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Neurotransmitter signaling in postnatal neurogenesis: the first leg

Jean-Claude Platel, Séverine Stambouliau, Ivy Nguyen, and Angélique Bordey

Departments of Neurosurgery, and Cellular & Molecular Physiology, Yale University School of Medicine, New Haven, Connecticut, USA

Abstract

Like the liver or other peripheral organs, two regions of the adult brain possess the ability of self-renewal through a process called neurogenesis. This raises tremendous hope for repairing the damaged brain and has stimulated research on identifying signals controlling neurogenesis. Neurogenesis involves several stages from fate determination to synaptic integration via proliferation, migration, and maturation. While fate determination primarily depends on a genetic signature, other stages are controlled by the interplay between genes and micro-environmental signals. Here, we propose that neurotransmitters are master regulators of the different stages of neurogenesis. In favor of this idea, a description of selective neurotransmitter signaling and their functions in the largest neurogenic zone, the subventricular zone (SVZ), is provided. In particular, we emphasize the interactions between neuroblasts and astrocyte-like cells that release gamma-aminobutyric acid (GABA) and glutamate, respectively. However, we also raise several limitations to our knowledge on neurotransmitters in neurogenesis. The function of neurotransmitters *in vivo* remains largely unexplored. Neurotransmitter signaling has been viewed as uniform which dramatically contrasts with the cellular and molecular mosaic nature of the SVZ. How neurotransmitters are integrated with other well-conserved molecules, such as sonic hedgehog, is poorly understood. In an effort to reconcile these differences, we discuss how specificity of neurotransmitter functions can be provided through their multitude of receptors and intracellular pathways in different cell types, and their possible interactions with sonic hedgehog.

Keywords

SVZ; adult neurogenesis; neurotransmitter; GABA; sonic hedgehog; wnt; glutamatergic signaling; vesicular release; astrocytes; glial cell; development; proliferation; survival; GLAST; glutamate transporter; dopamine; serotonin; stem cells; progenitor cells; migration; NMDA receptor

1. Introduction

Until recently, the generation of neurons was thought to occur only during the embryonic period while the brain was considered relatively “stable” after birth. It is now clear that the mammalian brain is more plastic than previously perceived and has a remarkable ability to adapt to environmental stimuli or stress by modifying its structural and physiological characteristics. New neurons continue to be produced in two regions of the adult forebrain, the subgranular

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Corresponding Author: Angélique Bordey, PhD Departments of Neurosurgery, and Cellular and Molecular Physiology, Yale School of Medicine New Haven, CT 06520-8082 angelique.bordey@yale.edu Phone: 203-737-2515; Fax: 203-737-2159.

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zone (SGZ) of the dentate gyrus and the subventricular zone (SVZ) lining the lateral wall of the lateral ventricle. This review focuses on the SVZ, the largest neurogenic zone, characterized by a well-defined migratory route, called the rostral migratory stream (RMS), taken by neuroblasts to reach the olfactory bulb where they integrate as interneurons.

The production of adult neurons is an ongoing process. While the extent of SVZ neurogenesis remains controversial in humans (Sanai et al., 2004; Curtis et al., 2007), in rodents estimates suggest that 10,000 to 30,000 neurons are produced every day (Lledo et al., 2006). This high turnover rate requires profound homeostatic mechanisms that regulate and coordinate the different stages of neurogenesis to prevent neuron overproduction. Homeostasis is ensured through interplay between genetic (intrinsic) program and micro-environmental (extrinsic) signals. Here, we propose that among the micro-environmental signals neurotransmitters are master regulators of neurogenesis.

Neurotransmitters are classically released from synaptic terminals and are the basis of the chemical communication between neurons. Pioneering studies have suggested that these small diffusible molecules play important functions on cell development during embryonic life (Behar et al., 1994; LoTurco et al., 1995; Behar et al., 1998; Haydar et al., 2000; Platel et al., 2005) as well as adult SGZ neurogenesis (Gould et al., 1994; Cameron et al., 1995). More recently a series of elegant studies reported that several neurotransmitters regulate postnatal neurogenesis in the SVZ. Findings from these studies are described here and divided into two groups; we first discuss the signaling of two monoamines, dopamine and serotonin, which are released by neuronal inputs in the SVZ. We next discuss the signaling by the metabolically related amino acids GABA and glutamate which are locally released by neuroblasts and astrocyte-like cells in the SVZ, respectively. The SVZ is composed of different cell types including astrocyte-like cells, transit amplifying cells, and neuroblasts. Some of these astrocyte-like cells act as neural stem cells. GABA and glutamate signaling have been described between astrocyte-like cells and neuroblasts providing a bidirectional communication between these two cell types. For each neurotransmitter, we describe the signaling mechanisms, including receptor, source(s) of release, and transporter. We then discuss the functions of each neurotransmitter on neurogenesis.

