Notch Signaling Balances Adult Neural Stem Cell Quiescence and Heterogeneity

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Summary

Adult neurogenesis continues throughout life in the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) of mammals. At the base of adult neurogenesis lie adult neural stem cells (NSCs). These cells can either be found in a dormant, non-dividing state (quiescent) or in a proliferating state (active). Over the last three decades the field of neurogenesis has expanded, but there are still open questions with regards to adult NSC maintenance and potential capacity. Over the course of my PhD studies I addressed three major questions of adult NSC maintenance.

(1) What are the differences between active and quiescent NSCs?

(2) Do NSCs have similar maintenance factors in the SVZ and the SGZ?

(3) What are the capacities of distinct subtypes of NSCs and progenitors to respond to external stimuli?

I was able to show that in the adult mouse brain, Notch2 is the gatekeeper of quiescent NSCs in both neurogenic niches, the SVZ and the SGZ. The loss of this Notch paralogue led to the activation of quiescent NSCS and a prolonged and abnormal activation, followed by NSCs exhaustion in the long term. If Notch1 was deleted in addition to Notch2, quiescent and active NSCs are no longer maintained properly and will differentiate to a neural fate. Thus an intricate interplay between Notch1 and Notch2 is needed for adult NSC maintenance in both neurogenic niches.

In the SVZ the receptors Notch1 and Notch2 are coexpressed on NSCs. We addressed NSC identity also in the second neurogenic niche, the SGZ, where the receptors are also coexpressed by NSCs. The loss of Notch2 led to the activation of quiescent NSCs and an increased production of neuroblasts.

The differential signal requirement for the maintenance of quiescent and active NSCs raises the question, whether these distinct cell populations might have unique functions in response to external physiological and/or pathological stimuli. In order to address this question we characterized the SGZ in great detail at different ages. In the geriatric SGZ active NSCs were lost and the NSCs that remained were quiescent. These quiescent NSCs have the capacity to replenish the active NSC pool upon induction of epileptic seizures. On the other hand, administration of

antidepressants left the NSCs unaffected initially. It was the amplifying progenitor pool that responded. In long-chase experiments the NSCs were then reactivated by either the resulting induced changes from the amplifying progenitors or a delay in NSC response.

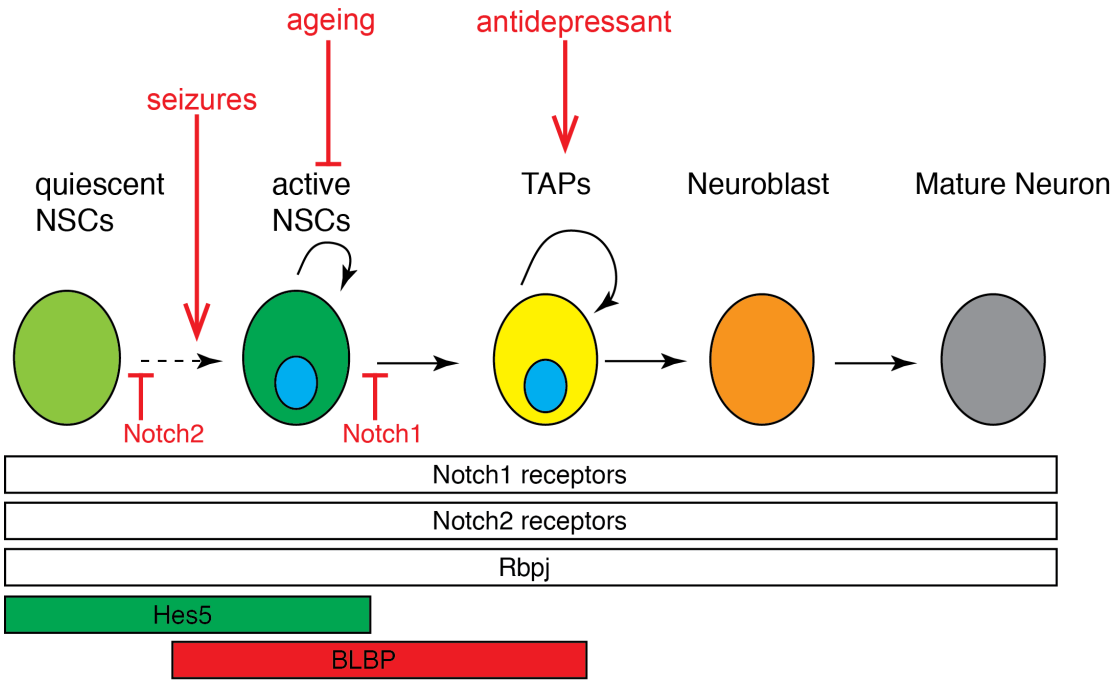

Figure 1: Graphical Summary; Cells of the neurogenic lineage express Notch receptors Notch1 and Notch2 and the Notch signaling mediator Rbpj. However, only NSCs exhibit active Notch signaling characterized by expression of Notch effector genes such as Hes5 and BLBP. Hes5 and BLBP allow for the discrimination between quiescent ($Hes5^{+}BLBP^{-}$) and active ($Hes5^{+}BLBP^{+}$) NSCs as well as transient amplifying progenitors (TAPs/IPs) ($Hes5^{-}BLBP^{+}$). The distinct cell populations in the early neurogenic lineage are maintained by different signals. Notch2 maintains quiescent NSCs, whereas Notch1 maintains active NSCs. Furthermore, the transition from quiescence to activity is fostered by induction of seizures, whereas ageing leads to a loss of active NSCs. The administration of antidepressants (namely Fluoxetine) is affecting the TAP cells, however not the NSCs.

NSC maintenance in the adult murine brain is an intricate mechanism highly dependent on the proper internal and external mechanisms. In the work presented here, I will illustrate the importance of Notch signaling in NSC maintenance and the high level of heterogeneity within the NSC pool and the NSC niche.

Lay Summary (German)

Das Gehirn von Säugetieren enthält bis ins hohe Alter Stammzellen, welche die Fähigkeit haben, die unterschiedlichsten neuronalen Zelltypen zu bilden. Die neuronalen Stammzellen (nSZ) liegen in zwei Zuständen vor: Die nSZ, welche sich selten teilen und in einem Ruhezustand befinden und die zweite Art nSZ, welche mehrere Zellteilungen durchläuft und schnell teilende Tochterzellen generiert. Diese Tochterzellen produzieren die Vorläuferzellen für vollständig entwickelte Neuronen und Glia, welche neu integriert werden können. Die nSZ werden in zwei klar definierten Regionen des Hirns gefunden, in der subventrikulären Zone zwischen Striatum und Seitenventrikel (SVZ) und in der subgranulären Zone (SGZ) des Gyrus Dentatus des Hippocampus (DG).

Die natürliche Balance zwischen ruhenden und aktiven Stammzellen, sowie deren direkten Nachkommen, ist essentiell für den Erhalt dieser Zellen bis ins hohe Alter. Während meines Doktorats habe ich mich mit den Mechanismen beschäftigt, welche Stammzellen regulieren und zur Generation neuer Zellen führen. Ich habe mich speziell mit drei Fragen beschäftigt:

(1) Wie werden nSZ im Ruhezustand und aktiven Zustand korrekt erhalten?
(2) Sind nSZ in den neurogenen Zonen SVZ und DG vergleichbar?
(3) Wie reagieren nSZ im Ruhezustand bis zuweilen aktiven Zustand auf externe Stimuli?

Wir konnten zeigen, dass der Ruhezustand durch einen speziellen Zellrezeptor, Notch2, aufrecht erhalten wird. Ein weiterer Verwandter in dieser Zellrezeptorfamilie, Notch1, ist essentiell für den Erhalt des aktiven Zustands. Das Fehlen von Notch1 und Notch2 führt zum Verlust der nSZ. Dieses Verhalten konnten wir sowohl in der SVZ als auch im DG beobachten.

Um die nSZ zu testen, wurden verschiedene Stimuli verabreicht und wir konnten feststellen, dass die einzelnen nSZ Typen unterschiedlich reagierten. Während die nSZ im Ruhezustand ein Reservoir darstellten, sind die aktiven Zellen die funktionale Einheit. Die hier präsentierten Erkenntnisse sind wichtig für die Entwicklung von neuen, gezielten Therapiemöglichkeiten.

Publication List and Contributions

(1) Neurogenic Stem Cells in a Dormant Niche are Activated by Antidepressant Fluoxetine and Suppressed by Notch2 signaling;
Anna Engler, Chiara Rolando, Claudio Giachino, Andrea Erni, Ichiko Saotome, Runrui Zhang, Philipp Berninger, Erik van Nimwegen, Ursula Zimber-Strobl, Freddy Radtke, Spyros Artavanis-Tsakonas, Angeliki Louvi and Verdon Taylor; submitted Cell Stem Cell (2016)

Contribution: I planned, conducted and analyzed all the experiments, prepared the figures and the manuscript.

(2) Notch2 Maintains Adult Neural Stem Cell Quiescence in the Hippocampal Subgranular Zone; Runrui Zhang, **Anna Engler**, Claudio Giachino, Ichiko Saotome, Angeliki Louvi, Ursula Zimber-Strobl, Verdon Taylor; in preparation

Contribution: I planned, conducted and analyzed the Notch levels in the DG and prepared the corresponding figure. I planned and conducted the Tamoxifen experiments as well as the FACS experiments.

(3) Adult Hippocampal Heterogeneity and its Modulation in Physiological and Pathological Conditions; Anna Engler[*], Chiara Rolando[*], Claudio Giachino, Andrea Erni, Onur Basak, Verdon Taylor; prepared Glia (2016)

Contribution: I planned and analyzed all the experiments and prepared the figures and manuscript. CR conducted the Fluoxetine experiments and edited the figures and manuscript.

(4) Multipotency of Adult Hippocampal NSCs In Vivo Is Restricted by Drosha/NFIB; Chiara Rolando[*], Andrea Erni[*], Alice Grison, Robert Beattie, **Anna Engler**, Paul J. Gokhale, Marta Milo, Thomas Wegleiter, Sebastian Jessberger and Verdon Taylor; Cell Stem Cell (2016), In Press Corrected Proof; DOI: http://dx.doi.org/10.1016/j.stem.2016.07.003

Contribution: I assisted with the FACS and animal experiments and edited the manuscript.

Introduction

Cell diversification in the body is largely completed by birth, or shortly thereafter, but organs possess mechanisms to replace lost cells throughout life. To be able to maintain this repairing capacity many developing organs set aside somatic stem cells (SC). These adult stem cells (aSC) maintain some of the features of embryonic stem cells (eSC), such as the capacity to self-renew. aSC remain within specific regions of the organ and are able to differentiate into one (unipotent) but more typically many (multipotent) lineages (Fuchs, 2004; Schofield, 1978). As organs differ in size, architecture and function they are subject to different biological and physical challenges and therefore have different regenerative needs. Thus different ways to restore cell numbers have evolved. Today it is known that aSC are not only found in high turnover organs, such as the bone marrow, which harbors hematopoietic stem cells (HSCs). They are also present in organs where cell replacement is slower, including the brain where neural stem cells (NSCs) generate restricted neuronal cell types (Gage, 2000; Kempermann et al., 2015).

Neurogenesis

The development of the central nervous system (CNS) is an intricate process precisely regulated in time and space. In rodents, the majority of the cells present in the adult brain are produced during embryogenesis. The SCs responsible for building the brain are retained in the ventricular zone (VZ) These SCs give rise to all cells of the developing and mature CNS, including NSCs (Fuentealba et al., 2015; Kazanis et al., 2008). The process by which new neurons are formed from NSCs is termed neurogenesis.

Neurogenesis in mammals is a complex process, which needs to be controlled and regulated properly as it is an energetically expensive process that bears risks (Kempermann, 2015). In the last years various populations of stem and progenitor cells have been identified in the developing and the adult brain. These distinct stem cells in turn can be found within specific regions of the brain. The regional specificity and the distinct intrinsic properties of NSCs illustrate the high complexity and heterogeneity of neurogenesis. Improper regulation, due to extrinsic injury or intrinsic genetics, can lead to aberrant wiring of newborn neurons both in the embryo and the adult, contributing to pathologies (Dietrich and Kempermann, 2006).

Embryonic neurogenesis

Around embryonic day 8 (E8) neurulation is initiated through a combination of released growth factors and inhibitory signals secreted by the notochord, the dorsal ectoderm and the Spemann organizer (Tam and Loebel, 2007). During neurulation the neural plate folds and forms the neural tube, the very early precursor of the CNS.

The neural tube is lined exteriorly by neural crest cells (NCCs) and interiorly by neuroepithelial progenitors (NEPs). NCCs give rise to the majority of the peripheral nervous system (PNS); they also generate smooth muscle cells, pigment cells and the cranium bone (Bhatt et al., 2013; Sauka-Spengler and Bronner, 2010). While NCCs give rise to the PNS, it is the NEPs in the neuroepithelium of the neural tube that are essential in the formation of the CNS. The neural tube follows sequential, competing patterning steps during brain development. An interplay of morphogen gradients and signaling pathways, including sonic hedgehog (Shh), retinoic acid (RA), fibroblast growth factor (FGF), wingless (Wnt) and bone morphogenic protein (BMP) (Lupo et al., 2006) regionalize the neural tube. Due to this patterning the NEPS of the neural tube become more specified and defined structural domains appear.

The four most important segments of the regionalized tube are the forebrain, the midbrain, the hindbrain and the spinal cord. The forebrain contains two cortical structures – the neocortex and the hippocampus. Both of these structures are derived embryonically and early postnatally. The neocortex starts to be formed by E11.5 and is finished by birth whereas the hippocampus starts to be formed by E17.5 and is finished around postnatal day 14 (P14). These two regions harbor the NSC niches in the adult brain.

Development of the Neocortex

At E9 the neuroepithelium is a single layer of NEPs. As the progenitors proliferate and increase in number, some will become radial glia cells (RGC), that constitute the ventricular zone (VZ) and will function as NSCs (Noctor et al., 2004) (Figure 2, adapted from Greig et al., 2013). The precursor and progenitor populations have distinct features. RGCs span the thickness of the cortex from the apical to the basal surface with their radial processes whereas intermediate progenitor cells (IPCs) are not connected to the surfaces. RGCs have a polarized organization, initially they undergo more symmetric division to increase their numbers but switch to an asymmetric division mode later on, with a horizontal cleavage plane, thus dividing

apical and basal positioning, to self-renew and produce IPCs. The IPCs are multipolar and lack the apical and basal process. They rapidly divide and amplify the precursor pool (Noctor et al., 2007). The number of divisions IPCs can undergo is limited and they will mostly divide symmetrically to produce neurons (Noctor et al., 2004). Alternatively the produced daughter cell of the RGCs is differentiated, will no longer divide and migrate out of the ventricular zone, along the RGC process (Greig et al., 2013).

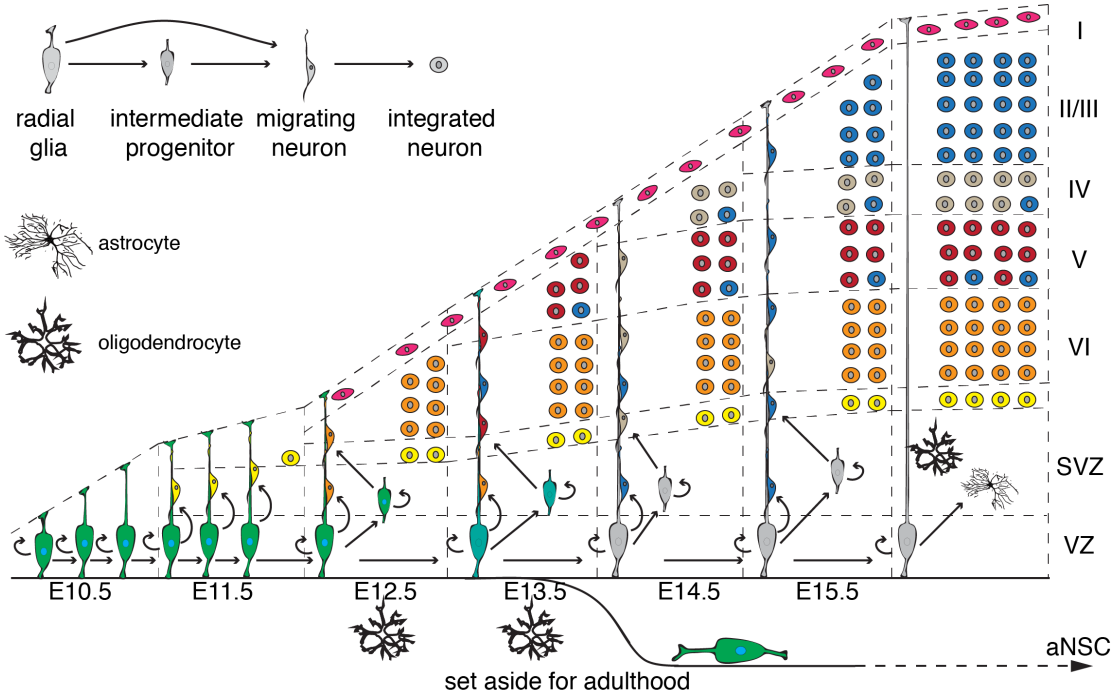

Figure 2: Development of the Neocortex; This scheme shows the sequential generation of neocortical neuron subtypes and their migration to the appropriate layers during embryonic neurogenesis. Around E11.5 radial glia cells start to give rise to intermediate progenitors or directly to migrating neurons. Shortly after this initiation of embryonic neurogenesis NSCs are set aside for adult neurogenesis that will not divide until the animal reaches adulthood. The distinct projection neuron subtypes are born in sequential waves. During embryonic neurogenesis the newly generated neurons migrate to their dedicated layer where they will integrate. Neocortical layering is complete around E16.5. After this the remaining NSCs are thought to take on a more gliogenic fate, giving rise to astrocytes and oligodendrocytes.

Neurogenesis starts at E10.5 in the dorsal telencephalon and from the beginning excitatory neurons are being produced. These neurons are produced in a sequential manner, whether this is happening from one common RGC or whether distinct subtypes of RGCs mediate the generation of the individual layers is currently debated (Franco and Muller, 2013; Guo et al., 2013). It is accepted that the first neurons produced migrate away from the progenitors to form the preplate, which will form a boundary for neurogenesis (Marin-Padilla, 1978). Subsequently the newly born neurons migrate into the cortical plate in an “inside-out” fashion – early born

neurons will be found in the deep layer, late born neurons migrate pass them and will be found in the superficial layers. At E17.5 cortical development is largely finished, the VZ will disappear and the SVZ will remain as neurogenic zone postnatally (Figure 2, adapted from Greig et al., 2013).

Much like neurogenesis, gliogenesis is a complex mechanism crucially depending on the right temporospatial input. Glial cells carry out a diverse range of critical functions in the brain, including nutrient supply, removal of cellular debris, providing a scaffold and axonal insulation (Auld and Robitaille, 2003). The two major types of glial cells are oligodendrocytes and astrocytes. The production of astrocytes, a process termed astrogliogenesis, occurs most likely from the same pool of stem cells that gives rise to neurons. Astrogliogenesis is thought to be a default mode of differentiation of the IPCs if they do not obtain the proper proneural input (Kanski et al., 2014). The production of oligodendrocytes, a process termed oligodendrogenesis occurs in two sequential, competitive waves beginning in the embryo around E12.5, continuing into the early postnatal brain. Whether any of the oligodendrocytes produced in the first embryonic waves, survive is unclear, the ones from the postnatal wave however are maintained (Kessaris et al., 2006). Both, oligodendrocytes and astrocytes produced early in life are retained into adulthood.

