are severe, leading to extensive neuronal loss (Arvidsson et al 2002; Parent et al 2002). Degeneration in the stroke area is not limited to a specific population; it involves

different neuronal populations, glial cell types and endothelial cells. In addition, white matter is often damaged leading to disruption of pathways that pass through the damaged area (Locatelli et al 2009). The ischemic area is surrounded by an area of less severe damage, the ischemic penumbra. It is to be expected that repair of such a complicated environment is challenging. The early stages after stroke are characterized by excitotoxicity, generation of free radicals, disruption of the blood brain barrier, inflammation, necrosis and apoptosis (Durukan & Tatlisumak 2007). The subsequent release of growth factors and other trophic factors stimulates the survival and integration of new neurons. The initiation of angiogenic processes also aid in the migration and differentiation of neural precursors. Thus, it seems that even though the initial stages after stroke do not favor neurogenesis, the dynamic stroke area changes to promote neuronal differentiation, survival and integration.

Mature neurons generated after stroke have repeatedly been found in the ischemic and surrounding area (Jin et al 2003; Parent et al 2002; Zhang et al 2004a). Stroke induces a 31-fold increase of newborn neurons in the striatum four weeks after the last BrdU injection (BrdU administered four to six days after stroke). Furthermore, two weeks after stroke 20% of the newborn neuroblasts in the striatum express the neuronal marker NeuN. Four weeks later the number of newborn NeuN cells was five- to ten-fold higher. This indicates that over time increasingly more newborn progenitors differentiate into a neuronal cell type. However, it was estimated that only about 0.2% of the total number of lost striatal neurons after ischemic stroke are replaced by new neurons (Arvidsson et al 2002).

In the ischemic cortex, a portion of the newborn cells that are found around the infarct area survive long-term. A small percentage of these newborn cells express the neuronal markers MAP-2, β-tubulin-III and NeuN at three months after stroke (Gu et al 2000; Jiang et al 2001). However, the origin of these newborn neurons was not shown. Neuroblasts migrate via the corpus callosum toward the ischemic cortex after stroke (Jin et al 2003). However, very little of these neuroblasts have been found to differentiate into mature neurons (Arvidsson et al 2002; Parent et al 2002). This suggests that the cortical ischemic area is less supportive of neurogenesis than the ischemic striatum. It has been suggested that the environment of the corpus callosum or cortex could lack signals necessary for differentiation, or contain signals that prohibit neuroblasts to differentiate (Wiltrout et al 2007).

Over time, the numbers of newborn neurons that survive in the ischemic striatum declines. At six weeks poststroke roughly 40% of the newborn neurons found at two weeks poststroke have survived. At four months poststroke, there remain only about 10% of the total newborn neurons found at two weeks after stroke. This however, is not the full story, because new neurons are continuously generated. So, there is the 10% of new neurons that survives over the period between two weeks and four months after stroke. In addition to that, of the neurons generated for example at two months poststroke, some 20% remain at four months poststroke (Thored et al 2006). This shows that neuroblasts are not only generated for extended periods after stroke, they also retain the capacity to form mature neurons, although the efficiency to do so appears to decrease over time.

Since not all neuroblasts give rise to mature neurons, the question arises what happens to the rest of these neuroblasts. The possibility of neuroblasts remaining in an undifferentiated state is highly unlikely, since no newborn neuroblasts were present four weeks after BrdU injection (Thored et al 2006). Neuroblasts giving rise to glial cell types is also highly unlikely, since no neuroblasts have been found expressing glial markers (Arvidsson et al 2002; Thored et al 2006). Neuroblasts scattered in the striatum on the ischemic side have been found to express the caspase-marker PARP. Also, neuroblast numbers were increased after treatment with caspase inhibitors (Thored et al 2006). This

demonstrates that neuroblasts undergo apoptosis in a caspase-mediated way. Inhibiting apoptosis has been shown to increase the number of neuroblasts that survive in the ischemic striatum up to one month after stroke (Zhang et al 2006a).

The striatum is comprised of many neuronal cell types, but the large majority (90-95%) of them is GABAergic medium-sized spiny projection neuron. A smaller population (~5%) consists of local interneurons (Liu et al 2009). Striatal medium-sized spiny projection neurons can be marked by DARPP-32 and calbindin immunohistochemistry (Liu et al 2009; Liu & Graybiel 1992). Striatal interneurons exist of four distinct populations, marked by parvalbumin, calretinin, somatostatin, and choline acetyltransferase (ChAT; Kawaguchi et al 1995). Stroke models in rodents do not lead to uniform death of all cell types in the ischemic area. Some neuronal subtypes are more susceptible to hypoxic damage than others. Ischemic damage leads to the loss of DARPP-32-positive spiny projection neurons (Andsberg et al 1998) and parvalbumin-positive interneurons (Andsberg et al 2002), but there is relative preservation of ChAT- and somatostatin-positive interneurons (Chesselet et al 1990).