Despite a growing wealth of information on neurotransmitter signaling and function in adult neurogenesis, there are clear limitations to the present work. First, the *in vivo* impact of selectively perturbing neurotransmitter signaling remains poorly addressed. Addressing this issue has been limited for technical reasons as will be discussed here. Neurotransmitter signaling has been described as uniform across the SVZ which dramatically contrasts with the mosaic cellular and molecular nature of the SVZ. It is unknown whether GABA and glutamate signaling differ among astrocyte-like cells considering that some of these cells act as neural progenitor cells. These neural progenitor cells located in different regions of the SVZ generate different types of interneurons (Merkle et al., 2007; Young et al., 2007). However, it remains unclear, whether and if so how, neurotransmitter signaling selectively regulates some subpopulations of neural progenitor cells. It is also hard to reconcile the uniformity of neurotransmitter signaling with their multiple actions such as regulators of migration and proliferation. One possible explanation is that each neurotransmitter activates different receptor subtypes that are developmentally regulated and composed of different subunits, and thus activate different intracellular pathways. Finally, our perhaps egocentric view of neurotransmitter signaling needs to be revisited in light of the presence of a multitude of other well-conserved and critical signals. We will speculate how neurotransmitters and one crucial neurogenic signal, Sonic hedgehog (Shh), may interact, providing even more selectivity to neurotransmitter signaling as well as amplifying their diversity of actions. After addressing each limitation, we propose future directions and emerging concepts that may reconcile these different views.

2. Cellular and Functional Organization of the SVZ

Organization of the SVZ has been well-described and extensively reviewed (Doetsch et al., 1997; Mercier et al., 2002; Quinones-Hinojosa et al., 2006; Mirzadeh et al., 2008a) (for a few reviews see (Privat and Leblond, 1972; Privat, 1977; Alvarez-Buylla and Lim, 2004; Bordey, 2006)). Neural progenitor cells (also called neural stem cells, type B cells, SVZ astrocytes) are scattered along the SVZ and RMS (Doetsch et al., 1999; Gritti et al., 2002). They self-renew and generate transit amplifying cells (called type C cells) that also asymmetrically divide to give birth to neuroblasts (type A cells). Neuroblasts remain proliferative along the SVZ and migrate to the olfactory bulb via long distance, tangential migration throughout the SVZ and RMS (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Peretto et al., 1997) (Fig. 1a). Once in the olfactory bulb, they leave the RMS and migrate radially to the different neuronal layers. Neuroblasts will mature into two main interneuron types, granule and periglomerular cells that are located in the granule cell and glomerular layer, respectively (Fig. 1a).

Antigenically all SVZ cells express nestin, a marker of neural progenitor cells (Hockfield and McKay, 1985; Doetsch et al., 1997; Platel et al., 2009). Astrocyte-like cells of the SVZ inherited such a name from the expression of astrocytic markers such as glial fibrillary acidic protein (GFAP) and glutamate-aspartate transporter (GLAST, Braun et al., 2003; Bolteus and Bordey, 2004; Platel et al., 2009). In fact, they express other astrocytic markers including gap junction connexin 43, Aldh1L1 (in neonates, see www.gensat.org), Lex (Cd15), the other glial glutamate transporter GLT-1, glial GABA transporter GAT3/4, and glutamine synthase (Capela and Temple, 2002; Bolteus and Bordey, 2004, and see Platel JC and Bordey A, unpublished observations for glutamine synthase). Transit amplifying cells express epidermal growth factor receptor (EGFR, Platel et al., 2009; Cesetti et al., 2009; Pastrana et al., 2009), *dlx2* and *mash1* (Aguirre et al., 2004; Parras et al., 2004). Astrocyte-like cells and some EGFR-expressing cells also share some markers such as brain lipid basic protein and GLAST (Platel et al., 2009). Neuroblasts express doublecortin (DCX), β -III tubulin, and PSA-NCAM (Bonfanti and Theodosis, 1994; Rousselot et al., 1995; Nacher et al., 2001; Brown et al., 2003). Findings regarding the astrocyte-like cells and transit amplifying cells have raised several issues. First, it is unclear whether all astrocyte-like cells or only a subset of them can behave as neural progenitor cells during their lifetime. In other words, is there a unique population of astrocyte-like cells with stem cell characteristics or is there a turnover of cells that behave as neural progenitor cells? A related issue concerns the astrocytic nature of these neural progenitor cells. These SVZ astrocytes express properties of mature astrocytes that are fully differentiated cells. It thus remains unknown what makes them so different to behave as neural progenitor cells. It is even more confusing considering that based on a transcriptome analysis mature astrocytes have recently been shown to express transcription factors such as *Sox2*, *Pax6*, *Id1*, and *Id3*, thought to be enriched in neural progenitor cells (Cahoy et al., 2008). This is addressed in a detailed manner in the review by Berninger et al. in this Special issue. SVZ astrocytes derive from radial glia, the embryonic neural progenitor cells (Paterson et al., 1973; Malatesta et al., 2000; Noctor et al., 2001; Merkle et al., 2004), and perhaps the presence of embryonic extracellular matrix allows these astrocytes to retain their stemness (Gates et al., 1995). Finally, transit amplifying cells may contain distinct populations with different fates, neuronal, glial or both that need to be further clarified.