Besides neurons, astrocytes and oligodendrocytes that are retained from embryonic neocortical development also the postnatal NSCs become regionally specified and put aside. These set aside NSCs remain largely quiescent until reactivation in the adult. The remaining embryonic NSCs diverge their lineage during their development. The set aside adult NSCs share a common origin with the embryonic NSCs (Fuentealba et al., 2015; Furutachi et al., 2015; Greig et al., 2013; Gridley, 1996). Similar mechanisms can be observed in the second developing niche, the SGZ of the hippocampal DG.

Development of the Hippocampal Dentate Gyrus

In hippocampal development, RGCs detach from the embryonic ventricular wall and move into the subgranular zone (SGZ) where they transform into elongated cells, similar to the RGCs in cortical development, which generate neurons of the granule layer (Seri, 2001; Seri et al., 2004). The granule cell layer and subgranular layer of the DG of the hippocampus are only fully established late into postnatal development around P14 (Nicola et al., 2015). The formation of the dentate gyrus occurs in two stages – migration to the future DG and formation of the neurogenic zone of the adult SGZ (Figure 3, adapted from Rolando and Taylor, 2014).

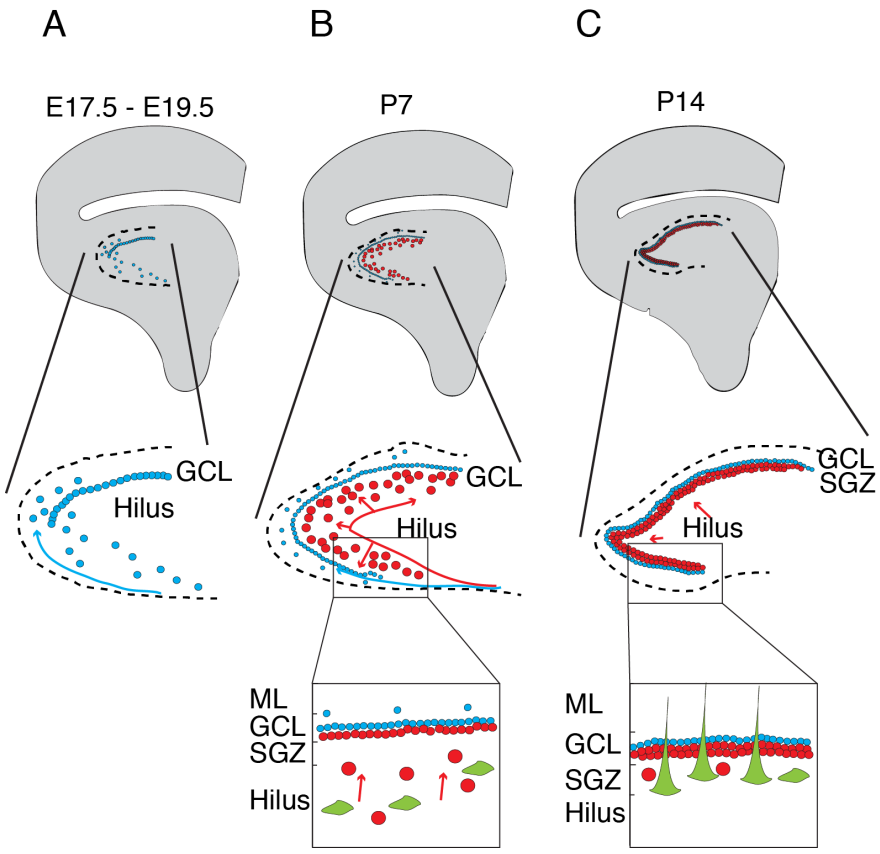

Figure 3: Development of the SGZ; Around E17.5 precursor cells from the VZ migrate into the hippocampal hilus **(A).** Migration, integration and maturation continue in the postnatal brain, the designated NSCs (green) do not display radial type morphology, the granule cell layer (GCL, red) and molecular layer (ML, blue) is being formed **(B).** Only around P14 the formation of the SGZ is finalized with NSCs (green) present in the SGZ, projecting through the GCL into the ML **(C).**

The first stage occurs during embryonic development. The precursor cells are led from the hippocampal hem to the area of the future DG. Around E17.5-E19.5 $GFAP^{+}$ precursors cells, originating from the VZ, migrate and accumulate in the hippocampal hilus and future SGZ. The granule cell migration is marked by Tbr2 and is broadly distributed in the developing DG. At this time point, the newly settled cells do not have a radial orientation (Rolando and Taylor, 2014).

The second stage occurs postnatally. The embryonically formed scaffold transforms into the neurogenic zone of the adult SGZ. The cells start to obtain their typical NSC characteristics around P7 and present their typical radial type morphology at P14. At this time point, the expression of Tbr2 becomes more restricted to the SGZ (Nicola et al., 2015). The development of the DG is completed just in time when young mice start to open their eyes and explore freely (Rakic,

2002). The remaining stem/progenitor cells in the SGZ are retained life-long and continue to produce new neurons throughout adulthood (Altman and Bayer, 1990; Gage, 2000; Kempermann et al., 2015).

From both the SVZ and the SGZ, developmental NSCs endure into adulthood. These spatially restricted zones are the ones where, under physiological conditions, new neurons can be formed even in the adult. The production of functioning, new neurons and proper integration into the adult CNS is termed as adult neurogenesis. Adult neurogenesis recapitulates many aspects of embryonic neurogenesis and is conserved among mammalian species (Faigle and Song, 2013).

Adult Neurogenesis

At the base of adult neurogenesis are adult NSCs, which are a rare population of cells that divide infrequently. The maintenance of NSCs in the adult is a life-long process, ensured by highly regulated control mechanisms that keep proliferation and differentiation in check (Faigle and Song, 2013). In order to assure a life-long reservoir of NSCs, the cells can be found as two distinct populations, quiescent and actively proliferating. This way the NSC pool renews itself while an adequate number of differentiated cells can be provided (Fuchs, 2009). In the adult brain we can find quiescent NSCs, giving rise to active NSCs, which in turn give rise to dividing daughter progenitors that become progressively postmitotic, and eventually provide various cell types such as neurons, astrocytes and oligodendrocytes to the adult brain (Bonaguidi et al., 2011).

Neural Stem Cell Hierarchy

In order to avoid stem cell depletion, an intricate hierarchy can be found in NSC lineage. At the beginning of the lineage are the NSCs. These can either be found as quiescent NSCs, potentially functioning as reserve pool or in an active, more frequently dividing form. The active NSCs can divide symmetrically to give rise to two NSCs or asymmetrically, to give rise to a stem cell and an amplifying progenitor. The amplifying progenitors will give rise to rarely dividing fate committed progenitors which in turn will give rise to the differentiated cells, either neurons or glia cells. It is proposed that there is an initial bias in the stem cell pools (Bonaguidi et al., 2012), meaning, the precursors and the amplifying progenitors will already have an intrinsic mechanism for either a glial fate or a neuronal fate.

At the beginning of the lineage are the NSCs, which are rarely dividing and exhibit either longer (10 - 28 days) and shorter (48 hours) cell cycle times (Encinas et al., 2011; Ihrie and Alvarez-Buylla, 2011). It is assumed that the stem cells with longer cell cycle (quiescent) are functioning as a reservoir. The more actively dividing cells have shorter cell cycles however maintain their stemness. Whether the active cells have the capacity to go back to a quiescent state or will eventually deplete is currently debated (Cavallucci et al., 2016; Urban et al., 2016). It is accepted that active NSCs give rise to transient amplifying progenitors (TAPs, called IPs in the DG SGZ), that are dividing faster, with a cell-cycle time of about 12 hours (Morshead, 1994). This shorter cell cycle allows them to amplify the cell pool of the early progenitor state. It is under investigation whether the TAPs are already biased towards a fate commitment or whether they only commit at a later progenitor stage (Taylor, 2011). The committed progenitors, called neuroblasts in the neuronal lineage, will rarely divide, become postmitotic and will develop into mature neurons (van Praag et al., 2005). The newly integrated, matured cells are morphologically and physiologically indistinguishable from the embryonically developed cells (Figure 4).

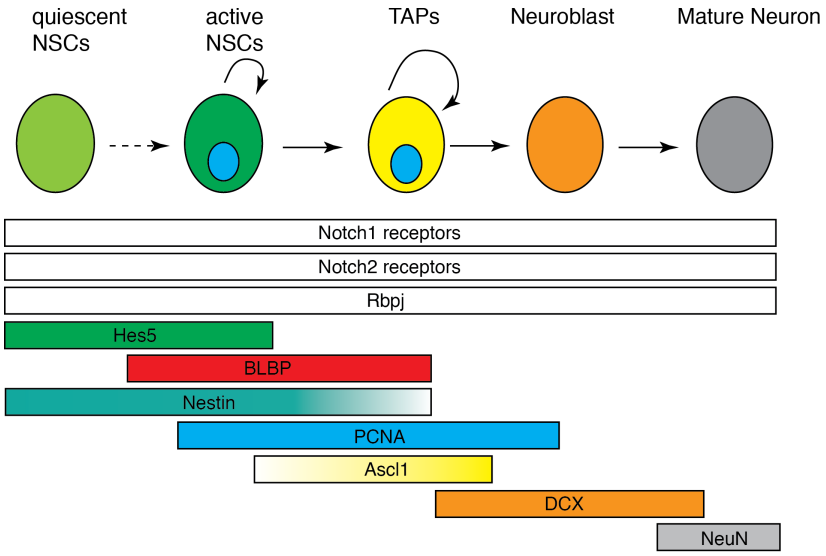

Figure 4: NSC Hierarchy; At the beginning of neurogenesis are the NSCs. NSCs can be divided in quiescent and active NSCs. NSCs give rise to transient amplifying progenitor cells (TAPs) which amplify the pool and give rise to fate committed neuroblasts. Neuroblasts undergo maximally one more division and become postmitotic hereafter. If they obtain the correct signals they can become mature neurons and integrate into existing circuits. The newly born neurons are indistinguishable from the embryonically generated neurons. The lineage can be analysed using distinct markers for the cell stages. Hes5 marks quiescent and active NSCs, BLBP marks active NSCs and TAPs. Nestin marks quiescent and active NSCs as well as TAPs. The proliferation marker PCNA is found in all dividing cells, namely the active NSCs, TAPs and few neuroblasts. Ascl1 is a marker for active NSCs and TAPs. Doublecortin (Dcx) labels late TAPs, neuroblasts and goes into the early neuron lineage. NeuN is a nuclear antigen for neurons.

Although adult NSCs have an intrinsic property to provide new cells throughout life, it is a delicate balance that ought to be tightly controlled. The SVZ and SGZ are stem cell niches with a defined microenvironment to avoid NSC exhaustion (Conover and Notti, 2008). NSC fate is regulated through cues provided by the niche, such as cell-cell contacts and secreted factors (Schofield, 1978; Voog and Jones, 2010). The specific cytoarchitectural properties found in the SVZ and the SGZ (Figure 5) maintain the NSC population, guide cell fate decisions and ultimately regulate the regenerative potential of the niche (Fuchs, 2004).

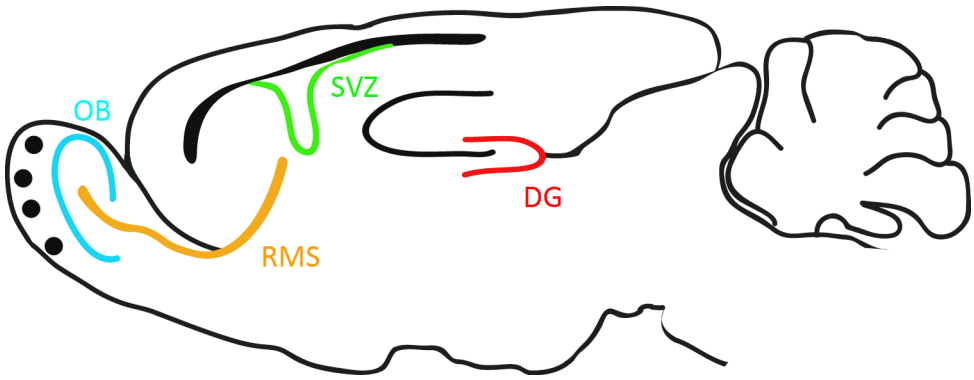

Figure 5: Adult neurogenic niches of the murine brain; Schematic representation of a sagittal mouse brain section. Neurogenesis occurs in the SGZ of the DG (red) and the LW of the SVZ (green). The SGZ is a stationary niche with NSCs and progeny found in the DG. The LW of the SVZ contains NSCs, however the daughter cells will migrate out of the SVZ along the rostral migratory stream (RMS; orange) into the olfactory bulb (OB; blue). If the cells obtain the correct signals they can functionally integrate into the OB.

Cytoarchitecture of the Adult Subventricular Zone

The NSCs in the SVZ are found between the lateral ventricle (LV) and the striatum. A single layer of ependymal cells separates the SVZ from the cerebral spinal fluid (CSF) in the LV (Ihrie and Alvarez-Buylla, 2011). New neurons originating in the SVZ will migrate along the rostral migratory stream (RMS) to the olfactory bulb (OB) (Figure 5). Under physiological conditions, the OB is provided continuously with new interneurons from the SVZ (Lois, 1996). The OB is the terminal location of the newborn neurons and thus an interesting region to look at the fate commitment of the cells originating in the SVZ niche.

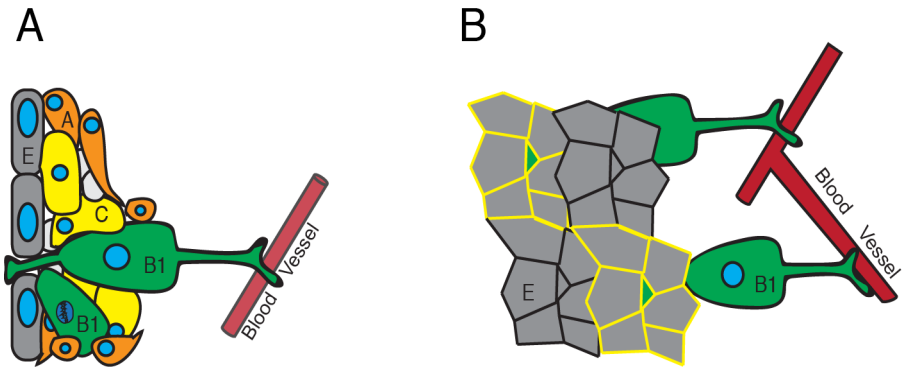

Figure 6: NSCs of the SVZ are organized in pinwheels; NSCs of the SVZ are divided into B-cells (NSCs, green), C-cells (TAPs, yellow) and A-cells (neuroblasts, orange). They are in a tight scaffold with each other. The A-cells will migrate out of the SVZ, along the RMS into the OB. The radial B1 cells, projecting to blood vessels and through the ependyma (E, grey) are the quiescent NSCs **(A)**. The radial B1-cells are characterized by their typical pinwheel morphology. In whole mount preparations the process projecting through the ependyma is generating this NSC typical morphology (yellow trace) **(B)**.

The NSCs found in the SVZ project bidirectionally through the ependyma into the CSF and radially to blood vessels (BV) to obtain systemic inputs (Merkle et al., 2007) (Figure 6A). Electron microscopy has revealed that the SVZ niche consists of four major cell types, E- (ependymal), B- (SVZ astrocytes and NSCs), C- (transitory amplifying) and A-cells (neuroblasts) (Figure 6A). Using whole mount techniques it was observed that B-cells of the lateral wall with NSC properties, defined as B1 cells (Ihrie and Alvarez-Buylla, 2011), can be found in a typical pinwheel structure (Mirzadeh et al., 2008). The core of the pinwheel contains the apical ending of a radial B1-cells and in its periphery are ependymal cells (Figure 6B). This typical embedding of B-cells within ependymal wall, blood vessels, immediate surrounding and own progeny allows for distinct response mechanisms of the stem cells. Comprised, these response mechanisms can be put in four categories.

First, the apical ending in the core of the pinwheel contains sensory cilia that respond to signals in the CSF and flow of the CSF. The CSF, for example, contains gradients of Slit2. These gradients are partially regulated by the movement of the mechanocilia on the ependymal cells (Sawamoto et al., 2006). Additionally, a cellular response might be triggered mechanically via the flow of the CSF passing the cilia (Banizs et al., 2005), due to shear forces activating ion channels and Ca^{2+} influx (Yamamoto et al., 2000). The role of cilia in neurogenesis is proposed to be crucial, as misregulation of this dual response system potentially has severe implications for NSC maintenance and progenitor migration (Goetz and Stricker, 2006).

Second, the B-cells are connected to another supply of extrinsic signals the BVs, which have a dual function. On one hand, the blood-brain barrier (BBB) in close proximity to the SVZ is more permeable than in the rest of the brain (Cheung and Rando, 2013; Shen et al., 2008), thus releasing blood-born factors including pigment epithelium-derived factor (Andreu-Agullo et al., 2009) and β−cellulin (Gomez-Gaviro et al., 2012) that are proposed to be involved in maintenance, proliferation and differentiation. On the other hand NSCs directly contact the epithelial cells of the BVs with their processes, getting input from surface receptors such as Delta-like (Dll) and Jagged ligands (Temple, 2001). These juxtacrine signals play a pivotal role in maintenance of NSCs in a quiescent and undifferentiated state (Ottone et al., 2014).

Third, the NSCs are located in regionalized portions of the SVZ innervated by distinct nuclei. Distinct OB interneuron subtypes are produced in finely patterned progenitor domains of the SVZ. These microdomains of the SVZ correlate with expression domains of distinct transcription factors such as Nkx6.2 and Zic-family members. These domains are potentially defined by the nuclei they are innervated by (Merkle et al., 2014). Axons from defined nuclei, such as the raphe or the pons can form an extensive plexus in close proximity to adult NSCs. NSCs express different receptors of neurotransmitters, which makes them susceptible to neuronal stimuli (Tong et al., 2014b). These microdomains, potentially regulated by innervation from CNS nuclei, exemplify the interconnectivity of NSCs and the niche.

Fourth, the immediate progenitors are in direct contact with the NSCs, allowing for direct cell-cell interactions. In the SVZ, mother and daughter cells are in close proximity. It is presumed that this direct interaction balances the populations of NSCs and TAPs in the niche. Both NSCs and TAPs present and secrete a vast array of proteins involved in regulating neurogenesis (Drago et al., 2013; Hermann et al., 2014). Some of the presented receptors are endodermal growth factor receptor (Doetsch, 2003) and Notch receptors (Aguirre et al., 2010) Notch ligands Jagged (Basak et al., 2012; Nyfeler et al., 2005). In parallel some of the secreted soluble growth factors are FGF and EGF (Deleyrolle et al., 2006; Türeyen et al., 2005). These paracrine mechanisms provide a feedback loop to keep neurogenesis and stem cell maintenance in tight control.