Since stroke leads to the specific loss of spiny projection neurons and a specific subset of interneurons, it would arguably be most beneficial for a functional recovery after stroke if neuroblasts differentiate into these types of neurons. Indeed, it was shown that almost all neuroblasts found in the ischemic striatum after stroke are Meis2-positive. Meis2 is a transcription factor normally expressed in striatal neuronal precursors, as well as in the adult striatum where it shows great overlap with DARPP-32 expression. Furthermore, a correlation between newborn NeuN-positive and newborn DARPP-32-positive cells in the ischemic striatum suggests that some neuroblasts differentiate into spiny projection neurons (Arvidsson et al 2002). Since DARPP-32 is expressed by virtually all major types of striatal medium spiny projection neurons (Anderson & Reiner 1991), these data suggest that neuroblasts differentiate into this subset of neurons lost after stroke. This was supported by Parent and colleagues (2002), who showed that five weeks after stroke many newborn neurons express the markers DARPP-32 or calbindin, both markers for medium spiny projection neurons. No parvalbumin-positive newborn interneurons were found in the peri-infarct region. No other interneuronal markers were tested, so the differentiation into calretinin-, somatostatin-, and choline acetyltransferase-positive interneurons could not be excluded.

In addition, mature neurons derived from SVZ neuroblasts found three months after stroke showed evidence obtained by immunoelectron microscopy of abundant synaptic vesicles and synapse formation with other cells (Yamashita et al 2006). This finding suggests that newborn neurons make functional connections in the infarct area. Electrophysiological data has provided evidence that newborn neurons from the SVZ integrate in the ischemic area after stroke. Neural precursors were labeled in the SVZ and shown to integrate into the functional neuronal network of the ischemic cortex eight weeks after stroke. Using patch-clamp techniques it was demonstrated that these newborn neurons show Na+ action potentials and spontaneous post-synaptic currents (Lai et al 2008). These results provide compelling evidence for functional integration of SVZ-derived newborn neurons into the ischemic area.

The range of neuronal cell types generated after striatal stroke was further investigated by Liu and colleagues (2009). As expected, they found newborn neuroblasts in the ipsilateral SVZ and striatum two weeks after stroke. At this time, no mature neurons were found, but after six weeks stroke-induced mature neurons were found significantly more in the ipsilateral striatum compared to the contralateral side. Surprisingly, newborn medium-sized spiny neurons could not be identified in the damaged striatum by morphological or immunohistochemical (using DARPP-32 and calbindin)

techniques. When looking at different subtypes of interneurons, newborn calretinin-positive interneurons were found in high numbers, but no parvalbumin-, somatostatin- or ChAT-positive newborn interneurons (Liu et al 2009). Similarly, in neonatal rats two and five weeks after stroke newly born calretinin-positive interneurons, but no newly born DARPP-32-, calbindin-, parvalbumin-, somatostatin- or ChAT-positive neurons were found in the damaged striatum (Yang et al 2008). In adult rats the newborn NeuN- and calretinin-positive neurons are similar in numbers and these markers have often been found expressed by the same cells (Liu et al 2009). This supports the notion that only calretinin-positive neurons were generated after stroke.

As neuroblasts migrate through the striatum they start to express the mature neuronal marker NeuN and at two weeks poststroke more than 30% of them express calretinin (Liu et al 2009). This demonstrates that after stroke involving the striatum neuroblasts from the SVZ migrate into the striatum where they start to differentiate and mature into calretinin-positive interneurons.

The migrational path of stroke-induced neuroblasts differs from normal migration through the RMS, but the neuroblasts themselves and their modus of migration show much resemblance in both situations (Jin et al 2001). The timing, location and mechanisms of differentiation of stroke-induces migrating neuroblasts are important for understanding the potential of the neurogenic response after stroke. If we understand the controls over poststroke neuroblasts differentiation, this could provide tools to manipulate differentiation in a desired direction.

The transcription factor Sp8 is expressed by the majority of newly born neurons in the ischemic striatum (Liu et al 2009). Sp8 is also expressed in almost all neuroblasts normally migrating to the OB (Waclaw et al 2006). In different layers of the OB, these neuroblasts differentiate into calretinin-positive neurons. It was suggested that Sp8-expressing neuroblasts, intrinsically determined to give rise to a single type of neurons, take an early exit from the SVZ-RMS-OB pathway after stroke and migrate to the damaged striatum to form calretinin-positive interneurons (Liu et al 2009). This means that the differentiation of neuroblasts migrating to areas of ischemic damage follows a similar predetermined path as a subset of neuroblasts normally migrating to the OB. This is further supported by the finding that none of the neuroblasts migrating to the ischemic striatum express Ctip2, Foxp1 or Islet1, which are transcription factors expressed by medium spiny neuronal and cholinergic interneuronal progenitors (Arlotta et al 2008; Elshatory & Gan 2008; Liu et al 2009). This also suggests that the absence of medium spiny neurons and cholinergic interneurons in this particular study was not the result of lower survival chances of these cells compared with calretinin-positive interneurons. Rather, it suggests calretinin-positive interneurons are uniquely generated in response to striatal ischemic damage.

Further investigating the controls over neuroblast differentiation after stroke brings us to the factor C3a. C3a influences stroke-induced migration (Rahpeymai et al 2006). It also plays a role in nonstroke SDF-1-mediated migration and differentiation of neural progenitor cells. C3a exerts it effects by binding to its receptor C3aR, which is present in the membranes of neural precursor cells and astrocytes. C3a promotes growth factor withdrawal-induced differentiation of neural precursor cells in culture, which leads to higher numbers of Map2-positive mature neurons with longer processes. In contrast, C3a inhibits SDF-1-induced neural progenitor differentiation into mature neuronal cell types, as measured by expression of the neuronal markers enolase2 and Map2 (Shinjyo et al 2009). Even though these roles for C3a in regulating differentiation of neural progenitors have not been confirmed in an ischemic model, the relationship with SDF-1, known for its role in stroke-induced migration (Imitola et al 2004; Kokovay et al 2010; Robin et al 2006; Thored et al 2006), suggests a possible role in stroke-induced differentiation. The controls over neuroblast

differentiation are only starting to be uncovered; more studies are needed, for example into the role of C3a on differentiation after stroke.