On a structural level, the SVZ is a thin region that spans the whole lateral wall of the lateral ventricle and contains tubes filled with neuroblasts also called chains of neuroblasts. Each chain composed of 4-5 neuroblasts is tightly ensheathed by processes of astrocyte-like cells (Lois and Alvarez-Buylla, 1994; Doetsch and Alvarez-Buylla, 1996; Peretto et al., 1997) (Fig. 1a and b). Astrocyte-like cells are located between these chains and their processes form the wall of the “tubes” containing neuroblasts. Astrocyte-like cells also seem to isolate the

neurogenic zone from the mature parenchyma (Platel et al., 2009). A subset of astrocyte-like cells also make contact with the ventricle on one side and the blood vessels on the other side and those SVZ astrocytes may be the neural progenitors (Shen et al., 2008; Tavazoie et al., 2008; Mirzadeh et al., 2008b; Lacar et al., 2009). Based on electron microscopic observations, the extracellular space between SVZ astrocytes and neuroblasts is between 20–50 nm (Privat and Leblond, 1972), which is comparable in distance to a synaptic cleft. This led us hypothesize that signaling occurring in the SVZ may resemble that at the tri-partite synapse. In particular we described a novel signaling between astrocyte-like cells and neuroblasts in the glutamatergic signaling section below.

3. Functions of dopaminergic and serotonergic inputs in the SVZ

Both dopamine and serotonin are monoamines implicated in the regulation of mood, motivation and movement, and more recently have been shown to regulate adult SVZ neurogenesis (for recent reviews on dopamine in neurogenesis see (Borta and Hoglinger, 2007; O'Keeffe et al., 2009)).

a) Dopaminergic and serotonergic signaling mechanisms

Dopamine receptors are G protein-coupled and classified as D1-like (D1 and D5) and D2-like (D2, D3 and D4) receptors according to structural homologies (Callier et al., 2003). In the embryonic and adult SVZ, high levels of D3 receptor expression has been shown by *in situ* hybridization and autoradiography (Diaz et al., 1997). D2-like dopamine receptors have been found on transit amplifying cells, and both D1- and D2-like receptors have been reported in neuroblasts (Hoglinger et al., 2004). Expression on SVZ astrocytes has not been reported so far. Although some SVZ neuroblasts differentiate into dopaminergic interneurons in the olfactory bulb, they do not express tyrosine hydroxylase, the enzyme involved in the synthesis of dopamine (Baker et al., 2001). This enzyme was not found in any SVZ cell type. Nevertheless, dopamine was shown to be released from dopaminergic afferents that directly contact transient amplifying cells in the SVZ (Hoglinger et al., 2004). In non-human primates, some of those fibers were shown to come from the substantia nigra (Freundlieb et al., 2006). Concerning dopamine uptake mechanisms, dopamine transporters were found on the dopaminergic fibers entering the SVZ but not on SVZ cells using immunostaining (Hoglinger et al., 2004; Shibui et al., 2009).

Serotonin (5-HT) receptors, which are encoded by 14 genes, are G protein-coupled receptors with the exception of the 5-HT₃ receptor (Barnes and Sharp, 1999; Pauwels, 2000). 5-HT₃ receptor is a ligand-gated ion channel that is mainly permeable to sodium and potassium ions. Using ligands for these receptors and cell proliferation assay *in vivo*, four of these receptors have been found to be involved in the regulation of neurogenesis: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C} (Banasr et al., 2004). However, the cellular localization of these receptors in the SVZ remains unknown. Another study showed that transgenic mice expressing green fluorescent protein (GFP) under the 5-HT₃ receptor promoter showed a high expression on SVZ neuroblasts that was confirmed by *in situ* hybridization (Inta et al., 2008). A dense plexus of 5HT-immunoreactive fibers projects into the SVZ from the raphe nucleus and thus provides an extrinsic source of serotonin; the exact cell type targeted by these fibers remains to be examined (Simpson et al., 1998; Brezun and Daszuta, 1999; Banasr et al., 2004; Diaz et al., 2009). Serotonin transporters have been observed on either neuroblasts or 5-HT fibers entering the SVZ using immunostaining (Diaz et al., 2009; Shibui et al., 2009). Additional studies are required to further assess the pattern of serotonin transporter expression in the SVZ.

b) Functions

In individuals with Parkinson's disease where dopaminergic signaling is disturbed, proliferation in the SVZ is decreased (Hoglinger et al., 2004). Consistent with this finding, dopaminergic agonists increase neurogenesis in mouse models of Parkinson's disease (Yang et al., 2008) and removal of the dopaminergic projections decreases proliferation in the SVZ (Hoglinger et al., 2004; Baker et al., 2004) (for review see (Borta and Hoglinger, 2007; O'Keefe et al., 2009)). This effect is mediated by activation of D2-like receptors on transit amplifying cells most likely via the EGF receptor in conjunction with release of EGF in a PKC-dependent manner (Coronas et al., 2004) or via a ciliary neurotrophic factor-dependent mechanism (Yang et al., 2008). However, dopaminergic innervations of the SVZ and dopamine functions on cell proliferation depend on the strain of mice used, suggesting that caution is required when comparing from different studies and extrapolating from mice to humans (Baker et al., 2005). In addition, the physiological significance of these dopaminergic projections into the SVZ needs to be further explored. Similarly, the clinical relevance of changes in SVZ cell proliferation during the course of Parkinson's disease remains unclear (for review see (Borta and Hoglinger, 2007)).