Thus, the NSCs in the SVZ are controlled on a niche and hierarchical level by extrinsic (CSF and BVs) and intrinsic (axons and feedback loops) factors. A similar system can be found in the SGZ of the DG the second neurogenic niche.

Organization of the Adult Subgranular Zone

The DG of the hippocampus is part of the limbic system and plays a key role in memory consolidation and spatial navigation. Neurogenesis in the DG is found in the SGZ of adult rodents, primates as well as humans (Spalding et al., 2013) and ongoing neurogenesis in the adult SGZ has been proposed to be important in learning and memory (Zhao et al., 2008).

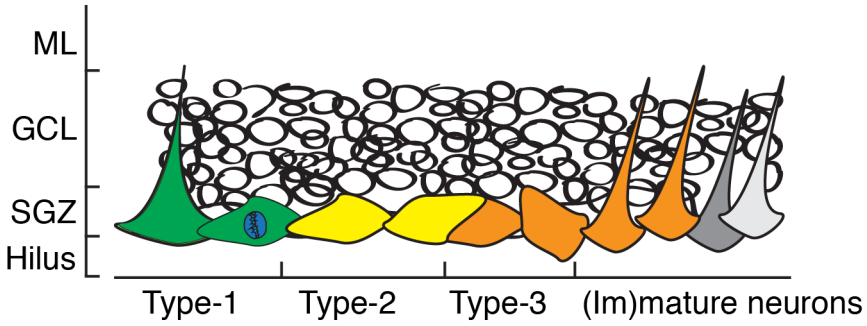

Figure 7: NSCs of the SGZ are in close proximity to their progeny; NSCs in the SGZ can be found as radial or horizontal cells (Type-1, green). The radial Type-1 cells, projecting through the granule cell layer (GCL) divide less frequently than the horizontal Type-1 cells. The radial cells are characterized as quiescent, the horizontal as active. They give rise to Type-2 cells, which are intermediate progenitors (IPs, yellow). The Type-3 cells are characterized as neuroblasts (orange), which are fate committed. They give rise to immature neurons that can mature and stably integrate into the DG circuits.

The nomenclature in the SGZ is different from the SVZ. One distinguishes Type-1, Type-2 and Type-3 cells (Figure 7, adapted from Kempermann 2004), and these types in turn are divided in further subtypes. Type-1 cells are divided into radial, quiescent, and horizontal, active, NSC (Lugert et al., 2010). The Type1 cells give rise to the Type-2 cells, which are divided into Type-2a (early progenitors) and Type-2b (late progenitors). Type-2 cells are intermediate precursor cells (IPs). The Type-2 cells give rise to Type-3 cells, which are fate-committed neuroblasts (Ehninger and Kempermann, 2008). Upon maturation they become neurons that potentially integrate into the DG circuits. In contrast to the SVZ, a single neuron-type – DG granule neurons - are produced (Seri et al., 2004). This population makes up 10% of the murine neural circuits (Kempermann et al., 2015) and 35% of the human neural circuits (Spalding et al., 2013). Newly generated neurons in the hippocampus integrate into established networks, making neurogenesis a unique form of neuronal plasticity. Although the neurogenic niches have distinct architectures and exhibit high levels of heterogeneity, the stem cells found in the SGZ and SVZ have, besides their differences also commonalities.

Due to lack of contact the SGZ NSCs do not obtain input from the CSF, however they are provided, just as in the SVZ with external stimuli via the vasculature. In the SGZ the NSCs are positioned close to endothelial cells of blood vessels. As in the SVZ it is presumed that the BBB in the SGZ might be more permeable, thus providing extrinsic signals (Cheung and Rando, 2013). Furthermore the endothelial cells might provide paracrine signals themselves that play into the signaling of direct or proximal cell-cell contact.

Similar to the patterned SVZ (Merkle et al., 2014), there are implications that there is a longitudinal regionalization of the SGZ, topographically separating dorsal and ventral blade of the DG (Kheirbek and Hen, 2011). Although no significant differences in dividing cells can be observed in the dorsal and ventral blade, the number of Type-1 cells seems to be less in the ventral blade as compared to the dorsal. Alongside the number of Doublecortin+ (Dcx+) neuroblasts in the dorsal blade is increased. Furthermore, the neuroblasts present in the ventral blade express less Calretinin (CR), a marker of immature granule cells (Jinno, 2011). This asymmetric density in hippocampal neurogenesis might affect the strength of the feedback loops generated by the nearby progeny (Snyder et al., 2009).

In the SGZ, just as the SVZ, the NSCs are in close contact with their progeny. One major difference of the two niches is that in the SGZ the neuroblasts and newborn neurons do not migrate out of the niche area. Thus, the regulation of NSCs by axonal inputs will be impacted additionally by a feedback of the newly generated neurons. Interneurons in the DG are critical niche components, coupling neuronal circuit activity to quiescent NSCs. The activation of NSCs is increased when the local circuit activity is low. Upon increase in activity of the circuit NSCs are maintained quiescent (Song et al., 2012). This implicates that neurogenesis can be impacted long-lasting if newly generated neurons are integrated wrongly, potentially causing pathological changes to the system.

Adult Neurogenesis Contributes During Aging and Pathologies

Various pathological conditions are associated with either an upregulation or a downregulation of adult neurogenesis (Abrous et al., 2005; Kempermann et al., 2015). A few pathologies associated with the downregulation of neurogenesis are depression (Bremner et al., 1995; Gurvits et al., 1996; MacQueen et al., 2003), schizophrenia (Heckers, 2001; Schmajuk, 2001), drug addiction (Koob and Le Moal, 2001; Nestler, 1997) as well as ageing and dementia (Ben Abdallah et al., 2010;

Lugert et al., 2010). On the other hand, diseases associated with up regulation of neurogenesis are epilepsy (Parent et al., 1997; Scott et al., 1998), ischemia (Kee et al., 2001; Liu et al., 1998; Zhang et al., 2004), Huntington's disease (Curtis et al., 2003; Eriksson et al., 1998), various traumatic brain injuries (Dash et al., 2001; Lu et al., 2003; Rice et al., 2003) as well as specific types of tumors (Giachino et al., 2015). Whether the change in neurogenesis is causative or a consequence of the pathologies depends on the individual disorder and very often it is not known.

Age-Related Decrease of Adult Neurogenesis

Neurogenesis diminishes with age. The age-related decline in neurogenesis might be a result of decreased activity of NSCs, and potentially quiescence. Decreased levels of proliferating stem cells in the hippocampus are associated with impaired aspects of learning and memory. Ageing is associated with a 6-fold decrease in the number of neurons generated in the adult murine brain. Conversely, exercise elicits beneficial effects on the aged brain, and affects NSC function increasing the number of newborn neurons some 3-fold (van Praag et al., 1999b). Exercise-induced increases in neurogenesis correlate with a better performance of mice in spatial learning (Creer et al., 2010) and memory tasks (van Praag et al., 1999a). These results are supported by studies in humans.

In a large-scale investigation, 631 individuals between the ages of 60 and 77 years underwent a 2-year multi-domain intervention, consisting of a change in diet, physical exercise, cognitive training and vascular risk monitoring. The physically active participants in the study performed significantly better than controls (n=629) with regards to working memory, task flexibility, problem solving and planning as well as processing speed (Hawkins et al., 1992; Ngandu et al., 2015). Thus, the neuroplasticity caused by neurogenesis itself is crucial for certain forms of learning and memory in the murine brain (Zhao et al., 2008) as well as the human brain (Ngandu et al., 2015).

Neurogenesis and Mood Related Disorders

In the brains of depressed patients monoamines, such as 5-hydroxytryptamine (5-HT, Serotonin), have a tendency to be reduced. Conventional antidepressants enhance the 5-HT transmission, for example by inhibiting the reuptake of the neurotransmitter. Problematically, a decrease in 5-HT does not immediately cause major depression (Mahar et al., 2014) and the administration of drugs, which increase 5-HT levels rapidly after administration, are not sufficient for depressive

amelioration immediately after intake (Hasler, 2010). These observations indicate that long-term mechanisms are involved in major depression. The cause for 5-HT impairment in patients suffering from depression has intrinsic, for example genetics and gender, but also extrinsic, for example drug use or stress, factors. Stress is being viewed as one of the most potent factors for developing major depression. Chronic stress has been shown to negatively regulate adult neurogenesis in the DG. Brain images of patients with major depression have shown hippocampal atrophy (Bremner et al., 1995). Decreased neurogenesis seems to underlie symptoms of depression (Kempermann, 2002). The high neuronal turnover in humans in the hippocampus supports the possibility that hippocampal neurogenesis can be causative in depression and/or the response to stress or antidepressants (Spalding et al., 2013). Hippocampal neurogenesis is regulated by monoamines (Diaz et al., 2012) and neurotrophic factors (Waterhouse et al., 2012) and chronic antidepressant treatment increases neurogenesis (Dranovsky and Hen, 2006). The selective 5-HT reuptake inhibitor Fluoxetine has been tested for its effects on both the SVZ (Tong et al., 2014b) and the SGZ (Encinas et al., 2006). NSCs seem to be in close proximity to serotonergic axons. In both neurogenic niches the antidepressant causes an increase in symmetric divisions of early progenitor cells.

Aberrant Neurogenesis and Epilepsy

While exercise is associated with a healthy increase in DG neurons, epileptic seizures (SE) are associated with a pathological increase. It is known that epilepsy stimulates proliferation in the DG (Parent, 2007). The DG responds shortly after SE with an increased cell proliferation in the subgranular zone (Parent et al., 1997). Seizures increase the activation of quiescent cells, recruiting them into an active state (Lugert et al., 2010). Upon SE abnormal mossy fiber sprouting and abnormal basal dendrite development, as well as migration of dentate granule cells are observed (Jessberger et al., 2007a). This abnormal integration might cause an imbalance in inhibition. Making abnormal neurogenesis the potential cause for epileptogenesis, leading to reoccurring, acute seizures (Di Maio, 2014; Pierce et al., 2005). In acute seizures this precocious NSC activation comes at an expense of long-term exhaustion for short-term plasticity (Sierra et al., 2015).

Adult NSCs and Tumor Biology

The idea that tumors contain a rare subset of stem-like cells capable of self-renewal, indefinite division and differentiation is gaining acceptance (Pierfelice et al., 2008) – this hypothesis is called the cancer-stem-cell theory. Adult neurogenesis

implicates the presence of undifferentiated, active stem and progenitor cells. Disruption of the regulatory mechanism either of the SCs or the rapidly dividing daughter cells is probably one cause for the formation of cancer initiating stem-like cells (Reya et al., 2001) also in the brain. This was underlined when neurosphere forming precursors with characteristic NSCs genes, such as Sox2, Musashi (Hemmati et al., 2003) were obtained from a human glioblastoma biopsy (Ignatova et al., 2002) and a human medulloblastoma (Singh et al., 2003). It appears as though the tumor initiating cells with neural precursor features respond to the same mitogens, possess some of the molecular features and seem to express similar markers as adult NSCs (Tamaki et al., 2002). Many tumors develop near the neurogenic SVZ indicating that they might derive from transformed undifferentiated precursor cells (Sanai et al., 2005).

Recently it was shown that NSCs in the SVZ with deleted p53, a cell cycle control gene, and deleted Rbpj, the Notch signaling mediator, form tumors in the brain. Loss of proper NSC maintenance and additionally the cell cycle disturbance leads to the formation of brain tumors (Giachino et al., 2015), highlighting the essentiality of temporospatial proper NSC maintenance.

Stem Cell Maintenance

Deregulation of NSC maintenance can lead to an early exhaustion of the NSC pool or worse, as previously highlighted, in various pathologies. Thus, adult NSCs are tightly regulated and controlled in order to achieve proper physiological functioning and maintenance. Three crucial features characterize proper maintenance: proper self-renewal, controlled fate determination and preservation of stemness.

Self-renewal depends on a cells capacity to undergo either symmetric or asymmetric cell division. While a symmetric cell division gives rise to two identical daughter cells, asymmetric division produces an exact copy of itself and a distinct daughter cell that will eventually terminally differentiate (Gotz and Huttner, 2005). Fate determination of stem and progenitor cells is subject to intrinsic and extrinsic factors. Besides the presence of a receptors on the cell surface (intrinsic) also the presence, timing and concentration of the extrinsic ligand will influence the cellular response (Fuchs, 2004). Stemness is preserved by the specific factors provided by the niche. These are local and environmental factors such as cytokines, growth factors, adhesion and signaling molecules, which are crucial for proper NSC

functioning and maintenance (Conover and Notti, 2008). There are multiple factors known to orchestrate these maintenance tasks. The best studied in terms of adult neurogenesis and adult NSCs maintenance are Shh, (Fuccillo et al., 2006), Wnt, (Zechner et al., 2003) and Notch signaling (Ables et al., 2011). These three pathways are implicated in regulating adult neurogenesis and potentially even crosstalk.

Shh has been implicated in adult neurogenesis and is important in stem cell proliferation and progenitor specification (Alvarez-Buylla and Ihrie, 2014). Shh signaling functions via a surface receptor complex consisting of Patched (Ptc) and its G-protein-coupled co-receptor Smoothened (Smo). Ptc inhibits signal transduction of Smo in the absence of Shh. Once Shh binds Ptc, Smo is disinhibited, leading to the activation of the Shh signaling cascade. This results in the disinhibition of Gli2/3. Gli 2/3 then function as transcription factors whose nuclear-cytoplasmic distribution is regulated via a protein-protein interaction with suppressor of fused (Su(Fu)) (Kogerman et al., 1999). Activation of proper Shh cascade leads to the transcription of further Gli-proteins (Gli1/7) and other Shh target genes (Philipp and Caron, 2009). Some known target genes of Shh signaling in the brain are Nkx2.2, Pax6 and Ptc1 (Shahi et al., 2010). Genetic manipulation of the Shh signaling cascade via deletion of Ptc leads to an increase of NSC divisions and symmetric NSC divisions in adult neurogenesis in the SVZ (Ferent et al., 2014).

Wnt signaling is highly conserved and has been implicated in CNS development and NSC differentiation (Zechner et al., 2003). In the absence of Wnt, Glycogen-Synthetase-kinase-3 (GSK3) is forming a complex with Axin and other cofactors. This complex ultimately phosphorylates and ubiquitinates β-catenin, thus keeping a low β-catenin level in the cell. Once Wnt is binding Frizzled receptor a tertiary complex with Lrp6 is formed. Axin is recruited to the intracellular domain of Lrp6, sequestering GSK-3 away and β-catenin is no longer tagged for degradation, thus accumulates and can migrate into the nucleus where it is acting as a transcription factor (Komiya and Habas, 2008) regulating for example the expression of Neurogenin1 (Hirabayashi et al., 2004), Six3 (Braun et al., 2003) and NeuroD1 (Kuwabara et al., 2009). Wnt signaling has mostly been proposed in proliferation and differentiation of neuronal progenitor cells. It has been shown that NeuroD1, a proneurogenic transcription factor, is a downstream mediator of Wnt-induced neurogenesis (Kuwabara et al., 2009). Inhibition of Wnt signaling via the secretion of Dickkopf or Secreted Frizzled-related Protein 3 in the adult SGZ has been implicated in downregulation of adult NSC proliferation and neuronal maturation. Interestingly,

Dickkopf expression is naturally increased with age, implicating a role of Wnt signaling in stem cell quiescence with progressed age (Wu and Hen, 2013)

The third, crucial signaling pathway is Notch signaling. The remainder of this work will be focusing on the Notch signaling pathway and the role of Notch in NSC maintenance in the adult murine brain.

Notch Signaling: a Summary of History

In 1914 John S. Dexter noticed a “notched” phenotype in the wings of Drosophila melanogaster. The responsible allele was then found by T.H. Morgan’s group in 1917 and through to the cloning of the gene in the 1980s (Artavanis-Tsakonas, 1983), the Notch family members are now recognized as essential signaling molecules that control a diverse array of cellular responses ranging from normal development to the maintenance of homeostasis in metazoans. Notch signaling components are evolutionarily conserved in all metazoan organisms - with a single receptor present in Drosophila, two in C. elegans and four in mammals (Kopan and Ilagan, 2009).

The Notch signaling pathway, compared to Shh and Wnt signaling, is highly dependent on direct cell-cell interactions and niche architecture. Notch signaling affects a wide range of cellular processes (Andersson et al., 2011) both during development (Artavanis-Tsakonas et al., 1999; Harper et al., 2003) and adulthood including stem cell maintenance (Borggrefe and Oswald, 2009; Koch et al., 2013), cell proliferation (Androutsellis-Theotokis et al., 2006), differentiation (Bigas and Espinosa, 2012; Gaiano and Fishell, 2002) and apoptosis (Gotte et al., 2011).

Notch Receptors and Ligands

The four mammalian Notch receptors (Notch1-Notch4) reside on the cell surface as non-covalently linked heterodimers (HD) and are Type I transmembrane receptors (Figure 8A, adapted from Mumm and Kopan 2000). They are comprised of an extracellular domain, which functions as receiver and an intracellular domain which functions as sender of signal information. The Notch extracellular part is comprised of numerous EGF-like repeats. The extracellular EGF-like repeats contain Thr/Ser amino-acid residues that prone for Fringe-mediated O-glycosylation. These sugar modifications are proposed to modulate signaling outcome influencing the interactions of different ligands (Takeuchi and Haltiwanger, 2014).

The extracellular and intracellular parts of Notch are combined at the heterodimerization (HD) domain. Two cleavage sites (S1 and S2) are found within

the HD domain (Mumm and Kopan, 2000). In order for all four Notch receptors to become mature, they need to be cleaved at the S1 site in the Golgi before integration into the membrane. The S2 extracellular cleavage, mediated by the metalloprotease Adam10 under physiological conditions (Alabi et al., 2016), and the intracellular S3, mediated by γ-secretase, cleavages are needed for proper signaling (Figure 8B, adapted from Mumm and Kopan 2000).