If we aim to improve neuronal survival and integration after stroke, it is important to know what factors control these processes.

VEGF is known to promote poststroke angio- and neurogenesis. In addition, it has a neurotrophic effect *in vitro*, where it stimulates axonal outgrowth and improves survival of several neuronal cell types (Sanchez et al 2010). This is in line with the earlier *in vivo* finding that VEGF has a positive effect on the long-term survival of newly generated neurons in the stroke area, as was measured 28 days poststroke by motor and sensory tests (Sun et al 2003). Furthermore, VEGF promotes the maturation and integration of newborn neurons in the ischemic region. After VEGF overexpression, newborn cells show increased neuronal differentiation toward a GABAergic neuronal cell type at four and eight weeks after stroke, visualized by immunostaining for the immature neuronal marker MAP2 and the GABAergic marker glutamate deacetylase (GAD67) in the ischemic cortex. Dendritic development was also enhanced, as total dendritic length and branch numbers of newborn neurons was increased (Wang et al 2009b). These results suggest that VEGF has a positive effect on long-term neurological function, possibly through its effect on neuronal maturation, survival and integration. How VEGF exerts these effects is not clear. VEGF could function through the stimulation of angiogenesis, another process or factor, or directly, more research is needed to elucidate this issue.

EGF is known to increase proliferation in the SVZ in healthy animals and after stroke (Baldauf & Reymann 2005; Palma et al 2005). Administration of this growth factor after stroke also increases the number of newborn mature neurons found in the ischemic striatum. Following stroke and continuous EGF infusion, between five and thirteen weeks poststroke the number of stroke-induced newborn neuroblasts decreased, whereas the number of newborn neurons detected in the striatal ischemic border increased. This suggests SVZ neuroblasts differentiate into a mature neuronal cell type in the ischemic border of the medial striatum. Around 65% of these newborn mature neurons were characterized as parvalbumin-positive interneurons. No DARPP-32-positive spiny projection neurons or somatostatin-positive interneurons were detected. Furthermore, 20% of the lost parvalbumin-positive neurons in the area were replaced, so that the total number of parvalbumin neurons in this area was 60% of that before ischemic damage. These results were found in EGF-treated animals, whereas non-treated animals displayed hundred-fold lower parvalbumin-positive neuronal replacement in the medial striatal ischemic border (Teramoto et al 2003). This suggests that EGF promotes neuronal differentiation after stroke.

The results found by Teramoto and colleagues (2003) differ from those found by Liu and colleagues (2009), but the reasons for these differences are unclear. More research will be needed to elucidate precisely what types of neurons are generated after stroke and on what conditions this is dependent.

In the ischemic striatum newborn GAD67-positive GABAergic neurons and ChAT-positive cholinergic neurons have been found (Zhang et al 2006a). Experiments in striatal slices show that these newborn neurons in the ischemic striatum develop increasing dendrite length and branch formation over time. Furthermore, they form synapses, fire action potentials, and receive input from surrounding cells (Lai et al 2008). These results indicate that newborn GABAergic and cholinergic neurons can functionally integrate into the ischemic striatum, which could provide an explanation for the functional improvement found after stroke-induced neurogenesis.

Discussion

The aim of this paper was to give a detailed overview of the most important mechanisms involved in stroke-induced SVZ cell proliferation, neuroblast migration, differentiation and integration of newborn neurons. This was done with the goal to identify controls that might be manipulated for therapeutic ends in stroke treatment. A wide range of factors and processes that play a role in controlling these aspects of neurogenesis have been reviewed (see table 1 for a complete overview). Special attention has been given to factors that seem to be involved in multiple aspects of stroke-induced neurogenesis, including the role of astrocytes and the vasculature.

It was shown that the neurogenic environment in the healthy adult is tightly regulated. Neural stem cells reside in the SVZ neurovascular niche where they give rise to transitory amplifying progenitor cells, which in turn give rise to neuroblasts (Curtis et al 2007; Doetsch & Alvarez-Buylla 1996; Doetsch et al 1999; Sanai et al 2004). In the neurovascular niche neural stem cells and transitory amplifying progenitor cells are closely associated with the vasculature. Neuroblasts also stay closely associated with the vasculature as they travel via the RMS to the OB (Shen et al 2008; Tavazoie et al 2008). Furthermore, neural precursors are in close contact with other neural precursors, ependymal cells, astrocytes and other cell types (Jiao & Chen 2008; Lim & Alvarez-Buylla 1999; Ma et al 2005; Song et al 2002). These tissue interactions and other sources provide a wide range of factors that play a role in SVZ neurogenesis. Such factors include ephrin (Conover et al 2000), Shh (Ahn & Joyner 2005), Notch (Wang et al 2009a), EPO (Tsai et al 2006; Wang et al 2007a) and EGF (Palma et al 2005).

EPO seems to exert its effect on neurogenesis through a pathway which involves Shh. EPO administration induces Shh expression in rats (Wu et al 2010), and inhibition of Shh signaling attenuates the effects of EPO on SVZ neurogenesis (Palma et al 2005; Wang et al 2007a). Furthermore, this pathway may involve astrocytes, which are a source of Shh (Jiao & Chen 2008). Thus, these studies suggest the existence of a signaling pathway involving EPO, Shh and astrocytes in regulating normal neurogenesis.