Regarding serotonergic signaling, activation of 5-HT_{1A} and 2C receptors *in vivo* increased proliferation in the SVZ while activation of 5-HT_{1B} decreased cell proliferation (Banasr et al., 2004). Activation and inhibition of 5-HT_{1B} receptors decreased and increased cell proliferation, respectively, suggesting an opposite effect compared to the other receptors. The increase in cell proliferation with 5-HT_{1A} and 2C agonists resulted in an increased number of adult born BrdU-labeled granule cells several weeks after a single dose of agonist injection (Banasr et al., 2004). Consistent with these findings, inhibition of serotonin synthesis or lesions of the serotonergic raphe neurons reduce neurogenesis by approximately 60% (Brezun and Daszuta, 1999). The effects of serotonin seems to be mediated in part by its stimulation of BDNF expression (Mattson et al., 2004). Overall, serotonin is considered as a positive regulator of adult neurogenesis in the SVZ.

4. GABAergic and glutamatergic signaling in the SVZ

The neurotransmitter GABA (gamma-aminobutyric acid) and glutamate have been extensively studied in the context of development and more recently neonatal/adult neurogenesis (for reviews see (Nguyen et al., 2001; Owens and Kriegstein, 2002; Schlett, 2006; Bordey, 2007; Manent and Represa, 2007; Henschel et al., 2008; Platel et al., 2008a)). In adult neurogenesis GABA had received particular attention compared to glutamate perhaps due to its ubiquitous expression of GABA receptors in immature cells.

a. GABAergic signaling and functions

GABA's action is mediated by at least three types of GABA receptors (GABARs) (Chebib and Johnston, 1999): the ligand-gated chloride (Cl⁻) channels, GABA_AR and GABA_CR, and the G protein-coupled receptor GABA_BR. GABA_BRs are functionally expressed in SVZ cells based on calcium imaging (Platel JC and Bordey A, personal observation). It is unknown whether GABA_CRs are expressed in the SVZ. GABA_ARs are functionally expressed on both neuroblasts and astrocyte-like cells based on calcium imaging and patch clamp recordings (Wang et al., 2003; Nguyen et al., 2003; Bolteus and Bordey, 2004; Liu et al., 2005). The expression of these receptors on transient amplifying cells still needs to be investigated. One limitation in studying transit amplifying cells has been the difficulty in identifying them in the live SVZ (*e.g.* acute slices). Using a viral vector encoding a green or red fluorescent protein under the EGFR promoter may provide one method to visualize them in live tissue. GABA_ARs are composed of multiple subunits (16 total) conferring different properties (Henschel et al., 2008). However, very little is known about the expression of these subunits

in SVZ cells. Neonatal neuroblasts expressed $\alpha 2$, 3, 4, $\beta 1$, 2 and 3, and $\gamma 2S$ mRNA *in vitro* (Stewart et al., 2002) while neonatal PSA-NCAM-positive progenitors from neurospheres (which may include glioblasts) expressed $\alpha 2$, 4, 5, $\beta 1$ and 3, and $\gamma 1$, 2, 3, and δ subunit transcripts (Nguyen et al., 2003). As mentioned later, identifying the sequential acquisition of the different subunits in the different SVZ cell types is critical to better understand GABA_AR specificity of action. In the SVZ and RMS, neuroblasts synthesize and release GABA as shown by immunostaining for GABA in slices (Wang et al., 2003; Bolteus and Bordey, 2004) and *in vitro* (Stewart et al., 2002), and by patch clamp recordings using GABA_ARs as sensors for GABA (Liu et al., 2005). Liu et al. (2005) reported that electrical stimulation or high potassium application induced GABA release from SVZ cells. The release was independent of action potentials, external calcium, and the SNARE-dependent vesicular machinery, but it was dependent on intracellular calcium changes. Together with the absence of synapses in the SVZ and RMS at the electron microscopic levels and the lack of immunostaining for the synaptic marker synapsin 1, it was concluded that GABA signaling was non-synaptic (*i.e.* paracrine). Recent data also showed that vesicular GABA transporters as well as a member of the SNARE complex synaptobrevin 2 (VAMP2) are not expressed in SVZ neuroblasts (Platel et al., 2007; Platel et al., 2010). The mechanism(s) of GABA release from neuroblasts remain unknown. Released GABA is taken up by the GABA transporters GAT3/4 in SVZ astrocytes (Bolteus and Bordey, 2004; Liu et al., 2005) while the presence of the neuronal GABA transporter GAT1 in neuroblasts remains unclear. It is unknown whether transit amplifying cells have the ability to release or take up GABA.