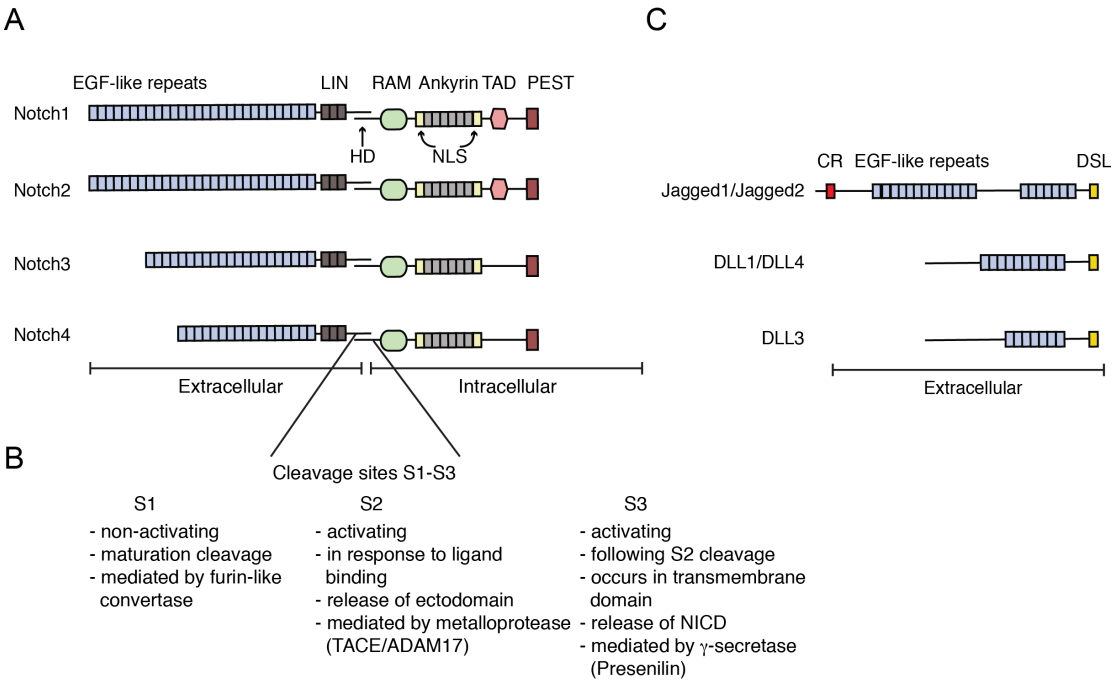

Figure 8 Notch receptors and ligands; Notch receptors are heterodimers with an extracellular and an intracellular domain. There are four Notch paralogues (Notch1-4) **(A)**. Notch receptors undergo three cleavages (S1-S3). S1 is a non-activating maturation cleavage occurring in the Golgi. S2 and S3 are activating cleavages necessary for canonical Notch signaling **(B)**. Notch ligands are composed of a large extracellular domain rich in EGF-repeats that interact with the extracellular domain of the Notch receptor. Upon interaction of ligand and receptor, the receptor undergoes a conformational change making the S2 cleavage site available. There are five Notch ligands, Jagged1/Jagged2, Dll1, Dll4 and Dll3 **(C)**.

The intracellular domains of all Notch paralogues contain an Rbpj associated molecule (RAM) domain, the nuclear localization signal (NLS), multiple Ankyrin (ANK) domains and the Proline-Glutamate-Serine-Threonine rich domain (PEST). Notch1 and Notch2 additionally contain a carboxy-terminal transactivation domain (TAD). The RAM domain is crucial for interaction with several cytosolic and nuclear proteins, including Rbpj, the transcriptional mediator of Notch signaling. The Ankyrin domain is important for further protein-protein interactions. The composition of

modulator proteins bound at the RAM and ANK domains lead to the formation of the Notch nuclear transcription complex. The TAD domain is important for transcriptional activation. The PEST domain, the most C-terminal component of the Notch protein, is essential in the regulation of Notch degradation (Kurooka et al., 1998) (Figure 8, adapted from Mumm and Kopan, 2000). Mutations in the HD, RAM, ANK or PEST domain can lead to severe phenotypes. Mutations in the HD domain can cause a ligand-independent activation of Notch receptors. Mutations in the RAM or ANK domain can cause improper or block of binding to the interaction partners (Mumm and Kopan, 2000). Mutations in the PEST domain can lead to an incorrect inactivation, and thus Notch signaling is prolonged (Chillakuri et al., 2012).

The two most related mammalian Notch paralogues are Notch1 and Notch2. These two are well characterized both genetically and functionally and share many structural features (Weinmaster et al., 1992). The extracellular domains of Notch1 and Notch2 have 57% amino acid conservation the intracellular 53%. It is worth to mention that the intracellular PEST and TAD domains are only 37%, while the ANK domain is 85% conserved at the amino acid level (Liu et al., 2015b).

The ligands of Notch signaling are receptors on the juxtapose cells. There are five mammalian Notch ligands: Jagged1, Jagged2, Delta-like 1 (DLL1), DLL3 and DLL4. These are Type I transmembrane ligands, and they provide short-range signals between directly opposed cells. The ligands possess a Delta/Serrate/LAG-2 (DSL) motif on their N-terminus as well as tandem EGF-repeats (Figure 8C, adapted from Mumm and Kopan 2000). The EGF-repeat regions mediate the short-range interaction of Notch and its ligands. The specificity is then ensured by O-Glycosylation, mediated by POFUT1 and Fringe, and by regulation of the availability of ligand and receptor in a temporospatial manner on the cell surfaces. Once short-range interaction of Notch and of its ligands, Delta, or Jagged occurs, the canonical Notch signaling pathway is activated.

The Notch Signaling Cascade

In the absence of Notch ligands, the receptor is not cleaved at the S2 and S3 sites and Rbpj, the nuclear mediator of Notch signaling in the nucleus is bound to Corepressors (CoR) and histone deacetylases (HDAc) at target genes (Figure 9-1). The transcriptional program in NSCs, in the absence of active Notch signaling, can be described as proneural, the NSCs are not maintained and potentially differentiate. In order to maintain NSCs, the Notch receptor needs to interact with one of its ligands, be activated and transduce a transcriptional signal to the nucleus.

Canonical Notch signaling is initiated by short-range signals between directly opposed cells (Figure 9–2a). Notch proteins and cell bound Notch ligands (DLL, Jagged) interact causing a conformational change, exposing the S2 cleavage site to a metalloprotease family (ADAM) (Figure 9-2b). The proteolytic release of the Notch extracellular domain leaves the Notch receptor truncated and exposed to intracellular γ-secretase mediated S3-cleavage (Figure 9-2c), which releases the Notch intracellular domain into the cytoplasm (Figure 9-2d). This active intracellular domain traverses to the nucleus and interacts with Rbpj. Upon interaction the nuclear Rbpj-complex the complex composition is changed (Figure 9-2e). CoR and HDAc are exchanged for Coactivators (CoA) and Histone acetyltransferases (HAcT). This change of the complex leads to the transcription of downstream Notch target genes, switching the function of Rbpj from a repressor to a transcriptional activator. Rbpj/NICD transcriptional complex activates a set of basic helix-loop-helix transcriptional repressors (Mumm and Kopan 2000).

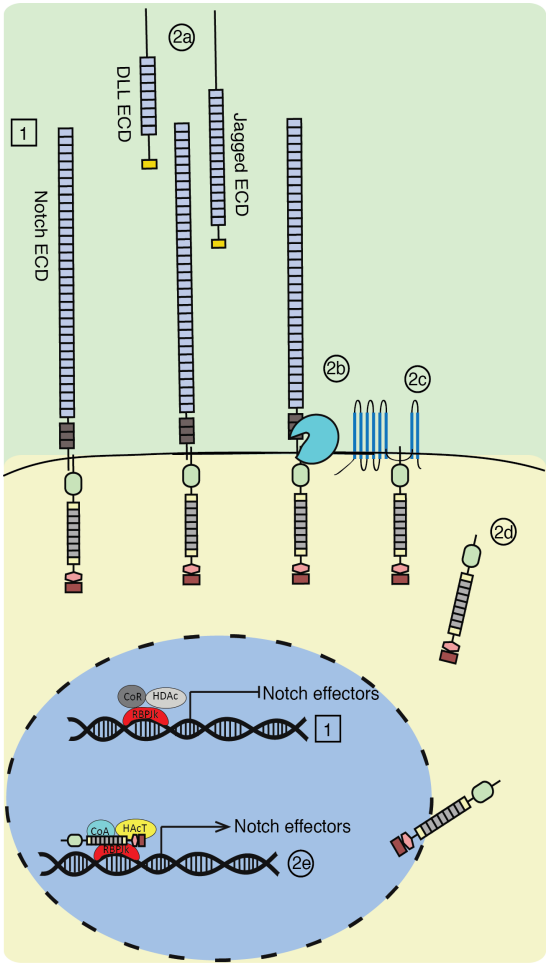

Figure 9: Canonical Notch signaling cascade; In the absence of ligand the Notch receptor is integrated as Type-I receptor in the membrane. In the absence of ligand, the nuclear Rbpj complex is bound to Corepressors (CoR) and Histone deacetylases (HDAc) (1). In the presence of ligand the Notch extracellular domain and the ligand extracellular domain interact, leading to a conformational change of the Notch receptor (2a). This conformational change leads to the exposure of the S2 site and a consecutive cleavage by a metalloprotease (2b). After the S2 cleavage the S3 cleavage site becomes available to a γ-secretase (2c). This cleavage releases the Notch intracellular domain (NICD) into the lumen (2d). The NICD will migrate into the nucleus where it can interact with Rbpj. The binding of NICD leads to the recruitment of members of the activated complex, exchanging the CoR through a Coactivator (CoA) and the HDAc with a Histone acetyltransferase (HAcT) resulting in transcription of Notch effector genes (2e).

This conserved cascade is repeatedly used in multiple developmental processes. The pathway appears simple, without any second messengers or apparent cytosolic interactions with a binary decision. However, Notch receptors and ligands are influenced by a broad spectrum of posttranslational modifications. Therefore, Notch signaling can drive numerous mechanisms, such as stem cell differentiation and maintenance both in the embryo and the adult (Koch et al., 2013).

Notch signaling is context and tissue dependent. In muscle stem cells, Notch has been implicated in maintenance of stem cells, self-renewal of progenitor cells and inhibition of terminal differentiation (Brack and Rando, 2012). In the intestine, Notch signaling is active in the intestinal stem cells and regulates their proliferation and the terminal differentiation (Barker et al., 2007). In the bone marrow HSCs, Notch does not seem to be essential for physiological HSC maintenance, however constitutive expression can lead to an expansion of these cells (Bigas and Espinosa, 2012). In NSCs Notch has been implicated in NSCs maintenance (Basak et al., 2012; Ehm et al., 2010; Imayoshi et al., 2010), inhibition of neuronal differentiation and even terminal differentiation into an astrocyte lineage (Gaiano and Fishell, 2002).

Notch signaling in Neural Stem Cells

Accumulating evidence underlines the importance of Notch signaling in NSC maintenance, differentiation and fate choice (Artavanis-Tsakonas et al., 1999). The dependence of NSCs on Notch signaling becomes evident when Rbpj, the downstream mediator of Notch signaling, is deleted specifically from NSCs in the adult murine brain. The NSCs are no longer maintained properly, this leads to an initial activation of the stem cell pool and an expansion of the progenitor population, however in the long run caused a depletion of the quiescent and active NSCs from the SVZ (Imayoshi et al., 2010). Interestingly when Notch1 was deleted from the same SC population only the active NSCs were affected (Basak et al., 2012). In Zebrafish, a similar observation was made - Notch signaling levels are crucial for maintenance of quiescent NSCs and recruitment to activity (Chapouton et al., 2010b). In a follow-up analysis it was shown that Notch1 is dispensable in the maintenance of quiescent NSCs also in Zebrafish, however Notch3 is required (Alunni et al., 2013).

When looking at the expression levels of Notch on NSCs in the SVZ, it appears as though the Notch paralogues Notch1 and Notch2 are coexpressed on all cells of the neurogenic lineage (Basak et al., 2012). Interestingly, Notch signaling is only

active, as determined by expression of Notch effector genes, in NSCs and TAPs (Giachino et al., 2014b). Two very prominent direct Notch target and effector genes, crucial for NSC maintenance are hairy-enhancer-of-split (Hes) and brain lipid binding protein (BLBP) genes. Hes genes are basic helix-loop-helix (bHLH) genes and essential effectors of Notch signaling for maintaining undifferentiated cells (Artavanis-Tsakonas et al., 1999; Gaiano and Fishell, 2002). The single deletion of either Hes1 or Hes5 has no apparent defects in embryonic development, thus illustrating a compensatory mechanism. Parts of this compensation might come from different upstream regulators, such as BMP4 (Kageyama et al., 2007). However, the double deletion causes severe phenotypes leading to disorganization of the neural tube, premature neuronal differentiation and loss of radial glia in the embryo (Hatakeyama et al., 2004).

Another well-known direct Notch target gene is BLBP. BLBP is broadly expressed throughout the brain of the embryo to the adult. It is proposed to be involved in neuronal–glial signaling. Antibody blocking experiments have shown that BLBP is required for NSC morphological changes in response to neuronal cues in the embryo (Anton et al., 1997) and loss of BLBP in the adult leads to precocious differentiation and loss of the adult NSCs (Matsumata et al., 2012).

Although the role of Notch signaling in NSCs is widely accepted as crucial, the distinct role of the Notch paralogues in maintenance of quiescent and active NSCs is only poorly understood. The goal of this thesis thus was to investigate the role of Notch signaling in balancing between adult NSC quiescence and heterogeneity.

Questions and Aims

Multiple lines of evidence indicate that Notch1 and Notch2 might have redundant biological functions in certain cellular, developmental or disease context (Liu et al., 2015b), however, have distinct functions in other contexts (Boulay et al., 2007; Chu et al., 2011; Kumano et al., 2003). In the intestine (Riccio et al., 2008) Notch1 and Notch2 seem to have redundant functions. In contrast, Notch1 and Notch2 have different roles in the commitment and lineage differentiation of the olfactory epithelium during development (Carson et al., 2006) and rather Notch1 than Notch2 is required for differentiation in the cerebellum (Lütolf, 2002). Also in adult NSCs a discrepancy between the deletion of the mediator of Notch signaling Rbpj (Imayoshi et al., 2010) and loss of Notch1 (Basak et al., 2012) have been observed. Notch1 maintains NSCs in their active state (Basak et al., 2012) whereas Rbpj is needed for maintenance of all, quiescent and active, NSCs.

What is the role of Notch in NSC quiescence and activity?

The factors maintaining quiescent NSCs are only sparsely understood. In order to assess the maintenance signals involved in quiescent NSCs we have analyzed the effects of active and quiescent NSCs upon the loss of Notch signaling components. We have been able to show that Notch2 is important in quiescent NSC maintenance in the SVZ and also in the dorsal medial wall (dMW), a non-neurogenic region under physiological conditions (Engler et al.; in preparation a). The SGZ contains NSCs albeit to a lesser extend than the SVZ.

What is the function of Notch2 in the SGZ and SVZ?

To address the importance of Notch2 in a known system, we have analyzed the effects of loss of Notch2 also in the SGZ of the DG (Zhang et al, in preparation). We showed that both the SVZ and the SGZ contain quiescent and active NSCs that are Notch dependent. Thus, in both niches Notch1 and Notch2 are coexpressed on the two NSC types. Previous studies form our lab (Giachino et al., 2014b) have shown the high level of heterogeneity within the stem cell populations in the SVZ niche. Therefore, we wanted to address the individual functions of quiescent and active NSCs in physiological and pathological conditions.

What is the level of response to physiological and pathological stimuli of quiescent and active NSCs?

We addressed the physiological properties of NSCs using Notch signaling reporter mice, *Hes5::GFP, BLBP::mCherry* (Giachino et al., 2014b). We characterized the lineage in great detail and illustrated the high complexity and heterogeneity of the SGZ (Engler et al, in preparation b). We have analyzed the NSCs' capacity to respond to seizures, antidepressant treatment and ageing in the SGZ of the DG. We distinguished the different subpopulations of NSCs and TAPs, which gave rise to the observed pathophysiological phenotypes.

Results

Neurogenic Stem Cells in a Dormant Niche are Activated by Antidepressant Fluoxetine and Suppressed by Notch2 Signaling

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Contribution: I planned and analyzed all the experiments, prepared the figures and the manuscript. The Rbpj trace was contributed by PB & EN, and the Notch2-CreER$^{T2\text{-}SAT}$ animals were provided by IS, AL & SAT.

Summary

Active Notch signaling maintains the NSC state thereby preventing neurogenesis. The loss of Rbpj, the downstream mediator of all Notch signaling, leads to the loss of active and quiescent NSCs (Imayoshi et al., 2010). The loss of Notch1 on the other hand only leads to the loss of active NSCs (Basak et al., 2012). This implies that Notch signaling is crucial for both promoting of proliferation and quiescence, however Notch1 in particular might be dispensable during quiescence. However, the nature of Notch quiescence signal is unknown. We hypothesize that another member of the Notch family provides the maintenance signal or Notch receptors have an intrinsic redundancy. We demonstrated, using a uniform, combinatorial, conditional knockout approach, that the deletion of Notch2 from adult NSCs causes the activation of quiescent NSCs and therefore an increase in proliferation in all neurogenic niches. We recapitulated the previously observed Notch1 phenotype, the loss of active NSCs, but not quiescent NSCs. Loss of Notch1 and Notch2 phenocopied the loss of Rbpj, implicating that these two Notch family members are the main players in NSC maintenance in the adult murine brain.

Surprisingly, loss of Notch2 leads to the appearance of neuroblasts in an otherwise non-neurogenic region, the dorsal medial wall of the subventricular zone, lining the lateral septum. We found that this particular area is a source of quiescent stem cells with a latent neurogenic potential activated by Notch2 deletion. Interestingly enough, these quiescent NSCs, with intact Notch2 signal, can also respond to Fluoxetine treatment.

Manuscript

Neurogenic Stem Cells in a Dormant Niche are Activated by Antidepressant Fluoxetine and Suppressed by Notch2 Signaling

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Short Title: Notch2 maintains Quiescent Neural Stem Cells

Key Words: quiescence, neural stem cells, stem cell niche, Notch2, neurogenesis

SUMMARY

Age-associated declines in tissue homeostasis and regeneration correlate with reduced stem cell activity. In most regions of the mammalian brain, neuron production stops soon after birth. Here, we find that the adult brain contains *bona fide* neural stem cells (NSCs) outside the classical neurogenic zones and identify a novel population of NSCs in their niche, the dorsal septum. Resident septal NSCs are held in a dormant state but retain neurogenic potential, responding to antidepressants to generate new neurons *in vivo*. Notch2 but not Notch1 signaling conveys quiescence to these stem cells and their subventricular zone counterparts, repressing cell cycle-related genes and neurogenesis. Loss of Notch2 activates quiescent NSCs to proliferate and generate new neurons. Thus, NSCs outside the classic germinal zones of the brain are held in a reversible, inactive state by Notch2 signals.

HIGHLIGHTS

- The mammalian brain contains dormant stem cells outside the normal neurogenic niches
- Notch1 Notch2 double knock-out phenocopies Rbpj knock-out
- Notch2 induces NSC quiescence, Notch1 promotes maintenance of activated NSCs
- Dormant septal NSCs are activated by antidepressants

eTOC

In Brief

Using a combinatorial knockout approach Engler and colleagues systematically analyzed Notch signaling mutants. Their study showed the role of Notch2 in maintenance of quiescent NSCs in the adult murine brain not only in known neurogenic zones but also in non-neurogenic regions of the brain.