Migration of neuroblasts through the SVZ-RMS-OB pathway is regulated by a wide range of signals, which include neurotransmitters (Bolteus & Bordey 2004; Platel et al 2010), cell adhesion molecules (Cremer et al 1994; Doetsch & Alvarez-Buylla 1996; Ono et al 1994), integrins (Jacques et al 1998), growth factors (Bagley & Belluscio 2010; Hurtado-Chong et al 2009), chemorepulsants (Kaneko et al 2010), MMP's (Bovetti et al 2007a) and extracellular proteins (Hack et al 2002).

In most cases, the exact signaling pathways through which these substances exert their effects on normal neurogenesis and neuroblast migration are not known. Unraveling the proteins and other factors involved in these signaling cascades would improve our understanding of neurogenesis and could possibly lead to therapeutic applications in stroke treatment.

In neuroblast migration an important role for the ECM protein laminin in the β1/laminin signaling pathway was found (Belvindrah et al 2007; Ohab & Carmichael 2008). Since ectopic laminin and disruption of β1/laminin signaling can cause redirection of neuroblasts (Emsley & Hagg 2003), it would be interesting to investigate if the β1/laminin signaling system is involved in stroke-induced migration. Interactions with the ECM are important in migration, and after stroke neuroblasts have to abandon their traditional path and overcome different types of ECM environments to travel to the ischemic area. Stroke causes loss of β1 integrin expression by endothelial cells and astrocytes, which is suggested to contribute to disruption of the blood brain barrier in the ischemic area (Milner et al

2008). The disruption seems to be caused by vascular changes, and a similar mechanism could be involved in adjusting integrin-expression on neuroblasts to direct their altered migration after stroke. Perhaps SDF-1 plays a role in controlling integrin expression by neuroblasts, thus guiding their migration, since SDF-1 was shown to have a differential effect on integrin expression by different precursors in the neural lineage (Kokovay et al 2010). The exact role of β1/laminin signaling on neuroblast migration is not clear yet, more research is needed to determine whether it may be used to direct more neuroblasts to the ischemic area after stroke.

Neuroblasts differentiate to form inhibitory interneurons in the OB (Doetsch & Hen 2005). GABA is one of the factors involved in differentiation in the OB (Gascon et al 2006). The role of GABA in neuroblast differentiation is interesting, because GABA is released by inhibitory neurons in the OB. Thus, it seems that the presence of inhibitory neurons directs differentiation into an inhibitory neuronal cell type. If so, the ischemic area may be a difficult environment for neuroblasts to differentiate, since many neurons have been destroyed by stroke. However, GABA is also secreted by neuroblasts migrating in the RMS (Bolteus & Bordey 2004), so perhaps neuroblasts can be their own source of GABA needed for differentiation. None the less, administration of GABA may be useful to promote differentiation into inhibitory neurons, whereas blocking GABA signaling could perhaps be used to promote differentiation into different types of neurons.

The stroke-induced upregulation of neurogenesis in the SVZ and subsequent migration of newborn neuroblasts to the ischemic site has been discussed in detail (Arvidsson et al 2002; Jin et al 2001; Jin et al 2003; Ohab et al 2006; Parent et al 2002; Zhang et al 2001). As in normal neurogenesis, the population of GFAP-positive neural stem cells in the SVZ gives rise neural precursors that in turn give rise to new neurons. In the ischemic area after stroke an inflammatory response is initiated (Durukan & Tatlisumak 2007), while at the same time factors are starting to be released that promote neurogenesis and cell survival (Locatelli et al 2009). A wide range of controls, such as epigenetic controls, growth factors, HDAC inhibitors, cytokines, transmembrane receptors and signaling molecules have been found to influence stroke-induced neurogenesis. These include SB (Kim et al 2009), BDNF (Li et al 2010), HB-EGF (Sugiura et al 2005), VEGF (Sun et al 2003), FGF2 (Leker et al 2007), EGF (Teramoto et al 2003), EPO (Tsai et al 2006; Wang et al 2004), Notch (Wang et al 2009a), TNF-α (Iosif et al 2008), BMP7 (Chou et al 2006), RA (Plane et al 2008), NO (Chen et al 2004) and C3a (Rahpeymai et al 2006). However, the high number of factors involved, the complexity of the mechanisms and the lack of knowledge on the origin of signals and of how they relate to each other all contribute to the difficulties encountered in trying to manipulate poststroke neurogenesis.

One possible relation is between Notch and Shh. Since one of the downstream targets of Notch is Shh (Androutsellis-Theotokis et al 2006), it could be that Notch influences neurogenesis after stroke through Shh. Interestingly, Shh was upregulated after stroke (Wang et al 2009a). This would be similar to the proposed mechanism in the normal SVZ, where EPO seems to exert its effect on neurogenesis through Shh signaling.

The same difficulties in controlling poststroke neurogenesis apply to attempts to control poststroke migration of neuroblasts. Astrocytes and the vasculature play an active role in directing migrating neuroblasts to the ischemic area (Parent et al 2002; Shen et al 2008; Tavazoie et al 2008; Teramoto et al 2003; Yamashita et al 2006). Factors that play an important role in stroke-induced migration include SDF-1/CXCR4 (Kokovay et al 2010), EPO (Wang et al 2004), EGF (Kolb et al 2007), BDNF (Schabitz et al 2007), RA (Plane et al 2008) and MMPs (Lee et al 2006). The positive effect of EPO on migration may be mediated through activation of endothelial cells, upon which they express MMPs, thus enhancing neuroblast migration (Bovetti et al 2007a; Wang et al 2006; Yang et al 2005).