GABA exerts a tonic inhibitory control on the proliferation of SVZ astrocytes in 30-40 day old transgenic mice expressing GFP under the human *GFAP* promoter (Liu et al., 2005) and striatal neuroblasts from neonatal mice (Nguyen et al., 2003). These studies used the S-phase mitotic marker bromodeoxyuridine (BrdU) in both organotypic slices as well as neurospheres. Ambient GABA was found to reduce the speed of neuroblast migration in acute sagittal slices from both juvenile and adult mice (Bolteus and Bordey, 2004; Platel et al., 2008b). GABA also increased dendritic growth of neuroblasts, which were integrating in the olfactory bulb in acute slices (Gascon et al., 2006). These studies suggest that GABA controls some of the early and late stages of cell development via paracrine signaling. The function of GABA on transit amplifying cells has not been explored although changes in their proliferation could profoundly impact the number of adult born neuroblasts.

b. Glutamatergic signaling and functions

Glutamatergic signals are conveyed by different glutamate receptor subtypes, namely ionotropic NMDA and AMPA/kainate receptors (Sommer and Seeburg, 1992; Hollman and Heinemann, 1994) and metabotropic glutamate receptors (mGluRs; groups I–III subtypes, mGluR1-8, (Conn and Pin, 1997; Coutinho and Knopfel, 2002)). The presence of mGluRs, and NMDA, AMPA, and kainate receptors were observed in cultured SVZ cells derived from neonates (Brazel et al., 2005). Using calcium imaging and electrophysiological recordings in acute slices, we found that neuroblasts express AMPA (Platel et al., 2007), kainate (Platel et al., 2008b) and NMDA receptors (Platel et al., 2010), and mGluR5 (Platel et al., 2008b). mGluR5 immunoreactivity was also reported in SVZ cells in fixed tissue (Di Giorgi Gerevini et al., 2004). It is important to emphasize that neuroblasts express a mosaic of glutamate receptors, but individual neuroblast does not express all four receptor types. It is unclear whether neuroblasts expressing different populations of receptors have different fates (*e.g.* periglomerular cells versus granule cells). In addition, at least for NMDA receptors it was clearly shown that neuroblasts acquire an increasing number of NMDA receptors during their migration along the SVZ and RMS prior to entering the olfactory bulb synaptic network (Platel et al., 2010). The characterization of glutamate receptor expression in astrocyte-like cells and transit amplifying cells is less clear. Astrocyte-like cells did not display any NMDA or AMPA-

induced currents or calcium increases even in the presence of an inhibitor of AMPA receptor desensitization (Liu et al., 2006; Platel et al., 2010). Whether transit amplifying cells express glutamate receptors is unknown. Regarding the source of glutamate in the SVZ, we recently found that astrocyte-like cells immunostain for glutamate and vesicular glutamate transporter 1 VGLUT1 (Platel et al., 2007; 2010). In addition, using post-embedding immunogold labeling for VGLUT1 we found gold-particles on vesicles including fusing vesicles in astrocyte-like cells of the SVZ (Platel et al., 2010). Importantly, to test for calcium-dependent glutamate release from astrocyte-like cells we used transgenic mice in which GFAP-expressing cells express a Gq-protein coupled receptor (called Mas-related gene A1, MrgA1) that is not endogenous to the brain and has no endogenous ligands. These mice were generated by Dr. Ken McCarthy using the inducible tet-off system (Fiacco et al., 2007). Mice expressing the tetracycline transactivator (tTA) under the human *GFAP* promoter were crossed to mice in which the MrgA1 receptor was transcribed off the tet (tetO) minimal promoter. In the SVZ of *hGFAP*-tTA $\times$ tetO-MrgA1 mice (referred MrgA1+ mice), GFP fused to MrgA1 was found to be selectively expressed in SVZ astrocytes but not in neuroblasts (Fig. 2a diagram). Application of the MrgA1 selective peptide agonist (phe-leu-argphe amide, FLRFa) resulted in calcium increases in GFP-fluorescent cells in the SVZ, *i.e.* astrocyte-like cells. In addition, FLRFa increased the frequency of NMDA receptor-mediated channel activity in neuroblasts recorded in the RMS of acute slices (Platel et al., 2010). It was concluded that astrocyte-like cells released glutamate in a calcium-dependent manner onto neuroblasts. It is noticeable that in acute hippocampal slices from MrgA1 mice, FLRFa application did not affect synaptic transmission (Fiacco et al., 2007). This emphasizes the importance of the glutamate signal from astrocyte-like cells to neuroblasts in the neurogenic zone. We also found that astrocyte-like cells in the SVZ, like mature astrocytes, immunostained for glutamine synthase, which is an enzyme involved in the conversion of glutamate into glutamine (Platel JC and Bordey A, unpublished observation). Although this enzyme may decrease the concentration of glutamate in astrocyte-like cells, the amount of vesicular glutamate may not be affected. In addition, it is possible that glutamate transporters expressed in SVZ astrocytes are specifically localized to load vesicles. However, these issues remain to be addressed in future studies. Another attractive question is whether SVZ astrocytes release glycine or D-serine, which when released from mature astrocytes was shown to activate NMDA receptors in surrounding neurons and modulate neuronal transmission (for review see Parpura and Zorec in this special issue). Finally, SVZ astrocytes also contain high affinity glutamate transporters, GLAST and GLT-1 (Bolteus and Bordey, 2004; Liu et al., 2005; Platel et al., 2009) (Fig. 2a). The neuronal glutamate transporter EAAT-3 (for excitatory amino acid transporter) was not found in the SVZ using immunostaining (Liu et al., 2005).