INTRODUCTION

Somatic stem cells in adult tissues are the source of cells for regeneration and repair (Li and Clevers, 2010). Adult somatic stem cells are regulated by their specialized niches, which control maintenance, activation and production of differentiated progeny (Cheung and Rando, 2013). Many tissues contain stem cells that divide infrequently and are thus mitotically quiescent (Li and Clevers, 2010). Stem cell quiescence preserves longevity of the progenitor pool, protects against acquisition and propagation of genetic mutations and counteracts hyperplasia and tumor formation. However, the interplay between signals that control quiescence and activation are not fully understood (Cheung and Rando, 2013). Radial glial stem cells produce most neurons and glia of the brain during embryonic development and temporospatial specification dictates their fate (Fuentealba et al., 2015; Furutachi et al., 2015; Greig et al., 2013; Malatesta et al., 2003; Merkle et al., 2007; Noctor et al., 2001). Towards the end of embryogenesis, neurogenesis ceases at most locations in the brain. It is unclear why, but it is thought that NSCs in these regions become exhausted and are lost. Prime exceptions are the ventricular-subventricular zone of the lateral ventricle walls (SVZ) and the subgranular zone of the hippocampal dentate gyrus where radial glia in the primordium generate adult NSCs that remain active and drive neurogenesis in rodents, non-human primates and humans into adulthood (Doetsch, 2003; Doetsch et al., 1999; Ernst et al., 2014; Fuentealba et al., 2015; Furutachi et al., 2015; Spalding et al., 2013). Adult NSCs (also known as B1-cells) in the SVZ intercalate between ependymal cells lining the lateral ventricle and extend radial processes that can contact blood vessels (Fuentealba et al., 2012; Mirzadeh et al., 2008). B1-cells are mitotically quiescent, sporadically enter cell division to generate C-cells, a transient and highly mitotic population that gives rise to neuroblasts (A-cells) (Fuentealba et al., 2012; Ihrie and Alvarez-Buylla, 2011). Neuroblasts generated in the SVZ migrate to the olfactory bulb and differentiate into different interneuron subtypes (Kirschenbaum et al., 1999; Lois et al., 1996). Within the neurogenic zones, NSCs may become dormant or are lost in aged animals resulting in a drastic reduction in neurogenic and regenerative potential (Giachino et al., 2014b; Shook et al., 2012).

Adult NSCs rely on Notch signaling, which regulates their maintenance and differentiation (Basak et al., 2012; Ehm et al., 2010; Giachino et al., 2014b; Imayoshi et al., 2010; Lugert et al., 2010; Nyfeler et al., 2005). Mammals have four Notch paralogues that regulate target gene expression, including those encoding the HES and HEY transcription factors (Hatakeyama et al., 2004; Zhu and Zhou, 2006). Adult NSCs can be isolated and genetically labeled using *Hes5::GFP* and *Hes5::CreER^{T2}* alleles (Giachino et al., 2014b; Lugert et al., 2010; Lugert et al., 2012). Deletion of *Rbpj*, which encodes the canonical transcriptional regulator of the Notch pathway, activates quiescent NSCs, blocks self-renewal and results in a collapse of neurogenesis (Basak et al., 2012; Imayoshi et al., 2010). Conversely, Notch1 regulates

maintenance and self-renewal of active NSCs but is dispensable during quiescence implying functional compensation by other Notch family members (Basak et al., 2012). We addressed how Notch signaling regulates quiescent NSCs by combinatorial conditional knockout (cKO) of Notch receptor genes. We deleted *Notch1* and *Notch2* from *Hes5::CreER*^{T2} expressing stem cells in the adult mouse and analyzed the forebrain. Our findings revealed that combinatorial cKO of *Notch1* and *Notch2* phenocopies a total loss of canonical Notch signaling in the forebrain and that Notch2 specifically regulates adult NSC quiescence. The loss of Notch2 function uncovered latent NSCs in the septal medial wall of the lateral ventricle. Septal NSCs activate and generate neuroblasts in response to loss of Rbpj, Notch2 and treatment with the antidepressant and selective serotonin reuptake inhibitor (SSRI) Fluoxetine. Thus, inactive stem cells in non-neurogenic regions of the brain can remain neurogenic and respond to selective signals *in vivo*.

RESULTS

Distinct functions of Notch paralogues in SVZ NSC

Notch signaling regulates SVZ NSC maintenance and cell fate (Androutsellis-Theotokis et al., 2006; Basak et al., 2012; Giachino et al., 2014b; Imayoshi et al., 2010). Both, conditional inactivation of *Rbpj*, to block canonical Notch signals, and inhibition of gamma-secretase, to block Notch activation, affect NSC activation and maintenance (Chapouton et al., 2010b; Imayoshi et al., 2010). In contrast, conditional deletion of *Notch1* results in a loss of active NSCs in the SVZ due to defective maintenance of self-renewal but does not affect quiescent NSCs (Basak et al., 2012). Notch1 and Notch2 expression overlaps in NSCs suggesting a potential functional redundancy in the quiescent NSC population (Basak et al., 2012). To date, overlapping, redundant versus specific functions for Notch receptors in the maintenance of neurogenic stem cells of the adult forebrain have not been addressed. The role of Notch receptors and Notch signaling is a major question in brain homeostasis and repair.

We took a combinatorial conditional gene knockout (cKO) approach in order to study the mechanisms controlling adult neurogenesis and unravel the role of Notch receptors in regulating adult forebrain NSCs (Figure 1A). We generated mutant mice deleting *Notch* receptors or *Rbpj* from *Hes5::CreER*$^{T2+}$ NSCs, and followed cell autonomous changes in the fate of the deleted stem/progenitor cells and their progeny with *Rosa26R::GFP* (GFP^{+}) (Figure 1A). GFP^{+} cells in the SVZ of Notch2 cKO animals were negative for Notch2 protein, whereas GFP^{-} cells still expressed Notch2 (Figure S1A). Acute ablation of *Notch2* (2-days post-Tamoxifen (TAM) treatment) resulted in an increase in proliferating (PCNA^{+}) *Hes5*-derived (GFP^{+}) GFAP^{+} NSCs without affecting the total number of progeny (GFP^{+}) akin to the deletion of *Rbpj* (Figure 1B and S1A-C) (Basak et al., 2012; Imayoshi et al., 2010). A similar increase in NSC proliferation was also observed following simultaneous deletion of *Notch1* and *Notch2* (Figure 1B and S1C). In line with previously published data, *Notch1* cKO reduced the number of GFP^{+} progeny (Figure S1B) without affecting proliferation of GFAP^{+} putative NSCs (Figure 1B and S1C) (Basak et al., 2012).

Although the number of GFP^{+}PCNA^{+}GFAP^{+} cells increased after gene deletion, the total density and number of GFP^{+}GFAP^{+} cells and overall proliferation (GFP^{+}PCNA^{+}) were not changed in the SVZ of any of the cKO mutants following a 2-day chase (Figure S1C). However, neuroblast production (GFP^{+}DCX^{+}) was increased specifically in the *Notch1Notch2* cKO and *Rbpj* cKO animals (Figure S1C). Thus, simultaneous cKO of *Notch1* and *Notch2* from NSCs in the SVZ had similar effects on proliferation and differentiation as the total loss of canonical Notch signaling (*Rbpj* cKO). In the single mutants, *Notch2*

cKO GFP^+GFAP^+ cells displayed an increased propensity to enter cell cycle ($GFP^+GFAP^+PCNA^+$) (Figure 1B).

Proliferation in the SVZ (GFP^+PCNA^+) of *Notch2*, *Notch1Notch2* and *Rbpj* cKO animals increased by 21-days post-TAM, as did the generation of neuroblasts (GFP^+DCX^+) (Figure 1C, D). In addition, *Notch1Notch2* cKO animals started to display a decrease in GFP^+GFAP^+ cells suggesting NSC loss (Figure S1D). Again, and in striking contrast, neurogenesis in the *Notch1* cKO mice was not induced compared to control animals showing a trend to reduction (Figure 1D, S1C, D) (Basak et al., 2012). Thus, although they have overlapping expression, Notch1 and Notch2 seem to play distinct roles in regulating neurogenic stem cells of the SVZ. However, *Notch1Notch2* cKO reveals that both receptors convey their signals and functions through Rbpj.

Loss of Notch2 leads to enhanced neuroblast production

Most NSCs of the SVZ are in a quiescent state and enter cell cycle when they initiate neuron production. The age-related decline in neurogenesis may be linked to stem cell exhaustion. We examined the SVZ 100- and 300-days after gene ablation. At 100-days, the number of *Hes5*-derived cells (GFP^+) were comparable between *Notch1* and *Notch2* cKO animals but the total number of GFP^+ progeny was reduced in the *Notch1Notch2* and *Rbpj* cKO mice (Figure 2A). The number of GFP^+GFAP^+ putative quiescent NSCs was not affected in the *Notch1* cKO (Figure S2A). Ablation of *Notch1, Notch1Notch2* and *Rbpj* caused a decrease in neuroblast production (GFP^+DCX^+; Figure 2B). The number of mitotic progeny (GFP^+PCNA^+) was not significantly changed in any mutants (Figure S2B). Surprisingly however, neuron production continued in the *Notch2* cKO at the same levels as in control mice even though GFP^+GFAP^+ cells were reduced to similar levels as in the *Notch1Notch2* and *Rbpj* cKO mice (Figure 2B and S2A). By 300-days post-ablation, all mutants showed a dramatic decline in the number of GFP^+ cells in the SVZ (Figure S2B) including proliferating progenitors (GFP^+PCNA^+) (Figure 2C) and newborn neuroblasts (Figure 2D). *Notch2* cKO either alone or in combination with *Notch1* deletion correlated with the strongest reduction in GFP^+GFAP^+ NSCs and neuroblasts (GFP^+DCX^+) (Figure 2D and S2B).

Although loss of Notch1 alone caused only a moderate reduction in GFP^+GFAP^+ NSCs, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice displayed a rapid decline in neurogenesis. This suggests that inactive $GFAP^+$ NSCs are unable to compensate for the reduction in active progenitors. In contrast, loss of Notch2 signaling resulted in a more rapid loss of $GFAP^+$ quiescent NSCs as a result of their potential activation and this initially sustained neuroblast production until both the quiescent and active NSC pools

became exhausted. This suggests that Notch2 plays a direct role in the maintenance of quiescent $GFAP^+$ NSCs but that Notch1 does not. Comparing single Notch mutants with *Notch1Notch2* and *Rbpj* cKO animals, we interpret the similarities and differences in phenotypes to indicate that NSCs enter an active state as a result of *Notch2* cKO and are then maintained by intact Notch1 signaling.

Notch2 gene regulation controls maintenance of quiescent NSCs

We addressed how Notch2 regulates NSC activity by isolating *Notch2* cKO cells from the SVZ early after ablation and analyzing genome-wide changes in gene expression (Figure 3A and S3A, S3B). We sorted *Notch2*-ablated, *Hes5::CreER^{T2}*-derived cells 1-day after TAM-treatment and performed microarray analysis (Figure 3A). Hierarchical gene clustering of gene expression in *Notch2* cKO versus control mRNA samples revealed significant differences (R^2=0.8289, 2'126 mRNAs 2-fold, 469 mRNAs 4-fold, and 71 mRNAs 8-fold changed; Figure 3B and Table S1). Gene ontology (GO) analysis of the 2-fold regulated genes showed strong correlations within cellular processes, biological regulation and single-organism processes (Figure 3C, Table S1). Within the top GO categories were genes involved in neurogenesis (P=3.92 10^{-28}), neurological processes (2.64 10^{-18}), Notch signaling pathway (P=9.21 10^{-15}) and cell cycle (P=1.47 10^{-8}) (Figure 3D). In agreement with the phenotypes observed in the SVZ of *Notch2* cKO mice, genes associated with stem cell maintenance (P=1.46 10^{-6}) and cell differentiation (P=2.8 10^{-6}) were also affected (Figure 3D and Table S1). Genes involved in cell division and cell growth were preferentially up regulated in the *Notch2* cKO cells whereas genes involved in stem cell maintenance, DNA repair and neuronal differentiation were down regulated (Figure S3C and Table S1). These global gene expression changes reflected the changes in cellular composition seen as a result of *Notch2* ablation. We defined genes with a 2-fold expression change and the presence of Rbpj recognition motifs proximal to their transcriptional start site as potential direct Notch targets. Refined Rbpj binding site predictions (ISMARA) were generated by combining multiple data sets including chromatin immunoprecipitation, and mapped these to the mouse genome (BED file Supplementary information) for the *in silico* definition of proximal promoters. Many of the regulated genes in our *Notch2* cKO microarray data set contained putative Rbpj recognition motifs. Within the panel of regulated genes were known Notch targets including Notch1, FABP7 (BLBP) and Cux2. Many of these genes, which fell within the GO terms cell cycle and stem cell maintenance, contained one or more Rbpj recognition motifs (Table S1). These *in silico* data suggest that Notch2 potentially regulated these genes directly in NSCs. Taken together, we interpret these results to indicate that loss of Notch2 induces changes in stem cell activation and differentiation, supporting the hypothesis that Notch2 is involved in maintenance of quiescent NSCs.

Non-neurogenic regions of the lateral ventricle wall contain dormant Notch2-expressing NSCs

NSCs of the lateral ventricle wall SVZ are embedded within a well-defined niche (Doetsch, 2003; Fuentealba et al., 2012; Ihrie and Alvarez-Buylla, 2011; Mirzadeh et al., 2008). NSCs (B1-cells) extend an apical process to the lateral ventricle and organize the ependymal cells of the lining into pinwheel structures (Mirzadeh et al., 2008). In addition, radial B1-cells contact blood vessels in the underlying parenchyma (Mirzadeh et al., 2008). We found $GFAP^+$ B1-cell like cells in the non-neurogenic dorsal medial ventricular wall (dMW) of the septum that displayed Notch activity (*Hes5::GFP*) with characteristic radial morphologies and blood vessel contact (Figure 4A, B). These B1-cell-like cells in the dMW contacted the ventricle through the ependymal lining that was organized into pinwheel-like structures (Figure 4C). Most *Hes5::GFP*$^+$ dMW B1-cells expressed Notch2 protein (Figure S4A), which we confirmed by acute conditional lineage tracing in *Notch2::CreER*$^{T2\text{-}SAT}$*Rosa26R::tdTomato* animals (Figure S4B). Genetically labeled dMW *Notch2::CreER*$^{T2\text{-}SAT}$ cells and their progeny expressed GFAP but not PCNA or DCX, which were almost absent in the dMW (Figure S4C) confirming the non-neurogenic nature of this part of the adult brain under homeostatic conditions. Thus, the dMW contain cells with Notch signaling and characteristics of NSCs (which we termed mB1-cells for medial wall B1-cells) that are embedded in a *bona fide* germinal niche-like structure (Figure 4D).

Notch2 represses a latent neurogenic potential of dormant dMW NSCs

NSC quiescence is a key character of maintained long-term neurogenesis (Beckervordersandforth et al., 2010; Furutachi et al., 2013; Giachino et al., 2014b; Pastrana et al., 2009). The role of Notch signaling in blocking neural commitment of self-renewing NSC by repressing proneural gene expression is well documented (Kageyama et al., 2007). Experimental data also indicate that Notch promotes mitotic quiescence of NSCs but the mechanism is unclear (Chapouton et al., 2010b). Therefore, long-term quiescence or dormancy of NSCs in non-neurogenic regions of the adult brain could explain the lack of neuron production outside the neurogenic zones.

As Notch2 regulates NSC activation in the SVZ and is expressed by NSC-like cells in the dorsal septal wall (Figure 5A), we addressed the functions of Notch2 in these mB1-cells by analyzing the *Notch2* cKO animals. *Notch2* cKO induced neuroblast (GFP^+DCX^+) production in the dMW (Figure 5B, C). The increase in neuroblasts in the dMW of *Notch2* cKO mice was accompanied by an increase in the total *Hes5::CreER*^{T2}-derived GFP^+ cells (Figure S5A) at the expense of GFP^+GFAP^+ mB1-cells (Figure 5C). Similarly, *Rbpj* cKO animals also showed activation of proliferation and neurogenesis in the dMW at the expense of GFP^+GFAP^+ mB1-cells (Figure S5B, C). Consistent with the hypothesized role of Notch1

in regulating active but not quiescent NSCs, *Notch1* cKO had no effect on proliferation (GFP^+PCNA^+) nor did it result in production of neuroblasts (GFP^+DCX^+) in the dMW (Figure S5D). The *Notch2* and *Rbpj* cKO dMW phenotypes were also evident in *Notch1Notch2* cKO animals at 21-days post-TAM treatment (Figure 5D and S5E). However, *Rbpj* and *Notch1Notch2* cKO dMW NSCs were exhausted by 100-days whereas proliferation and neurogenesis in the dMW of *Notch2* cKO animals persisted (Figure 5D and S5F). After *Notch2*-ablation, *Hes5::CreER^{T2}*-derived GFP^+NeuN^+ neurons were present in the septum adjacent to the ventricular wall and accumulated over time (Figure 5E, F). These newborn neurons settled into septal nuclei (Figure S5G) and many expressed Calbindin, Calretinin or Parvalbumin suggesting the formation of different neuron-subtypes (not shown).

Notch2-ablation induces neurogenesis from local NSCs

To confirm local neurogenesis in the dMW of *Notch2*, *Notch1Notch2* and *Rbpj* cKO animals, we analyzed the mice 2-days post-TAM treatment. Consistent with a local activation of NSCs in the dMW, proliferation increased in the region following ablation of *Notch2* (Figure S6A, B). Unlike in the *Notch1Notch2* cKO and *Rbpj* cKO animals where NSCs seemed to generate neurons directly without entering cell cycle, neuroblasts were not increased in the *Notch2* cKO (Figure S6B). Hence, even shortly after deleting *Notch2* or nuclear Notch signaling via ablation of *Rbpj*, proliferating cells and neuroblasts were already present in the dMW supporting that local mB1-cells were the likely origin of the neurogenesis.

We confirmed that the production of neuroblasts in the dMW of *Notch2* cKO mice was from local $GFAP^+$ mB1-cells and not neuroblasts aberrantly migrating from the lateral wall SVZ by restricting ablation of *Notch2* to $GFAP^+$ cells by stereotactic injection of adeno-*gfap::Cre* virus into the dorsal septum and lineage tracing the cells (*Rosa26R::GFP*) (Figure 6A) (Giachino et al., 2014b; Mirzadeh et al., 2008). Adeno-*gfap::Cre*-induced genetic recombination was restricted to $GFAP^+$ cells in the dMW (Figure 6B). *Notch2*-ablated $GFAP^+$ cells entered the cell cycle and generated neuroblasts confirming the dormant neurogenic potential of these local cells and the repressive effect of Notch2 (Figure 6C, D). Thus, the dMW contains NSCs with latent neurogenic potential, which are repressed by Notch2. Upon loss of Notch2 activity, these stem cells self-renew over prolonged periods and generate neurons. *Notch1Notch2* cKO and *Rbpj* cKO (complete loss of canonical Notch signaling) animals showed a similar but transient increase in initial neurogenesis in the dMW but subsequent progenitor exhaustion. One likely explanation for the persistent neurogenic activity in the *Notch2* cKO mice is that the Notch2-deficient NSCs entered an active, Notch1-dependent state and were maintained as neurogenic NSCs.