However, knowledge of the controls over stroke-induced migration seems to be lagging behind the understanding of normal migration through the RMS. This can partly be explained by the higher degree of complexity that arises from setting up experiments in stroke models. The knowledge of mechanisms involved in neuroblast migration in the healthy brain as well as from studies involving different kinds of brain trauma provides an opportunity for new experiments in stroke-induced migration. Neurogenesis and migration however, do not appear to be the bottleneck in poststroke brain repair. Many tens of thousands of new neuronal precursors are generated and they travel to the ischemic site in high numbers (Arvidsson et al 2002; Ohab et al 2006). What seems to be a more pressing issue is how to get these new neurons to survive and integrate into the damaged brain tissue.

The fact that inflammatory signals play important roles in poststroke neurogenesis (TNF-α and C3a) and neuroblast migration (SDF-1/CXCR4) suggests that possibly the inflammatory response after stroke is important for the recruitment of new neurons. This would also provide an explanation for the observation that many different types of brain injury invoke a neurogenic response (Kim & Szele 2008).

Stroke causes an angiogenic response in the stroke area, which is closely associated with the neurogenic response. This can be viewed as an extension of the close association between the vasculature and neurogenesis as well as migration. Also, many factors have been found that have an effect on both poststroke angiogenesis and neurogenesis. A difficulty that arises in explaining these findings lies in the fact that it is often not clear how these factors exert their effects. One possibility would be that a factor promotes angiogenesis, and that the angiogenic response subsequently promotes neurogenesis. Alternatively, a factor could promote both angiogenesis and neurogenesis through the same, or different signaling pathway. It could also be that a factor promotes neurogenesis, resulting in increased numbers of neural precursors and migrating neuroblasts. These newborn (migrating) neural precursors could in turn have a positive effect on angiogenesis. These options can often not be distinguished from the observed experimental results. Factors such as VEGF (Teng et al 2008) or Ang1 (Cui et al 2009) may promote angiogenesis upon which the newly formed endothelial cells secrete BDNF, which has been implicated in several neurogenic processes. Other factors that have been implicated in both neurogenic processes and angiogenesis include SDF-1 (Imitola et al 2004; Kokovay et al 2010), HB-EGF (Sugiura et al 2005) and MMPs (Wang et al 2006).

Neuroblasts differentiate into mature and region-appropriate neurons in the ischemic area (Arvidsson et al 2002; Liu et al 2009; Parent et al 2002). However, only a very small part of the neurons lost after stroke can be replaced by the normal neurogenic response after stroke (Arvidsson et al 2002). Since most neuroblasts undergo apoptosis (Thored et al 2006; Zhang et al 2006a), and thus fail to differentiate and survive long-term, survival of newborn neurons in the damaged area is a bottleneck in neurogenesis after stroke. This is not surprising, given the fact that also in the intact brain only part of the newborn neurons survive in the OB (Doetsch & Hen 2005). Given the high number of new neural precursors that migrate to the ischemic area, arguably the easiest way to improve neurogenesis in the damaged area is to manipulate these cells to differentiate and survive long-term. The failure to integrate has been proposed to be a reason for the lack of survival of newborn neurons in the ischemic area (Kernie & Parent 2010). This is similar to the healthy brain, since the survival of newborn neurons in the SVZ also depends on activity (Doetsch & Hen 2005). The mechanisms that promote integration have not been found for stroke-induced neurogenesis, but likely this is dependent on signals from neighboring cells and ECM. A couple of studies have investigated the controls over differentiation and integration after stroke. The transcription factor

Sp8 plays a role in the differentiation of neuroblasts into calretinin-positive neurons in the ischemic area (Liu et al 2009). C3a promotes differentiation of neuroblasts into mature neurons (Shinjyo et al 2009), but more research is needed to confirm this function of C3a in an ischemic model. Growth factors are also implicated in neuronal differentiation and survival. VEGF promotes neuronal differentiation into inhibitory neurons in the ischemic cortex (Wang et al 2009b) and EGF promotes neuronal survival in the ischemic striatum (Teramoto et al 2003). It is not clear how these growth factors exert their effects, but it is reasonable to suppose that this involves signaling through some of the canonical pathways used by these growth factors. Such pathways involve signaling through one of the different tyrosine kinase receptors, activating intracellular pathways ultimately resulting in cell differentiation and survival (Abe 2000; Leker et al 2009).

Several groups investigating the neuronal cell types generated by stroke-induced SVZ neurogenesis have reached contrasting conclusions. Some studies find parvalbumin-positive interneurons (Teramoto et al 2003), or calretinin-positive interneurons but no parvalbumin-positive interneurons (Liu et al 2009), other studies find only medium spiny neurons (Arvidsson et al 2002; Parent et al 2002). The reasons for these discrepancies remain largely unexplained. Lack of testing for all neuronal subtypes (Parent et al 2002) may have led to overlooking of neuronal subtypes. Differences in experimental setup, such as the use of BrdU or retroviral reporters for labeling SVZ progenitors, may have resulted in different subsets of progenitors being labeled. It has been speculated that region subtype specificity of neural progenitors may give rise to different types of mature neurons (Kernie & Parent 2010; Lledo et al 2008; Merkle et al 2007). For this to explain these disparate findings, it would be expected that different regions of the SVZ were labeled. BrdU injections were used in all studies to label dividing neural progenitors in the SVZ, so this would generally label similar neural progenitors across experiments. Another way these results could have arisen is by inter-species differences (Young et al 2010). Finally, it is possible that the administration of EGF favored the survival of parvalbumin-positive neurons over other types of neurons.