In the SVZ, we and others have recently observed that glutamate receptors carry out different functions. Using an acute whole mount of the lateral wall of the lateral ventricle we found that inhibition of GLU_{K5} kainate receptors (previously called GluR5 and now called GluK2) significantly increased the speed of neuroblast migration while mGluR5 inhibition had no effect (Platel et al., 2008b). Regarding mGluRs, adult mice lacking mGluR5 or treated with mGluR5 antagonists showed a dramatic reduction in the number of proliferating cells in the SVZ (Di Giorgi Gerevini et al., 2004; 2005). Recently, “single-cell” knock-out of NR1, a critical NMDA receptor subunit for receptor functionality, led to a significant increase in neuroblast apoptosis resulting in a dramatic reduction of neurogenesis in the olfactory bulb (Platel et al., 2010; Lin et al., 2010). Neuroblast apoptosis occurred during their migration in the SVZ and RMS, which is much earlier than previously thought and to some extent during synaptic integration (Platel et al., 2010), as expected based on data in the SGZ (Tashiro et al., 2006). Genetic removal *in vivo* or pharmacological blockade of NMDA receptors did not affect the speed of neuroblast migration in the RMS (Platel et al., 2010). To genetically remove NR1, a Cre recombinase-containing vector was either transduced using a viral vector or electroporated into SVZ cells in adult and neonatal transgenic mice, respectively, where the

NR1 subunit is flanked by loxP sites (*i.e.* floxed). Neonatal electroporation was recently described by two studies (Boutin et al., 2008; Chesler et al., 2008). In addition, for the electroporation experiments these mice were crossed with Rosa26 reporter mice so that Cre recombinase expression led to excision of both NR1 and a Stop sequence in front of yellow fluorescent protein (YFP) (Platel et al., 2010). As a result, a subset of neuroblasts were YFP-fluorescent and lost NR1 leading to loss of functional NMDA receptors, which was validated with both patch clamp recordings and calcium imaging. Data in Platel et al.'s study suggest that acquisition of NMDA receptors in neuroblasts is critical for their survival in an asynaptic environment where glutamate is provided from astrocyte-like cells. This study raises several questions; it was shown that astrocyte-like cells tonically release glutamate in a calcium-dependent manner. However, the signal(s) leading to intracellular calcium increases in SVZ astrocytes remain unclear. One possibility is the neurotransmitter GABA released from neuroblasts (Liu et al., 2005), but it is not known whether GABA increases calcium in SVZ astrocytes (Fig. 2b for diagram). The function of NMDA receptors on neuroblast proliferation remains to be examined. Finally, the *in vivo* function of glutamate release from astrocyte-like cells on neurogenesis remains to be explored. This would require a genetic conditional knockout of glutamate release from SVZ astrocytes for example using transgenic mice in which VGLUT1 is floxed.

5. Limitation 1: *In vivo* effects of manipulating signaling mechanisms on neurogenesis

Beside the studies on NMDA receptors in transgenic NR1^{floxed} mice and on mGluR5 in knockout mice, the work on neurotransmitters in neurogenesis has been performed in acute slices or following drug injection *in vivo*. The work in slices is hard to extrapolate to the process of neurogenesis *in vivo*. The use of *in vivo* drugs may lack specificity and involves indirect pathways such as hormonal responses. Nevertheless, despite the lack of specificity and mechanistic insight, testing the effects of drugs on neurogenesis is important to eventually use these drugs for therapeutic interventions aimed at boosting neurogenesis.

To address this limitation, we clearly need to use genetic manipulations of receptors as shown for NMDA receptors. Knockout mice are helpful and will provide valuable information, but compensation by other receptor subtypes is common and may bias the results and data interpretation. One of the best approaches in terms of selectivity and complete removal is using transgenic mice where a receptor or subunit flanked by loxP sites (*i.e.* floxed) is excised upon Cre recombinase expression. Fortunately the Knockout Mouse Project (KOMP) will soon be making scores of loxP conditional strains available to the research. Another approach is to use RNA interference (RNAi) technology. RNAi is now easy to design and deliver using viral constructs or electroporated plasmids encoding short hairpin RNA (shRNA). RNAi is less selective than using “floxed” transgenic mice due to off-target effects. Nevertheless, together with rescue experiments, the use of RNAi is becoming a standard and relatively simple approach. These two approaches (“floxed” mice or RNAi) require knowing which receptors or subunits are expressed in each SVZ cell type both at the transcript and protein levels. This is an imperative next step to advance our knowledge of neurotransmitter function on neurogenesis.