Dormant NSCs in the dMW activate in response to serotonin

Serotonin released by a plexus of axons coursing over the lateral ventricle wall has been shown to activate NSCs in the SVZ (Tong et al., 2014b). We found that the plexus of serotonergic axons also extends along the septal ependymal surface lining the ventricle and is in close proximity to the *Hes5::GFP*$^{+}$ mB1-cells (Figure 7A). Activation of NSCs correlates with their expression of BLBP, which, unlike Hes5, is retained by C-cells (Giachino et al., 2014b). In order to examine NSC activation, we treated *Hes5::GFP, BLBP::mCherry* mice with the antidepressant SSRI Fluoxetine for 7-days (Figure S7A). Fluoxetine treatment resulted in a rapid increase in proliferation and of *Hes5::GFP*$^{-}$ *BLBP::mCherry*$^{+}$ cells (C-cells) in the dMW (Figure 7B and S7B). The increased proliferation was accompanied by an increase in neuroblast production (DCX^{+}) (Figure 7B). In contrast, the number of *Hes5::GFP*$^{+}$ mB1-cells was reduced in response to Fluoxetine implying a transition from quiescent to active neurogenic progenitors at the expense of the NSC pool (Figure S7B). The Fluoxetine-induced reduction in *Hes5::GFP*$^{+}$ mB1-cells and increase in proliferation and neurogenesis continued for 3 weeks (Figure 7C). In order to confirm that the newly generated neuroblasts were generated by Hes5^{+} NSCs following Fluoxetine-treatment, we genetically labeled *Hes5::CreER*$^{T2+}$ cells (*Rosa26R::GFP*) with a 5-day TAM induction and subsequent treatment with Fluoxetine for 7-days. The neuroblasts generated in the septal wall were GFP^{+} and thus derived from the *Hes5::CreER*$^{T2+}$ NSCs (Figures S7C, D).

DISCUSSION

Notch signaling is a key mechanism in neurogenic niches to control NSC activity and differentiation. Canonical Notch signaling downstream of the four Notch paralogues is mediated by the transcription factor Rbpj. Ablation of *Rbpj* or *Notch1* abolishes neurogenesis in the adult SVZ, however, *Rbpj* and *Notch1* cKO mice display key differences in phenotype (Basak et al., 2012; Imayoshi et al., 2010). Loss of Rbpj leads to activation of quiescent NSCs, a wave of enhanced neurogenesis, and depletion of the NSC pool, whereas, loss of Notch1 abolishes self-renewal of activated NSCs without affecting the quiescent stem cell pool (Basak et al., 2012; Imayoshi et al., 2010). Thus, it was unclear whether Notch signaling controls quiescence or whether Rbpj acts as a transcriptional repressor independent of Notch activity in quiescent adult NSC. To address this, here we performed a detailed combinatorial analysis of Notch signaling knockouts in the adult mouse brain. We show that simultaneous ablation of *Notch1* and *Notch2* from forebrain stem cells phenocopies *Rbpj* cKO. We show that Notch2 is required by mitotically inactive and dormant NSCs both in the neurogenic SVZ and, by a novel stem cell population in the non-neurogenic dorsal septum of the adult brain. Therefore, Notch1 and Notch2 play discrete functions in

forebrain NSCs implying that activation of quiescent stem cells following *Rbpj* cKO reflects its signaling role downstream of Notch2 rather than a Notch independent function.

During development, radial glia stem cells initially generate neurons and then astrocytes (Malatesta et al., 2003; Noctor et al., 2001). Astrocytic differentiation is considered as the end-fate of NSCs in most brain regions and leads to exhaustion of the progenitor pool. Stem cells of the adult SVZ are set-aside during the peak of neurogenesis in the developing forebrain by some radial glia in the lateral ganglionic eminence, which stop dividing (Fuentealba et al., 2015; Furutachi et al., 2015). These perspective adult NSCs incorporate into the primordium of the adult lateral ventricle wall SVZ. Outside the neurogenic SVZ and dentate gyrus of the hippocampus, the mammalian brain has a poor capacity for regenerating neurons. This has been proposed to be partially due to a lack of neurogenic stem cells. Hence, our finding of dormant NSCs in the septal wall seems contradictory. It is unclear whether dMW NSCs are also set-aside during brain development. It is tempting to speculate that these cells are remnant of development.

However, analysis of proliferation indicates cell division throughout the adult brain and the production of oligodendrocytes and astrocytes but not new neurons outside the neurogenic zones under normal conditions. Further, neural progenitors can be isolated from non-neurogenic regions of the adult mammalian central nervous system including the spinal cord, optic nerve, cerebral cortex and hypothalamus (Palmer et al., 1999; Robins et al., 2013). Once isolated and expanded in the presence of growth factors, these progenitors can give rise to neurons *in vitro*. Therefore, their neurogenic potential *in vivo* remained questionable. However, our data indicate that neurogenic stem cells do exist in non-neurogenic regions of the adult brain and that the local environment in which these cells find themselves restricts their activation and neuronal determination. In support of a niche mediated fate restriction, some parenchyma progenitors are able to generate neurons once grafted into the DG indicating that adult germinal zones can instruct neuronal fate (Shihabuddin et al., 2000). Conversely, grafting of SVZ NSCs and putative parenchymal progenitors into ectopic non-neurogenic regions of the brain results in glial but not neuronal differentiation (Seidenfaden et al., 2006). Thus, local niche signals in the neurogenic zones contribute to maintained neurogenic potential and fate determination *in vivo* and Notch2 signals may mask their neurogenic potential *in situ* (Merkle et al., 2007).

Astrocytes in non-neurogenic brain regions that retain the ability to divide may be restricted from adopting a neuronal fate through lateral activation of Notch signaling in local niches. While the specific role of Notch2 in NSC quiescence was not described previously, there were indications of specific Notch-dependent regulation in other models for example in fish where Notch3 regulates NSC activation (Chapouton et al., 2010a). Loss of Notch signaling in astrocytes within the striatum after stroke results in

increased neurogenesis and ablation of Rbpj in striatal astrocytes initiates neuroblast production (Magnusson et al., 2014). These findings lend direct support to our results showing that dMW B1-cells, which have astrocytic characteristics, are repressed by Notch2, which prevents both entry into cell cycle and the generation of neurons even outside the classical neurogenic regions. Thus, it is intriguing that *Notch2* cKO resulted in the down regulation of stem cell associated genes and up regulation of cell cycle genes. A number of these regulated genes contain Rbpj recognition motifs in their proximal promoter regions opening up the possibility of a direct and selective regulation by Notch2 in quiescent NSCs. In addition, recent data indicate that mutations in Notch receptors, including Notch2, are found in human gliomas suggesting that loss of Notch signaling in brain parenchyma progenitors could be involved in early stages of brain tumor formation (Cancer Genome Atlas Research et al., 2015; Giachino et al., 2015; Suzuki et al., 2015).

The quiescent dMW NSCs are able to respond to environmental cues. The septal nuclei in the brains of humans receives input from many brain regions including the olfactory bulb, hippocampus, hypothalamus and thalamus and is part of the pleasure zone of the brain with a role in reward and reinforcement. Whether neurogenesis in the dMW is linked to pathophysiological stimuli that modulate neurogenesis in the classic neurogenic brain regions remains to be determined (Anthony et al., 2014). However, NSCs in the dorsal septal wall are in contact with a plexus of serotonin positive axons. SVZ NSCs rapidly divide and generate newborn neuroblasts in response to serotonin agonist (Tong et al., 2014a; Tong et al., 2014b). dMW mB1-cells respond similarly to increased serotonin levels following treatment with the SSRI and antidepressant Fluoxetine with increased progenitor production and newborn neuroblasts.

The crucial role of the niche is highlighted by recent advances in astrocyte reprogramming, in which astrocytes in the brain parenchyma can be driven to neurogenesis (Peron and Berninger, 2015). This can be induced by local tissue damage and by the forced expression of pro-neurogenic transcription factors including Ascl1, Neurog2 and NeuroD1 *in vitro* and *in vivo* (Berninger et al., 2007; Guo et al., 2014; Heinrich et al., 2010; Liu et al., 2015a; Masserdotti et al., 2015). Expression of the proneural transcription factors is repressed by Notch signaling thereby preventing NSCs adopting a neuronal fate (Kageyama et al., 2005). This partially explains Notch signaling control of the developmental switch in NSC fate, inhibiting neurogenesis whilst favoring glial fates (Zhong et al., 1997). However, proneural gene expression is also repressed by Notch in parenchymal astrocytes. Loss of Notch signaling in astrocytes within the striatum after stroke increases neurogenesis and ablation of Rbpj increases Ascl1 expression in striatal astrocytes and initiates their formation of neurons (Magnusson et al., 2014). We believe that our findings have important implications suggesting that even in regions of the adult mammalian brain which

no longer generate neurons, stem cells may be present in a Notch2-repressed dormant state and these can be rejuvenated to form new neurons.

EXPERIMENTAL PROCEDURES

Animals and husbandry

Hes5::GFP, Hes5::CreER^{T2}, Notch2::CreER$^{T2\text{-}SAT}$, Rosa25R::GFP, Rosa25R::tdTomato, floxed *Notch1,* floxed *Notch2,* floxed *Rbpj* mice have been described elsewhere (Basak et al., 2012; Basak and Taylor, 2007; Besseyrias et al., 2007; Fre et al., 2011; Lugert et al., 2012; Schouwey et al., 2007). Mice were kept according to Swiss Federal and Swiss Veterinary office regulations under license numbers 2537 and 2538 (Ethics commission Basel-Stadt, Basel Switzerland). For further information see Supplementary Materials and Methods.

Administration of TAM and Fluoxetine and tissue preparation

Adult mice 8-10 weeks of age were injected daily intraperitoneal with 2 mg TAM in sunflower oil for five consecutive days and killed 2, 21, 100 or 300-days after the end of the treatment. Fluoxetine (1.8 mg/kg) was administered intraoral for seven consecutive days. Animals were sacrificed 2 or 19-days after treatment. Animals were given a lethal dose of Ketamin-Xylazine and perfused transcardial. Tissue was sectioned at 30 µm and immunostained as floating sections (see Supplementary Materials and Methods) (Giachino and Taylor, 2009; Lugert et al., 2010).

Microarray analysis and quantitative RT-PCR

Animals were sacrificed 24 hours after TAM treatment. Tissue was prepared for FACS sorting as described previously (Lugert et al., 2010) and GFP^{+} cells sorted directly into Trizol reagent (Thermo Fisher Scientific). RNA extracted according to manufacturers recommendations. RNA quality was tested by Fragment Analyzer (Advanced Analytical). cDNA was prepared using BioScript (Bioline). qRT-PCR was performed using SensiMix SYBR kit (Bioline). Affymetrix expression profiling was performed on Affymetrix GeneChip Mouse Gene 1.0 ST arrays (ATLAS Biolabs). GO analysis was performed using DNASTAR Lasergene ArrayStar (DNASTAR). Large-scale target analysis was performed doing a cross comparison of differentially regulated genes and Rbpj promoter sites, using a ISMARA generated trace. For more detailed information see Supplementary Materials and

Stereotactic injection of adeno-*gfap*::Cre virus particles

Adeno-*gfap*::Cre virus was described previously (Merkle et al., 2007). Animals were injected with stereotactic coordinates (anterior/posterior 0 mm; medial/lateral 0 mm relative to Bregma and 2.5 mm below the skull) into the septum with 1 ml of adeno-*gfap*::Cre virus (titer 1 x 10^{12} infection particles per ml) in PBS. Animals were analyzed 21-days post-injection (see Supplementary Materials and Methods).

Quantification and statistical analysis

Stained sections were analyzed with fixed photomultiplier settings on a Zeiss Observer with Apotome (Zeiss). Images were processed with Photoshop or ImageJ. Data are presented as averages of a minimum of three sections per region and multiple animals (n in figure legends). Statistical significance was determined by two-tailed Student's T-test on mean values per animal, percentages were transformed into their arcsin value, Whitney-Mann U-test was used for distributions and two way ANOVA for cross-comparison of three and more data sets. Significance was determined at * - $P<0.05$, ** - $P<0.01$, *** - $P<0.001$ or P values are given in the graphs. Deviance from mean is displayed as standard deviation if not otherwise indicated. Complete data tables are provided in the supplementary information.

AUTHOR CONTRIBUTIONS

A.E., C.R., C.G., A.E., R.Z. and V.T., conceived experiments, analyzed and interpreted the data, wrote and edited the manuscript. U.Z-S, F.R. and S.A-T provided transgenic animals. I.S. and A.L., performed *Notch2::CreER*$^{T2\text{-}SAT}$ induction experiments and edited the manuscript. All authors approved the final manuscript.

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FIGURES AND LEGENDS

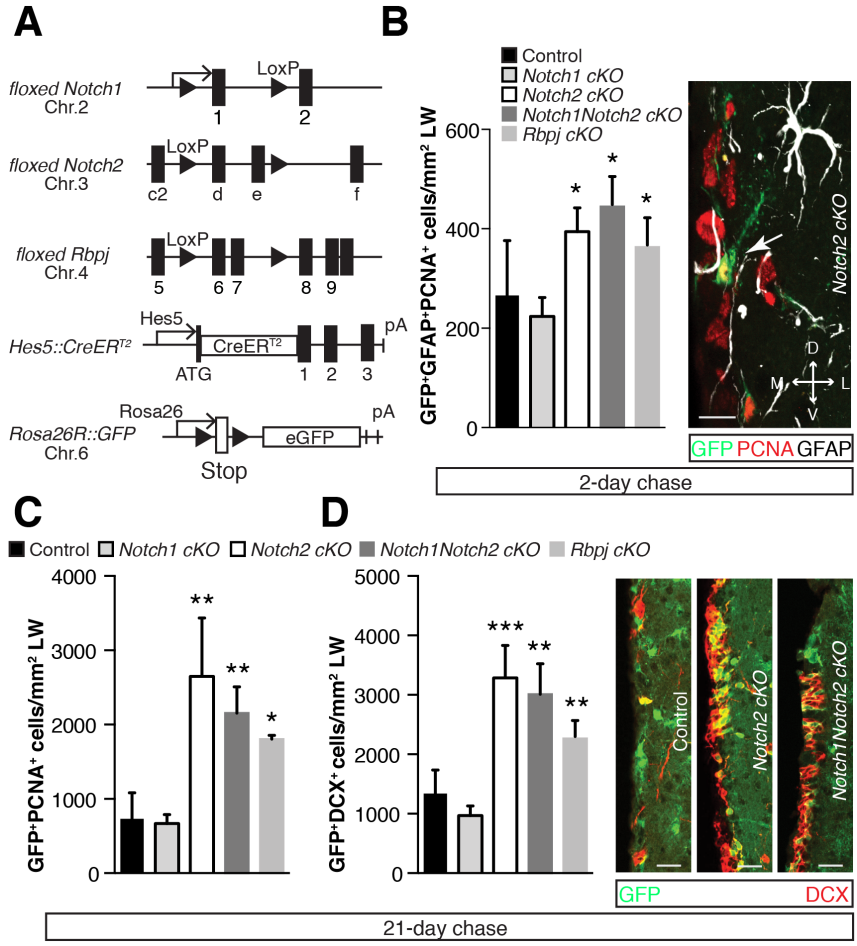


Figure 1. Notch paralogue knockouts have distinct SVZ phenotypes

A. Schemes of floxed *Notch1*, *Notch2* and *Rbpj* loci, *Hes5::CreER^{T2}* transgene and *Rosa26R::GFP* Cre-reporter allele with chromosome (Chr.), exons, LoxP, and poly-adenylation sites (pA). **B.** Quantification of *Hes5::CreER^{T2}*-derived (GFP$^+$) GFP$^+$GFAP$^+$PCNA$^+$ NSCs (B1-cells) in the SVZ of the lateral ventricle wall of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 2-days post-TAM induction; PCNA$^+$ mitotic radial NSC (B1-cell) in the SVZ in *Notch2* cKO mice (arrow). **C.** Quantification of GFP$^+$PCNA$^+$ cells in the SVZ of control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 21-days post-TAM induction. **D.** Quantification of GFP$^+$DCX$^+$ neuroblasts in the SVZ of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 21-days post-TAM induction.

Values are means ± SD; * - P<0.05, ** - P<0.01 ,*** - P<0.001, 2-day chase: Control n=4, *Notch1* cKO n=3, *Notch2* cKO n=4, *Notch1Notch2* cKO n=3 *Rbpj* cKO n=4, 21-day chase: Control n=6, *Notch1* cKO n=3, *Notch2* cKO n=5, , *Notch1Notch2* cKO n=6, *Rbpj* cKO n=4. Scale bars = 10µm in **B** and 25µm in **D**.

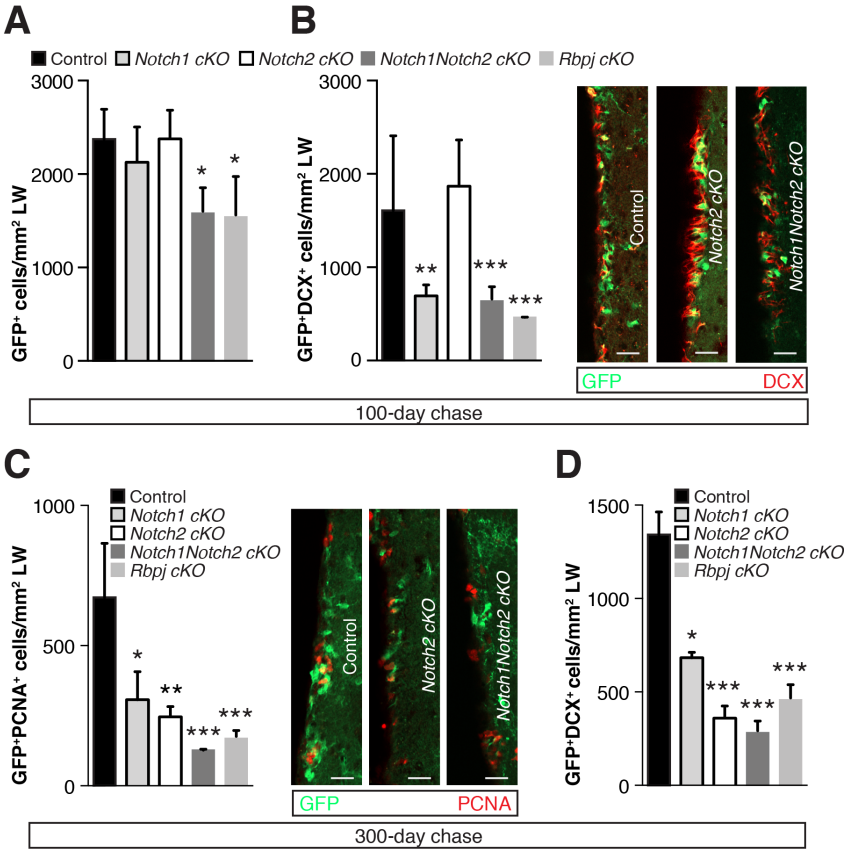


Figure 2. *Notch2* cKO animals display potentiated long-term neurogenesis compared to *Notch1*, and *Rbpj* mutants.

A. Quantification of *Hes5::CreER*^{T2}-derived (GFP$^+$) progeny in the SVZ of the lateral ventricle wall of control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 100-days post-TAM induction. **B.** Quantification of GFP$^+$DCX$^+$ neuroblasts in the SVZ of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 100-days post-TAM induction. Images of GFP$^+$DCX$^+$ neuroblasts in the SVZ of *Notch2* cKO compared to Control and *Notch1Notch2* cKO mice. **C.** Quantification of GFP$^+$PCNA$^+$ cells in the SVZ of the lateral ventricle wall of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 300-days post-TAM induction. Images of GFP$^+$PCNA$^+$ cells in the SVZ of Control, *Notch2* cKO and *Notch1Notch2* cKO mice 300-days post-TAM induction. **D.** Quantification of GFP$^+$DCX$^+$ neuroblasts in the SVZ of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 300-days post-TAM induction.