It is a general expectancy among researchers that adult neurogenesis may aid in the recovery after stroke, by replacing neurons lost because of stroke (Arvidsson et al 2002; Parent et al 2002; Teramoto et al 2003). The logic behind this line of reasoning is appealing. Since the loss of neurons after stroke leads to functional deficits, and mature neurons have been found in the ischemic area, it is logical to assume that the increased recovery is caused by functional replacement. However, some researchers have suggested that newborn neurons may not achieve functional recovery through neuronal integration and functional replacement after stroke (Ohab & Carmichael 2008). The neurogenesis-induced functional recovery after stroke starts early (two to four weeks after stroke; Sugiura et al 2005; Sun et al 2003), suggesting that integration of neurons could not have been the cause of this recovery, since neuronal integration had only been demonstrated at three months after stroke (Yamashita et al 2006). This has led researchers to question the effect of functional replacement on recovery, and suggest that the effect of the neurogenic response on recovery could be due to other functions of newborn neurons, such as supply of growth factors to the ischemic region (Ohab & Carmichael 2008; Ohab et al 2006). Others have pointed out that the limited types of neurons formed by newborn neurons after stroke seriously limits the possibility for neurogenesis to improve functional outcome by replacing lost neurons (Pirotte & Rogister 2008). As they see it, functional recovery where it is found after brain injury is the result of the plasticity of existing brain structures, somehow aided by the neurogenic response. In the ischemic area, newborn neurons and neural precursors may mediate neuroprotection and immune-modulation (Wiltrout et al 2007). Even though this may be the case, a functional recovery through functional integration is by no means

refuted by these suggestions. Signs of functional integration of GABAergic and cholinergic neurons have been found between six and eight weeks after stroke (Hou et al 2008). Since functional recovery has been shown to improve beyond this time-point (Thored et al 2006), functional replacement may very well play a causal role in improving functional recovery. It has been demonstrated that suppression of neurogenesis after stroke worsens stroke outcome (Jin et al 2010). However, a direct demonstration that functional neural replacement after stroke is responsible for functional recovery has not yet been provided. Perhaps experimental approaches that allow neurogenic processes, but somehow impair the integration of newborn neurons could in the future show a causal link between integration and functional recovery. However, the integration of new neurons is still being investigated itself, and has yet to become fully established. Therefore, experiments that impair integration are not feasible yet.

When investigating the possibilities of functional replacement, long-term neurogenesis after stroke is particularly interesting, since it could lead to long-term brain repair. Cell proliferation in the SVZ has a strong peak in the early stages after stroke (Jin et al 2001; Zhang et al 2001), but new neurons migrate to the ischemic area for up to four months after stroke (Thored et al 2006). Importantly, several factors have been found that influence the neurogenic response and could possibly be used for increasing the duration of the neurogenic response after stroke. In people recovering from stroke the initial period of recovery is marked by large regain in functionality, followed by a chronic phase with slower recovery (Jorgensen et al 1995). If the neurogenic response can be manipulated to persist for longer periods after stroke, this could lead to an improvement in recovery, especially during the chronic phase after stroke.

Possibly the neurogenic response after stroke is not just aimed at replacing lost neurons, but also of other cell types in the ischemic area. Newborn cells in the injured striatum were found to express markers for astrocytic (GFAP), endothelial (Glut-1), and activated microglial (ED1) cells at two to three weeks after stroke (Parent et al 2002). Also near the cortical stroke area newborn cells were found expressing markers suggesting astrocytic or endothelial lineage, but interestingly, in one study, no newborn cells were found expressing neuronal markers (Parent et al 2002). The newborn nature of these cells was established by the expression of BrdU, but it has to be taken into account that there is a small possibility these cells sustained DNA damage which caused them to incorporate BrdU. However, this is not likely since it was shown little DNA damage occurs as a result of stroke (Jin et al 2001). More likely, the endothelial cells are generated in the angiogenic response after stroke and the role of the newborn astrocytic and activated microglial cell types play a role in the neurotrophic and inflammatory responses. Given the myriad of newborn cell types found in the ischemic area, it must be considered whether they all contribute to the recovery of stroke. Perhaps even more important than replacing lost neurons, are capacities such as angiogenesis, trophic support and activation of a chemoattractive signal of new neurons.