6. Limitation 2: Mismatch between the uniform actions of neurotransmitters with the mosaic nature of the SVZ and the diversity of functions on cell development

Almost every study thus far has presented neurotransmitter signaling as uniform along the SVZ. This is particularly true for GABAergic signaling, which seems ubiquitous in the SVZ;

every neuroblast and astrocyte-like cell express GABA_A receptors (Wang et al., 2003; Liu et al., 2005; Platel et al., 2008b). This is in drastic contrast with the mosaic cellular and molecular nature of the SVZ and the diversity of neurotransmitter functions.

The SVZ contains several populations of cells. The population of SVZ astrocytes contains non-proliferative astrocyte-like cells and self-renewing neural progenitor cells (see section #2 on organization of the SVZ). At the molecular level, it has recently been reported that neural progenitor cells in the SVZ are heterogeneous with respect to their neurogenic fate and embryonic origin (Merkle et al., 2007; Young et al., 2007). This heterogeneity translates into subpopulations of neural progenitor cells each in a distinct location of the SVZ resulting in a mosaic appearance (Merkle et al., 2007). In addition, the same neurotransmitter may regulate every stage of cell development. The extreme example is for GABA, which regulates neuroblast proliferation, migration, and dendrite extension through the same type of receptor GABA_A receptors. The question is thus: how is neurotransmitter selectivity provided for distinct cellular populations, subpopulations, and stages of cell development? We provide three possible explanations: (1) The presence of distinct receptor subtypes or subunits allow for receptor heterogeneity, which may lead to activation of different intracellular pathways, thus regulating distinct stages of cell development or cell populations. In addition, it is suggested that receptor subtypes are developmentally regulated by the sequential acquisition of different glutamate receptor subtypes (Platel et al., 2008a; b; 2010). Even for GABA_A receptors, it is expected that different cell populations will express different subunits and that the subunit composition may change as cells mature. (2) The dopaminergic and serotonergic inputs into the SVZ may provide regional selectivity. For example, it is conceivable that dopaminergic inputs may predominate in the rostral section of the SVZ while serotonergic inputs may be more caudal. This, however, has not been investigated. (3) Neurotransmitters may interact with other signaling molecules that are critical for neurogenesis (e.g. Shh or Wnt signaling, see section 7 for additional details) and may be differentially expressed along the SVZ. These types of interactions would amplify the diversity of neurotransmitter action and also would provide regional and cellular specificity of functions.

Because each receptor subtype or subunit is expected to play unique roles during neurogenesis, it is important to knockout or knockdown every receptor subtype or subunit one by one. This would help tease out their selective role on different stages of cell development and cell type. In addition, stage-dependent conditional removal of receptor subtypes may reveal their sequential function during cell development. For example, the same receptor subtype or subunit may play a role on cell proliferation and later on cell integration through activation of different intracellular cascades. This could be explored using a promoter selective to maturing postmitotic neuronal precursors to drive Cre recombinase or shRNA expression in these cells once they enter the olfactory bulb. Analyzing the effect on the different types of interneurons should reveal whether certain genetic manipulations affect certain subpopulation of neural progenitor cells.

7. Limitation 3: How does neurotransmitter signaling integrate with other crucial signals?

It is perhaps egocentric to view neurotransmitters as master regulators of neurogenesis in light of the diversity of signals implicated in regulating the different stages of neurogenesis (for reviews see (Pozniak and Pleasure, 2006; Pathania et al., 2010)). However, neurotransmitters, such as GABA and glutamate, have conserved signaling functions across phyla. For example, GABA accumulates rapidly in plant tissues in response to biotic and abiotic stress. Recent evidence suggests that a gradient of GABA regulates plant growth possibly through regulation of intracellular calcium dynamics (for review see (Ma, 2003; Bouche and Fromm, 2004; Roberts, 2007)). Although genes highly homologous to the mammalian GABA receptors are