Values are means ± SD; * - P<0.05, ** - P<0.01, *** - P<0.001, 100-day chase: Control n=5, *Notch1* cKO n=3, *Notch2* cKO n=4, *Notch1Notch2* cKO n=3, *Rbpj* cKO n=4, 300-day chase: Control n=4, *Notch1* cKO n=3, *Notch2* cKO n=3, *Notch1Notch2* cKO n=3, *Rbpj* cKO n=3. Scale bars = 25µm.

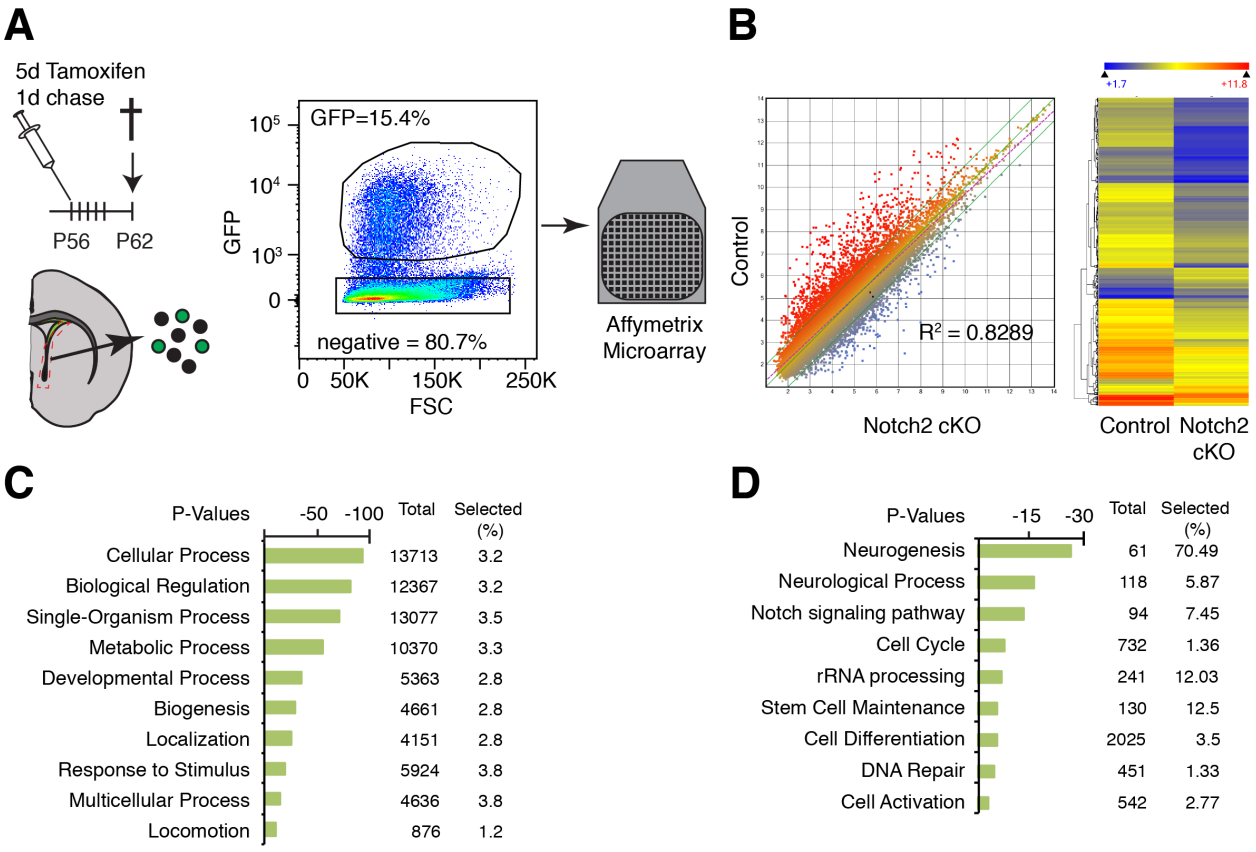


Figure 3. Gene ontology analysis of genes regulated after *Notch2* ablation.

A. Scheme of experimental setup. Following 5-days of TAM-induction mice were sacrificed 1-day later and *Hes5::CreER*^{T2}-derived (GFP^{+}) Control or *Notch2* cKO SVZ cells were isolated by FACS and RNA prepared for microarray analysis. **B.** Scatter plot of mean Control versus Notch2 cKO gene expression. **C.** Gene ontology analysis of differentially expressed genes in *Notch2* cKO versus Control with significance, total genes in category and percent differentially expressed. **D.** GEO analysis and top ten biological process of differentially expressed genes in *Notch2* cKO versus Control with significance, total genes in category and percent differentially expressed.

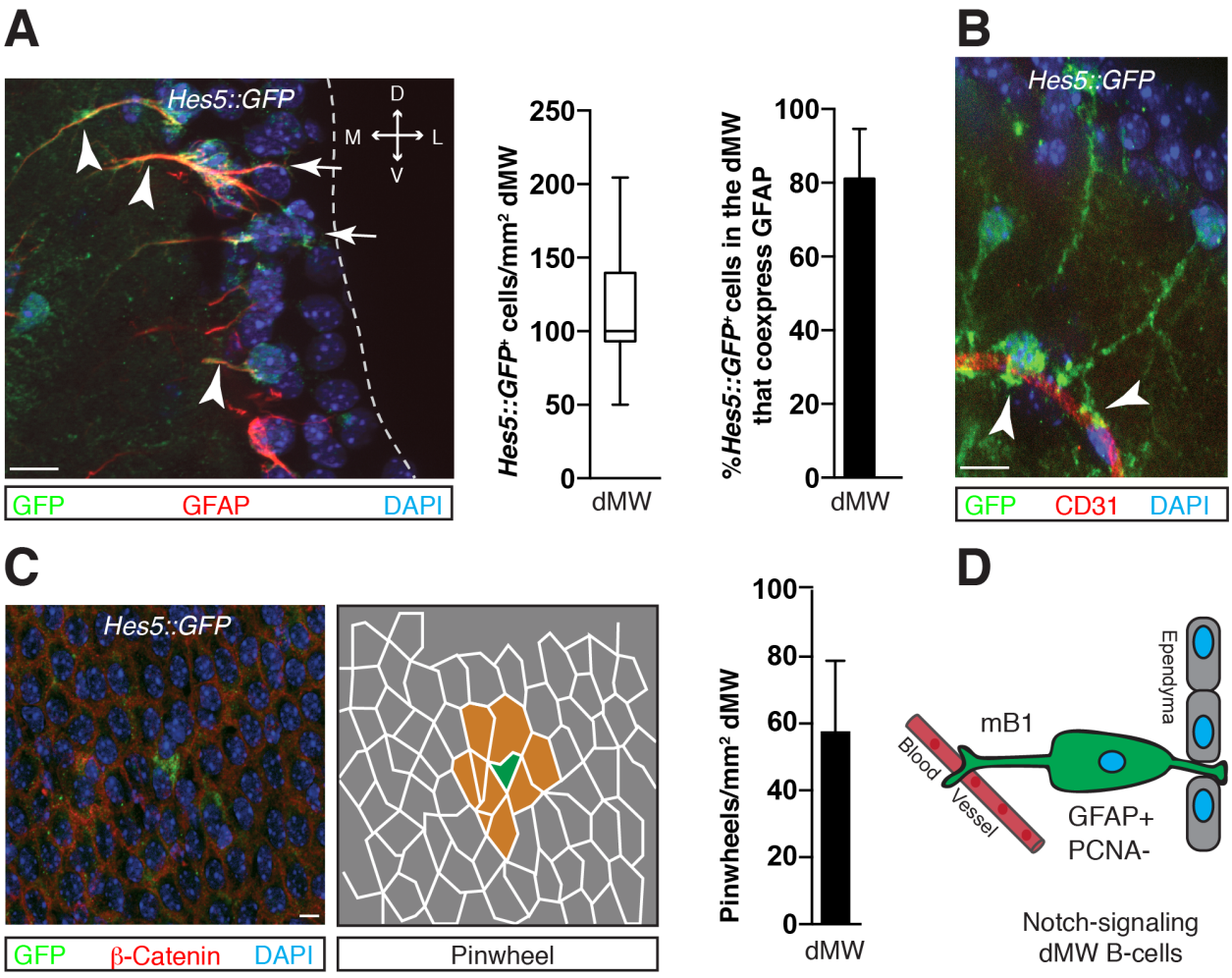


Figure 4. The dorsal wall of the septum contains putative dormant NSCs

A. Notch-signaling *Hes5::GFP*$^+$ cells in the dMW have a radial type morphology and express GFAP. Quantification of *Hes5::GFP*$^+$ cells per mm^2 of the dMW and their coexpression of GFAP. **B.** Radial *Hes5::GFP*$^+$ cells in the dMW project to underlying CD31$^+$ blood vessels. **C.** Whole mount preparation of the dorsomedial septal wall showing *Hes5::GFP*$^+$ cells protruding through the ependymal marked with the adherence junction protein b-catenin and organizing the ependymal cells into pinwheel structures. Quantification of *Hes5::GFP*$^+$ cells containing pinwheels per mm^2 of the dMW. **D.** Schematic representation of radial *Hes5::GFP*$^+$GFAP$^+$ medial wall mB1-cells and their interactions with the ependyma lining the lateral ventricle and blood vessels.

Values are mean ± SD, * - P<0.05, ** - P<0.01,*** - P<0.001. Box whisker plot shows the mean, IQR, 1st and 3rd quartiles. Quantifications of *Hes5::GFP*$^+$ cells n=5, quantifications of pinwheels n=6 animals. Scale bars 15 µm in **A** and **B**, 10 µm in **C**.

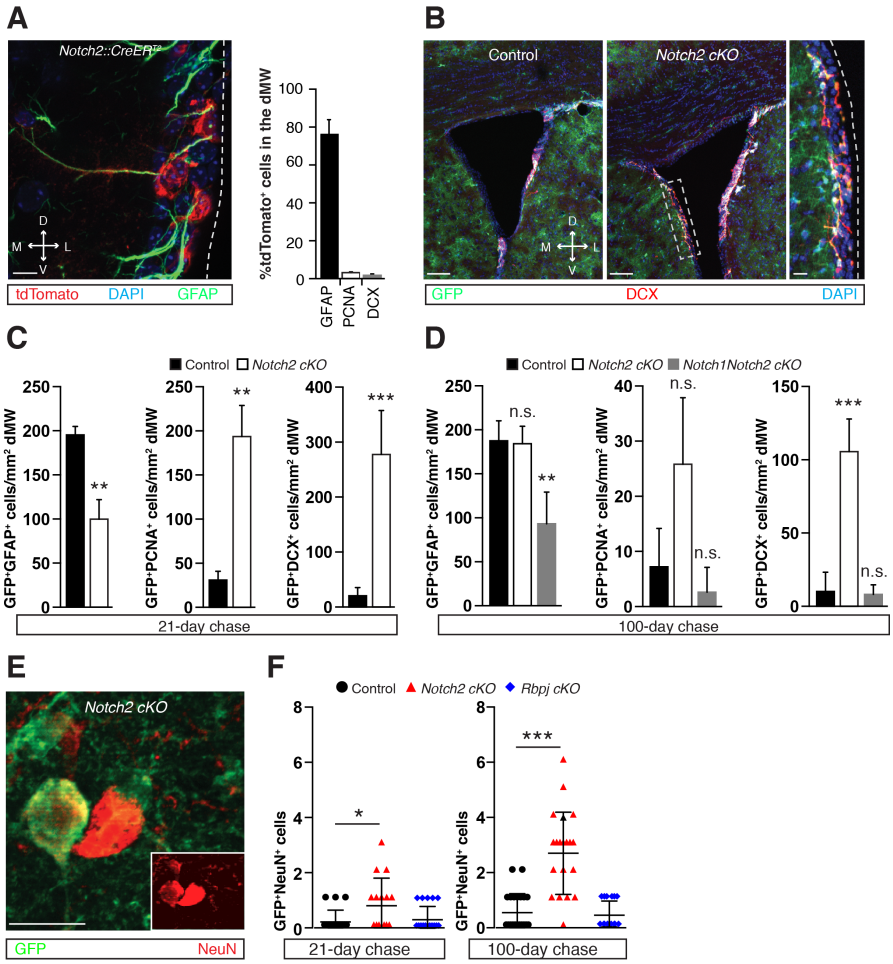


Figure 5. Notch2 deletion activates quiescent cells in the dMW

A. TAM-induced genetic labeling (*Rosa26R-tdTomato*) of Notch2$^+$ radial GFAP$^+$ mB1-cells in the dMW in *Notch2::CreER*$^{T2\text{-}SAT}$ animals. tdTomato$^+$ cells are mostly GFAP$^+$ and rarely PCNA$^+$ or DCX$^+$ under physiological conditions. **B.** Increased DCX expression in the dMW of *Notch2* cKO cells 21-days after TAM administration. Lineage tracing of *Hes5::CreER*^{T2}-derived (GFP$^+$) cells. **C.** Quantification of GFP$^+$GFAP$^+$, GFP$^+$PCNA$^+$, and GFP$^+$DCX$^+$ cells in the dMW of *Notch2* cKO compared to Control animals, 21-days after TAM administration. **D.** Quantification of GFP$^+$GFAP$^+$, GFP$^+$PCNA$^+$, and GFP$^+$DCX$^+$ cells in the dMW of *Notch2* cKO animals compared to *Notch1Notch2* cKO and control animals, 100-days after TAM administration. **E.** *Hes5::CreER*^{T2}-derived NeuN$^+$ newborn neurons in the septum of *Notch2* cKO animals 21-days after TAM administration. **F.** Quantification of GFP$^+$NeuN$^+$ neurons in the septum of Control, *Notch2* cKO, and *Rbpj* cKO animals, 21- and 100-days after TAM administration.

Values are mean ± SD, * - P<0.05, ** - P<0.01, *** - P<0.001. 21-day chase: Control n=6, *Notch2* cKO n=5, 100-day chase: Control n= 5, *Notch2* cKO n=5, *Notch1Notch2* cKO n=3. Scale bars 10 µm in **A** and 100 µm in **B** and 20 µm in **E**.

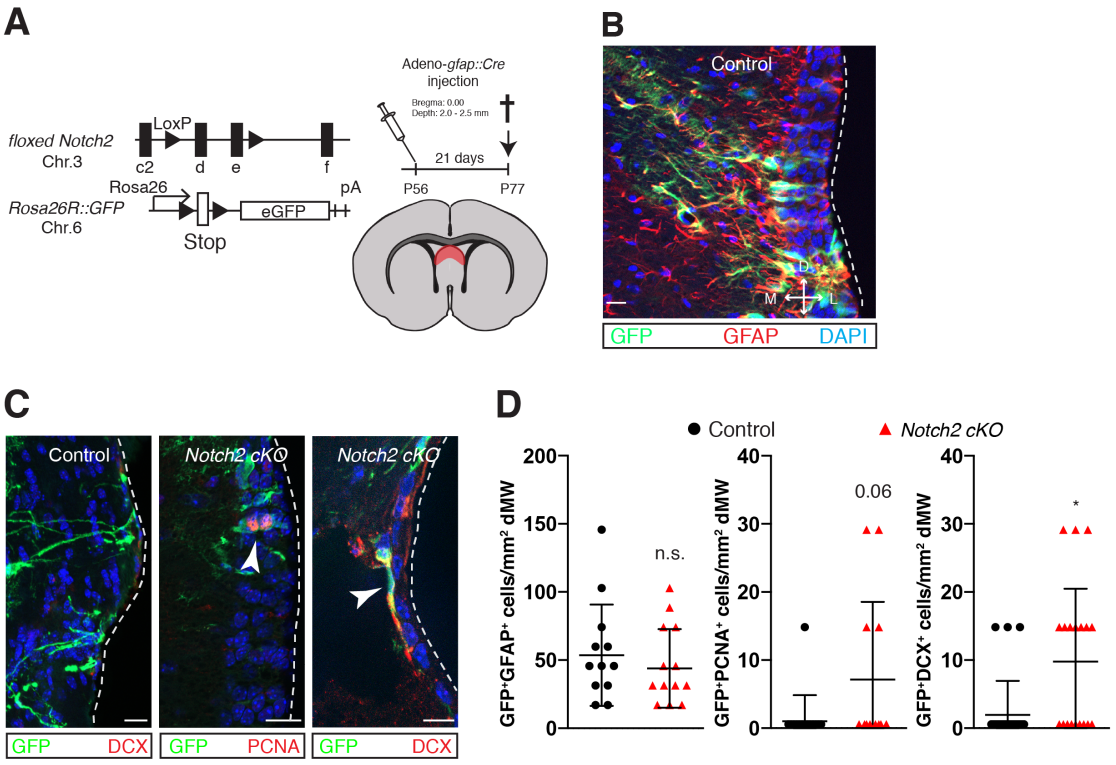


Figure 6. Neurogenesis in the dMW is mediated by local NSCs

A. Schematic representation of adeno-*gfap::Cre* infection (*Rosa26R-GFP*) of mB1-cells in the dMW in Control and Notch knockout animals. **B.** Stereotactic infection of radial mB1-cells in the dMW only in $GFAP^+$ cells. **C.** Stereotactic infection of radial mB1-cells in the dMW with adeno-*gfap*::Cre virus showing the generation and lineage tracing (*Rosa26R-GFP*) of mitotic cells (GFP^+PCNA^+) and neuroblasts (GFP^+DCX^+) in the *Notch2* cKO but not in Control animals. **D.** Quantification of GFP^+DCX^+ neuroblasts derived from adeno-*gfap*::Cre progenitors in the dMWs 21-days post-infection of *Notch2* cKO and Control animals.

Mean values are shown ± SD, P-values are shown * - $P<0.05$, n.s. – not significant. Control n= 3, *Notch2* cKO n=3. Scale bars 25 µm in **A**.