In most of the literature the neural progenitor cells are neatly divided into three cell types: neural stem cells, transitory amplifying progenitor cells and neuroblasts. Dcx is widely used as a marker for neuroblasts, which are generally regarded as a committed neural precursor (Arvidsson et al 2002; Ohab et al 2006). However, it has been reported that neuroblasts are not as differentiated as is sometimes suggested. Neuroblasts can, when transplanted into the striatum, differentiate into glial cell types in stead of neuronal cells. This shows that these neuronal precursors can undergo glial differentiation when placed in a different environment (Seidenfaden et al 2006). In another study, a transgenic mouse line expressing GFP under the Dcx promoter was used to select Dcx-positive neuroblasts. They found that Dcx-positive neuroblasts from the SVZ are largely neuronal precursors,

but that some retain stem cell-like capacities. Especially neuroblasts with lower levels of Dcx expression are multipotent and self-renewing (Walker et al 2007). From these results, the possibility arises of manipulating the ischemic target area of neuroblasts after stroke. In doing this, neuroblasts may be directed to not only differentiate into new neurons, but also undergo glial differentiation to provide glial cells that are necessary for a meaningful repair of the damaged area. This could improve functional recovery, since neurons need glial cells for their functioning and in the damaged area not only neurons, but also astrocytes are lost after stroke (Takano et al 2009). Cell morphology can be used as a cell type identifier, for example, migratory cells have been identified on the basis of cell morphology (Parent et al 2002). However, it has been shown that bipolar cells that appear motile can be stationary. Conversely, cells with non-motile-appearing morphology can be migratory (Nam et al 2007). This shows that cell morphology is not always a good predictor of cell behavior. A careful approach has to be taken when results on neuroblast migration are presented based on morphological observations.

It is important to keep in mind that neural stem cells have a slow cell cycle of 28 days (Morshead et al 1994). When using BrdU to label newborn cells, this has to be taken into account. Some studies do this by administering BrdU for fourteen days (Liu et al 2009), but other studies administer BrdU for shorter periods (Parent et al 2002; Teramoto et al 2003), increasing the chance they may miss some subpopulation of newborn cell types in their analysis.

In the use of pharmacological substances that can improve stroke outcome and recovery after stroke, the timely administration is of great importance. For example, VEGF administration starting one day after stroke was shown to improve neurogenesis, angiogenesis and stroke outcome (Sun et al 2003). However, VEGF also strongly influences vascular permeability and the blood brain barrier. Therefore, early VEGF administration can negatively affect the outcome of stroke. This has been shown by using a VEGF antagonist to inhibit VEGF function during and directly after stroke, thus reducing edema formation and infarct size (van Bruggen et al 1999). Another example is the combined treatment with MSC and NONOate promotes angiogenesis, neurogenesis as well as VEGF and bFGF expression in the SVZ and ischemic boundary (Chen et al 2004). Since VEGF stimulates eNOS expression, which results in enhanced generation of bioactive NO (Papapetropoulos et al 2001), there may be a positive feedback loop involved in the neurogenic and angiogenic effects of NO and VEGF. Furthermore, the timing and origin of NO administration is of importance, since it has been shown that NO from different sources at different stages after stroke can have opposing, even neurotoxic, effects after stroke (Niwa et al 2001). It thus becomes clear that successful stroke treatment depends on stringent time restraints. Mistakes in timing of application of pharmacological substances can have adverse effects on stroke outcome.

The findings of neurogenesis in animal stroke models presented in this paper would be of little interest if similar processes did not occur in humans. The demonstration in humans that newborn neurons are present in areas where ischemic damage due to stroke occurs was thus of great importance (Jin et al 2006). Similar to the situation in animal stroke models, neurogenesis in the human brain takes place in the vicinity of blood vessels, supporting the existence of a neurovascular niche (Ohab et al 2006). Several studies have also shown that proliferation of neural progenitors is upregulated in the ipsilateral SVZ after stroke in humans (Macas et al 2006; Marti-Fabregas et al 2010). Although this is not direct proof, these results suggest that newborn neurons found in ischemic areas originate from the SVZ. It is not clear whether SDF-1 and Ang1, factors involved in stroke-induced neurogenesis and angiogenesis, also play a role in the human ischemic brain. However, it has been shown that SDF-1 and Ang1 are upregulated after cerebrovascular insults

and ischemic muscle damage in human patients (Hohenstein et al 2005; van Weel et al 2007). Extrapolation of results in animal stroke models of course relies on the existence of similar mechanisms at work in humans. Therefore, these studies pave the way for more studies in humans that investigate whether Ang1 and SDF-1 play a similar role in the ischemic brain. Interestingly, the finding that EE increased neurogenesis in animals (Plane et al 2008) has in a way been confirmed in human patients. Patients who exercise more frequently after stroke were shown to have increased cerebral blood volume in the dentate gyrus, which was shown to be a correlate of increased neurogenesis (Pereira et al 2007).

Several therapeutic approaches have been suggesting in trying to take advantage of the neurogenic capacity of the brain after stroke. One approach is aimed at manipulating the brain's intrinsic neurogenic capacities, making use of the natural proliferation, migration and survival of new neurons after stroke. It is clear that these processes, although present after stroke, are not enough to repair the extensive damage caused by stroke. This paper has shown what is known about the neurogenic processes and how we may manipulate them. Several studies aimed at improving the neurogenic response after stroke to repair the brain have had promising results in animal models. However, the translation to human patients has not yet been successful. It remains to be seen whether the brains neurogenic response can be manipulated sufficiently to repair the damaged area. Rodent models have added hugely to our understanding of stroke-induced neurogenesis. Despite the discovery of many potential target mechanisms for stroke therapies and a diversity of pharmacological substances that can influence the neurogenic response, the translation from bench to bedside has been problematic (Braeuninger & Kleinschnitz 2009). The reason for this may be that the higher development of the olfactory and thus neurogenic system in rodents compared to human makes it hard to translate findings in rodents to human patients. However, it is exactly for this reason that attempts to understand the mechanisms controlling the neurogenic response are important, in order to ameliorate the neurogenic capacity that is available in humans (Curtis et al 2007; Jin et al 2006; Macas et al 2006; Marti-Fabregas et al 2010; Minger et al 2007). If the neurogenic response does not provide sufficient recovery, transplantation of neural stem cells provides a promising alternative. It has been shown that transplanted cells can integrate into the circuitry and help repair the brain (Locatelli et al 2009). A combined treatment using both the intrinsic neurogenic capacity of the brain as well as neural stem cell transplantation is also a possible line of treatment, perhaps combining best of both worlds. To date, no studies have been undertaken that investigate such a combined approach.