not present in the *Arabidopsis* genome, it has been proposed that GABA could interact with a family of proteins (designated atglrs for *Arabidopsis* glutamate receptors) that sequence and structural homology with the mammalian ionotropic glutamate receptors (iGluRs) (for review and references see (Bouche and Fromm, 2004)). Neurotransmitters may also interact with and perhaps control both the expression and function of other signals such as Sonic hedgehog (Shh), Wnt, and/or Notch signaling. We provide here a speculation on interactions between neurotransmitters and Shh. Shh homolog is one of three proteins in the signaling pathway family called hedgehog, the others being Desert and Indian hedgehog. Shh is well-known to be critical for vertebrate organogenesis. More recently, Shh was found to be a crucial signal for adult neurogenesis, particularly in maintaining stem cell self-renewal and acting as a mitogen in the SVZ and SGZ (Palma et al., 2005; Ahn and Joyner, 2005; Wang et al., 2007; Han et al., 2008). Changes in monoamine levels *in vivo* through chemical depletion have been shown to regulate the expression of the Shh signaling cascade in the adult rodent brain measured with *in situ* hybridization (Rajendran et al., 2009). The same group also reported that electroconvulsive seizures upregulated Shh signaling pathways at the mRNA level in the SGZ (Banerjee et al., 2005). These data suggest that neurotransmitters released during seizures may impact the expression of Shh and its receptors. It is thus conceivable that neurotransmitters regulate the expression of Shh components in the SVZ. Shh acts through a receptor complex associating Patched and Smoothened (for review see (Philipp and Caron, 2009)). Smoothened is structurally similar to G protein-coupled receptors and accumulating evidence suggests that Smoothened relies on heterotrimeric G proteins to transduce Shh signal. mGluRs, which are G protein coupled receptor could thus interact with Smoothened signaling cascade. Similarly changes in intracellular calcium levels may affect kinase activity and G protein phosphorylation. Further studies are clearly required to examine such types of interactions and may help better understand the specificity of neurotransmitter action.

8. Conclusion

A better picture of neurotransmitter signaling in the largest postnatal neurogenic zone, the SVZ, is beginning to emerge. The signaling in this region is more complex than we anticipated with intricate communication between the different cell types. In particular, bidirectional communication between astrocyte-like cells and neuroblasts has just been reported; neuroblasts release GABA, which activates GABA_ARs on themselves and astrocyte-like cells while astrocyte-like cells release glutamate, which activates glutamate receptors on neuroblasts. How these two signaling interact remain to be investigated. In this synaptic network astrocyte-like cells are in charge of glutamatergic signaling that is critical for neuroblast survival and ultimately proper neurogenesis. Glutamate release from astrocytes appears to be a conserved function from immature to mature networks with distinct impact on network activity.

Despite an increase in our understanding of neurotransmitters in neurogenesis regarding their signaling and function, this is clearly only the first leg of the research journey as discussed through the limitations above. In the coming years, it is critical to identify the role of every receptor subtype or subunit in neurogenesis using genetic manipulations and to address whether neurotransmitters interact with crucial neurogenic signals. In addition, there is increasing interest in studying the role of glutamatergic signaling on cell development because genetic studies suggest that mutations or epigenetic misregulation in receptors and their subunits (*e.g.* glutamate receptors) are associated with neurodevelopmental disorders such as autism spectrum disorder, bipolar disorders, epilepsy, and schizophrenia, to name a few (please see for example the following database: www.genecards.org, section disorders and mutations of the gene of interest). Studying the consequences of gain or loss-of-function of receptors and subunits will help us understand the etiology of developmental disorders. A better understanding of neurotransmitter signaling in neurogenesis will hopefully also help us design

better strategies to promote brain repair from endogenous progenitor cells or improve transplant efficiency.

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Abbreviations

EGFR	epidermal growth factor receptor
FLRFa	phe-leu-arg-phe amide
GABA	gamma-aminobutyric acid
GFAP	glial fibrillary acidic protein
GFP	green fluorescent protein
GLAST	glutamate-aspartate transporter
mGluR5	metabotropic glutamate receptor 5
MrgA1	Mas-related gene A1
NMDAR	NMDA receptors
RMS	rostral migratory stream
SHH	Sonic hedgehog
SGZ	subgranular zone
SVZ	subventricular zone
VGLUT1	vesicular glutamate transporter 1
YFP	yellow fluorescent protein

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Figure 1. Structure of the neurogenic subventricular zone

(a) Reconstruction of a sagittal slice of a postnatal (P) 25 mouse brain immunostained for doublecortin (DCX). Chains of DCX-expressing neuroblasts from the subventricular zone (SVZ) converge to form the rostral migratory stream (RMS), which terminates in the olfactory bulb. Scale bar: 500 μ m. **(b)** Simplified diagram illustrating the cellular composition of the SVZ. The processes of a SVZ astrocyte ensheath a cluster of neuroblasts (in a coronal plan).

Figure 2. GABAergic and glutamatergic signaling in the SVZ

(a) Confocal photograph displaying co-immunostaining for glutamate (green), GLAST (glutamate-aspartate transporter) which labels astrocytes (red), and DCX (a marker of neuroblasts, blue) in the rostral extension of the SVZ. Scale bar: 10 μm . (b) Model illustrating that FLRFa peptide induces calcium increases in RMS astrocytes leading to glutamate release and NMDA receptor activation in adjacent neuroblasts recorded with a patch clamp pipette. (c) Simplified diagram illustrating the expression of GABA and glutamate signaling molecules in the SVZ. Neuroblasts can release GABA into the extracellular space. GABA activates GABA_A receptors in SVZ astrocytes. Upon intracellular calcium increase, glutamate is released by SVZ astrocytes. Released glutamate diffuses and activates glutamate receptors on neuroblasts.