In this paper we have discussed the myriad of mechanisms that are involved in poststroke neurogenesis. We have tried to give a coherent overview of the most important controls over the neurogenic processes after stroke. We show that many signals are interconnected and are involved in several key processes, such as proliferation, migration, differentiation and angiogenesis. It has become clear that although animal models of stroke have provided many new insights, much has yet to be discovered. Translation of these results to human stroke therapies has proven difficult. Further progress in our understanding of the mechanisms involved in poststroke neurogenesis will result in better treatments for stroke.

Factor:	Stroke-induced SVZ neuro-genesis	Stroke-induced migration	Stroke-induced angio-genesis	Stroke-induced neuronal differenti-ation	Stroke-induced survival and inte-gration	Normal SVZ neuro-genesis	Normal migration	Normal differenti-ation	Normal survival and inte-gration
α6β1 integrin		+/- (Shen et al 2008)					+ (Jacques et al 1998; Shen et al 2008)		
Ang1/Tie2		+ (Cui et al 2009)	+ (Zacharek et al 2006)						
BDNF	+ (Li et al 2010)	+ (Schabitz et al 2007)	+ (Chen et al 2005; Wang et al 2004)			+ (Benraiss et al 2001)	+/- (Bagley & Belluscio 2010; Snapyan et al 2009)		
BMP7	+ (Chou et al 2006)								
C3a	+ (Rahpeym ai et al 2006)	+/- (Rahpeym ai et al 2006; Shinjyo et al 2009)		+ (?) (Kokovay et al 2010; Shinjyo et al 2009)				+ (Shinjyo et al 2009)	
CREB								+ (Giachino et al 2005)	
EGF	+ (Teramoto et al 2003)	+ (Teramoto et al 2003)		+ (Teramoto et al 2003)	+ (cortex in combinati on with EPO) (Kolb et al 2007)	+ (Palma et al 2005)	+ (Craig et al 1996)		
Ephrin						+ (Conover et al 2000)	+/- (Conover et al 2000)		
EPO	+ (Wang et al 2004)	+ (?) (Wang et al 2004)	+ (via VEGF) (Wang et al 2004)		+ (cortex, in combinati on with EGF) (Kolb et al 2007)	+ (Tsai et al 2006)			
GABA							- (Bolteus & Bordey 2004)	+ (Gascon et al 2006)	
HB-EGF	+ (Sugiura et al 2005)	+ (Sugiura et al 2005)	+ (via VEGF?) (Abramovi tch et al 1998; Sugiura et al 2005)			+ (Jin et al 2002b)			
IGF-I							+ (Hurtado-Chong et al 2009)		
MMPs		+ (Lee et al 2006)					+ (Bovetti et al 2007a; Yang et al 2005)		

Factor:	Stroke-induced SVZ neuro-genesis	Stroke-induced migration	Stroke-induced angio-genesis	Stroke-induced neuronal differenti-ation	Stroke-induced survival and inte-gration	Normal SVZ neuro-genesis	Normal migration	Normal differenti-ation	Normal survival and inte-gration
MSC	+ (Chen et al 2004)		+ (Chen et al 2003)						
Neuregu-lins						+ (Ghashgha ei et al 2006)	+ (Ghashgha ei et al 2006)		
NMDA								+ (Platel et al 2010)	+ (Platel et al 2010)
Notch1	+ (Wang et al 2009a)					+ (Wang et al 2009a)			
PSA-NCAM		+ (to cortex) (El Maarouf et al 2006)					+ (Cremer et al 1994; Ono et al 1994)		
RA	+ (in combi-nation with EE) (Plane et al 2008)	+ (in combi-nation with EE) (Plane et al 2008)							
Reelin		+ (Won et al 2006)					- (Hack et al 2002)		
Robo/Slit							- (Sawamot o et al 2006; Wu et al 1999)		
SDF-1/ CXCR4	+ (Robin et al 2006)	+/- (Kokovay et al 2010; Liu et al 2008)					+ (Peng et al 2004; Robin et al 2006)		
Shh	+(?) (Wang et al 2009a)					+ (Ahn & Joyner 2005)			
Sp8				+ (Liu et al 2009)				+ (Liu et al 2009)	
Tenascin-R							+ (OB) (Bovetti et al 2007b)		
VEGF	+ (Wang et al 2007b)	+ (?) (Chen et al 2005)	+ (Sun et al 2003; Wang et al 2009b; Zhang et al 2000)	+ (Wang et al 2009b)		+ (Jin et al 2002c)			

Table 1. Overview of the factors and pathways that influence neurogenic and angiogenic processes in normal and poststroke brain discussed in this paper. + means positive effect, - means negative effect, ? means doubts are present or it may induce another factor or process responsible for the observed effect, +/- means bidirectional effect or unclear whether the influence is positive or negative.

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