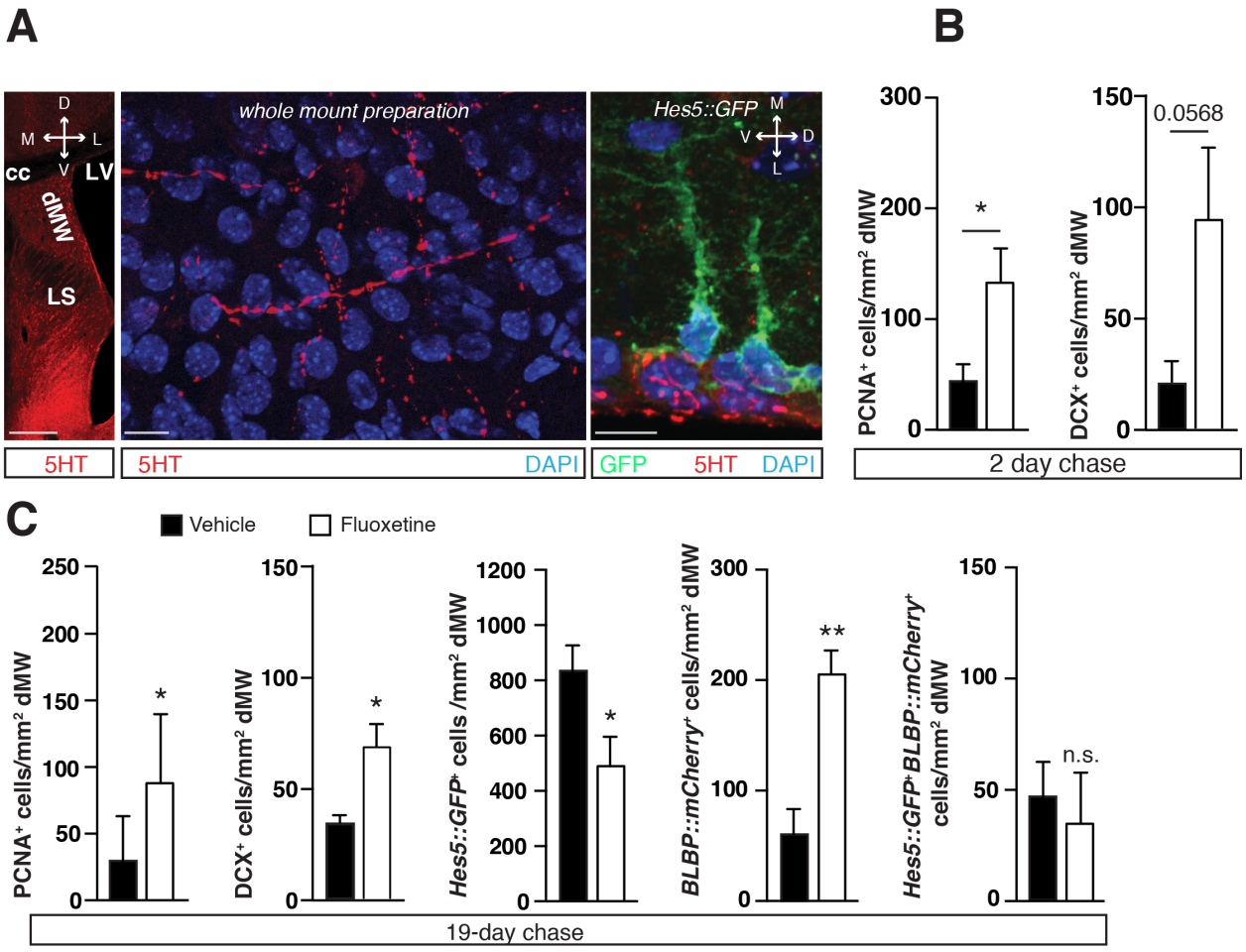


Figure 7. mB1-cells in the dMW are responsive to antidepressant SSRI treatment

A. Serotonergic afferents in the dMW coursing over the ependyma of the septal, medial ventricular wall in close proximity to the *Hes5::GFP*$^+$ mB1-cells. **B.** Quantification of mitotic (PCNA$^+$) cells in the dMW 2-days after administration of the SSRI Fluoxetine. **C.** Quantification of mitotic cells (PCNA$^+$), neuroblasts (DCX$^+$), *Hes5::GFP*$^+$ mB1-cells, *BLBP::mCherry*$^+$ activated progenitors and *Hes5::GFP*$^+$*BLBP::mCherry*$^+$ activated NSCs in the dMW 19-days post-Fluoxetine treatment.

Values are mean ± SD, * - P<0.05, ** - P<0.01, *** - P<0.001. 2-day chase: Vehicle n=3, Fluoxetine n=4, 19-day chase: Vehicle n=3, Fluoxetine n=3. Scale bars 100 µm in left panel, 15 µm in middle and right panels in **A**

Supplemental Information

Inventory of Supplemental Information

Figure S1, related to Figure 1
Figure S2, related to Figure 2
Figure S3, related to Figure 3
Figure S4, related to Figure 4
Figure S5, related to Figure 5
Figure S6, related to Figure 6
Figure S7, related to Figure 7

Supplemental Information on Thesis CD

Supplemental Data Tables
Supplemental Data GO and Rbpj trace, related to Figure 3 and Figure S3
Supplemental Experimental Procedures
Supplemental References

Supplemental Figures and Legends

Figure S1. Notch paralogue cKOs have distinct SVZ phenotypes

A. Immunohistochemistry and quantification of $GFP^+Notch2^+$ cells in Control *Hes5::CreER^{T2}* transgene and *Rosa26R::GFP* Cre-reporter and *Notch2* cKO animals. **B.** Quantification of *Hes5::CreER^{T2}*-derived GFP^+ cells in the SVZ of the lateral ventricle wall of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 2-days post-TAM induction. **C.** Quantification of GFP^+GFAP^+, GFP^+PCNA^+ and GFP^+DCX^+ cells in the SVZ of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 2-days post-TAM induction **D.** Quantification of GFP^+GFAP^+ B-cells in the SVZ of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 21-days post-TAM induction. Values are means ± SD; * - P<0.05, ** - P<0.01, *** - P<0.001, 2-day chase: Control n=4, *Notch1* cKO n=3, *Notch2* cKO n=4, *Notch1Notch2* cKO n=3 *Rbpj* cKO n=4, 21-day chase: Control n=6, *Notch1* cKO n=3, *Notch2* cKO n=5, *Notch1Notch2* cKO n=6, *Rbpj* cKO n=4. Scale bars = 10μm

Figure S2. *Notch2* cKO animals display potentiated long-term neurogenesis compared to *Notch1*, and *Rbpj* mutants.

A. Quantification of *Hes5::CreER^{T2}*-derived GFP$^+$GFAP$^+$ B-cells and GFP$^+$PCNA$^+$ dividing cells in the SVZ of the lateral ventricle wall of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 100-days post-TAM induction. **B.** Quantification of *Hes5::CreER^{T2}*-derived (GFP$^+$) progeny in the SVZ of the lateral ventricle wall of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 300-days post-TAM induction. Values are means ± SD; * - P<0.05, ** - P<0.01,*** - P<0.001, 100-day chase: Control n=5, *Notch1* cKO n=3, *Notch2* cKO n=4, *Notch1Notch2* cKO n=3, *Rbpj* cKO n=4, 300-day chase: Control n=4, *Notch1* cKO n=3, *Notch2* cKO n=3, *Notch1Notch2* cKO n=3, *Rbpj* cKO n=3.

Figure S3. Gene ontology analysis of genes regulated after *Notch2* ablation.

A. Schemes of floxed *Notch2*, *Hes5::CreER^{T2}* transgene and *Rosa26R::GFP* Cre-reporter allele with chromosome (Chr.), exons, LoxP, and poly-adenylation sites (pA).**B.** qPCR analysis of Control and *Notch2* cKO for *b-actin* and *Notch2*. **C.** GO analysis of differentially expressed genes in *Notch2* cKO versus Control with significance, percent differentially expressed up versus down regulated genes.

Figure S4. The dorsal wall of the septum contains putative dormant NSCs

A. Notch-signaling *Hes5::GFP$^+$* cells in the dMW have a typical radial type morphology and express Notch2. Quantification of *Hes5::GFP$^+$*Notch2$^+$ cells per mm^2 of the dMW **B.** Schemes of *Notch2::CreER$^{T2\text{-}SAT}$*, *Rosa26R::tdTomato* Cre-reporter allele with chromosome (Chr.), exons, LoxP, and poly-adenylation sites (pA). **C.** Images of Notch2::CreER$^{T2\text{-}SAT}$, *Rosa26R*::tdTomato co-stained with GFAP and DCX. Values are means ± SD; *Hes5::GFP* animals n=5, Scale bars 15 µm.

Figure S5. Notch signaling manipulation activates quiescent cells in the dMW

A. Quantification of *Hes5::CreER^{T2}*-derived GFP$^+$ cells in the SVZ of the dorsal medial wall of Control, *Notch1* cKO, *Notch2* cKO, *Notch1Notch2* cKO and *Rbpj* cKO mice 21-days post-TAM induction. **B.** TAM-induced genetic labeling (*Rosa26R-GFP*) of Hes5$^+$ radial GFAP$^+$ mB1-cells in the dMW in Control and knockout of *Rbpj* (*Rbpj* cKO) animals, stained for PCNA and DCX. Upon loss of Notch signal mediator cells in the dMW are activated. **C.** Quantification of GFP$^+$GFAP$^+$, GFP$^+$PCNA$^+$, and GFP$^+$DCX$^+$ cells in the dMW of *Rbpj* cKO compared to Control animals, 21-days after TAM administration. **D.** Quantification of GFP$^+$GFAP$^+$, GFP$^+$PCNA$^+$, and GFP$^+$DCX$^+$ cells in the dMW of *Notch1* cKO animals compared Control animals, 21-days after TAM administration. **E.** Quantification of

GFP^+GFAP^+, GFP^+PCNA^+, and GFP^+DCX^+ cells in the dMW of *Rbpj* cKO animals compared Control animals, 100-days after TAM administration. **F.** TAM-induced genetic labeling (*Rosa26R-GFP*) of *Noch1Notch2* knockout animals (*Notch1Notch2* cKO) animals. **G.** Overview image of septum of Notch2 cKO animals. Circles represent position of neurons. Values are mean ± SD, * - P<0.05, ** - P<0.01, *** - P<0.001. 21-day chase: Control n=6, *Notch2* cKO n=5, *Rbpj* cKO n= 4, 100-day chase: Control n= 5, *Notch2* cKO n=5, *Notch1Notch2* cKO n=3, *Rbpj* cKO n= 4; Scale bars 10 µm in **A, C, E** and 100 µm in **F**.

Figure S6. Neurogenesis in the dMW is mediated by local NSCs

A. TAM-induced genetic labeling (*Rosa26R-GFP*) of $Hes5^+$ mB1-cells in the dMW in Control and Notch knockout animals, stained for PCNA and DCX. **B.** Quantification of GFP^+GFAP^+ mB1-cells, GFP^+PCNA^+ proliferating cells and GFP^+DCX^+ neuroblasts 2-days post-TAM of different Notch knockouts and Control animals.

Mean values are shown ± SD, P-values are shown * - P<0.05, n.s. – not significant. Control n= 4, *Notch1* cKO n= 3, *Notch2* cKO n= 4, *Notch1Notch2* cKO n= 3, *Rbpj* cKO n= 4. Scale bars 25 µm in **A**.

Figure S7. mB1-cells in the dMW are responsive to antidepressant serotonin uptake inhibitor treatment

A. Schemes of *Hes5::GFP* and *BLBP::mCherry* transgenes with exons. Scheme of the induction of Fluoxetine and chase periods. **B.** Quantification of labeled quiescent ($Hes5::GFP^+$) and active ($Hes5::GFP^+BLBP::mCherry^+$) stem cells and progenitors ($BLBP::mCherry^+$) 2-days after administration of the serotonin uptake inhibitor Fluoxetine. **C.** Schemes of $Hes5::CreER^{T2}$ transgene and *Rosa26R::GFP* Cre-reporter allele with chromosome (Chr.), exons, LoxP, and poly-adenylation sites (pA). Representation of TAM-administration (5 days) and Fluoxetine (7-day) for GFP-reported lineage tracing. **D.** Administration of the serotonin uptake inhibitor Fluoxetine leads to proliferative activation of GFP^+ cells and generation of GFP^+DCX^+ cells in the dMW.

Values are mean ± SD, * - P<0.05, ** - P<0.01, *** - P<0.001. 2-day chase: Vehicle n=3, Fluoxetine n=4, 19-day chase: Vehicle n=3, Fluoxetine n=3, TAM-Vehicle n= 2, TAM-Fluoxetine n=2. Scale bars 25 µm in left and right panels, 10 µm in middle panels.

Supplemental Figures

Figure S1

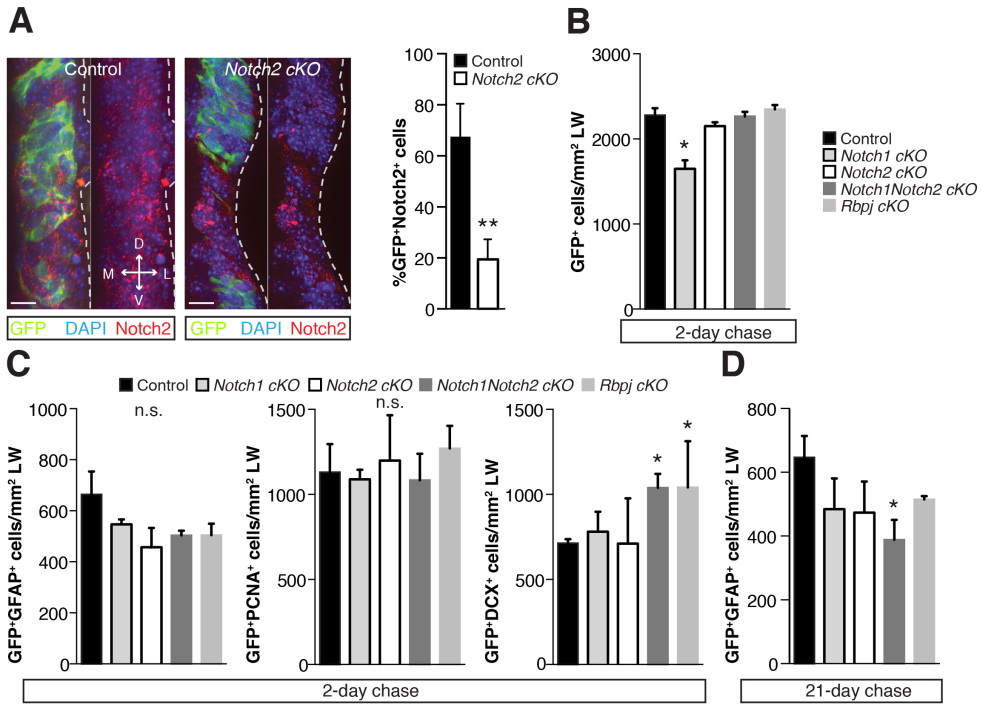
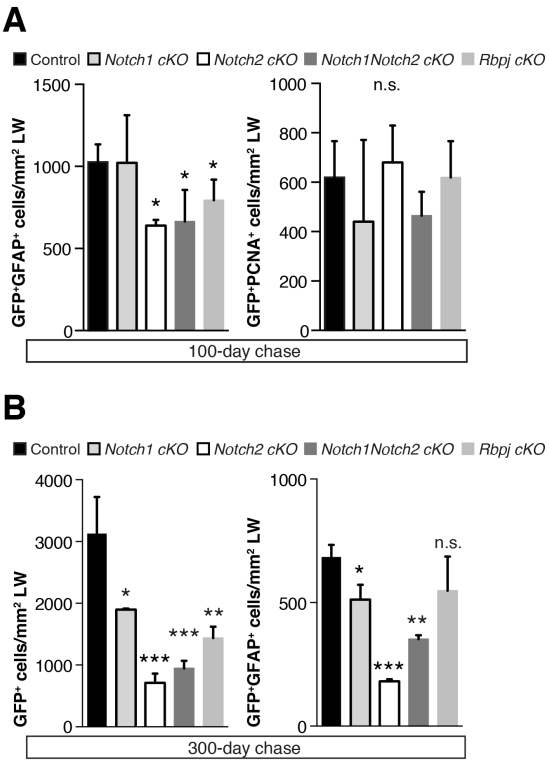


Figure S2



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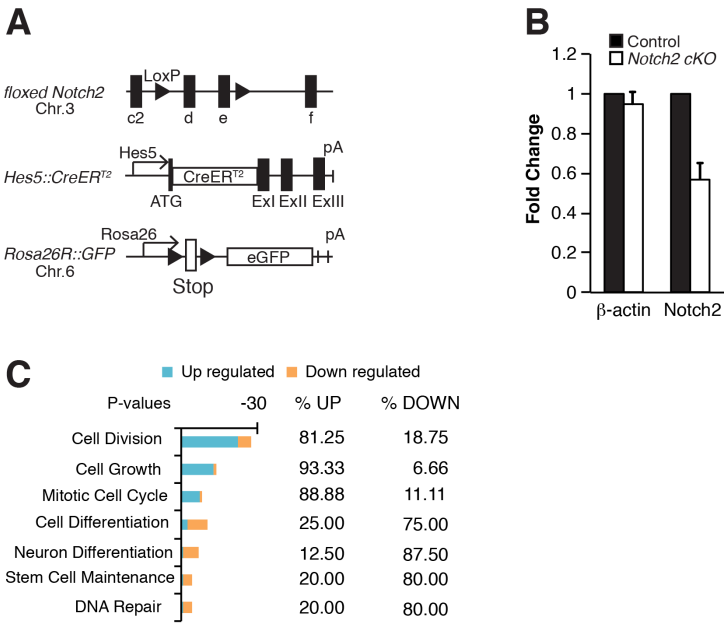


Figure S4

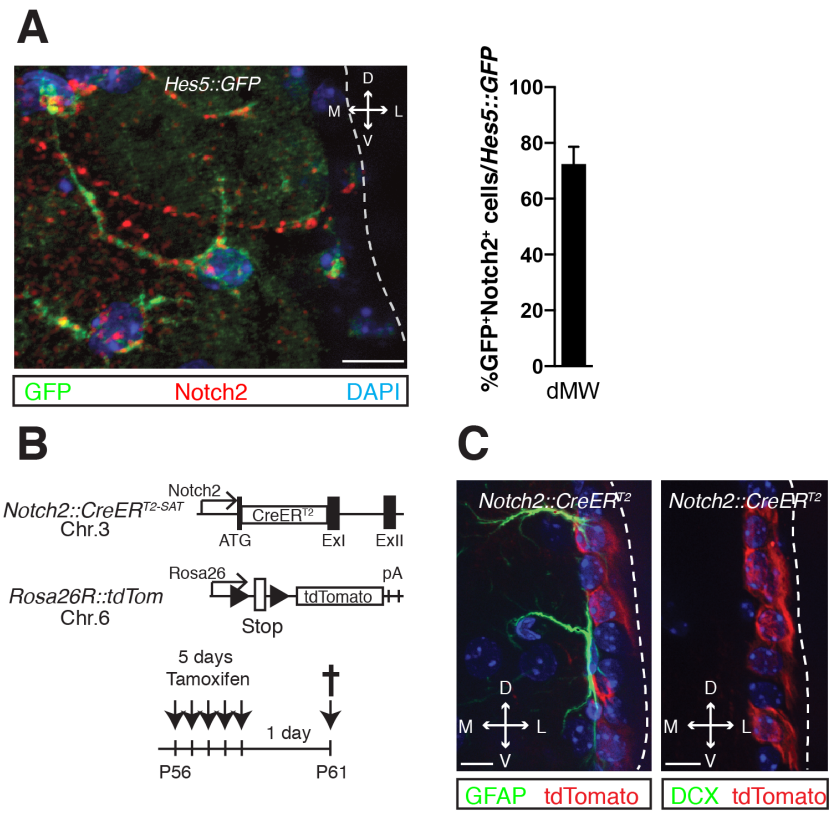


Figure S5

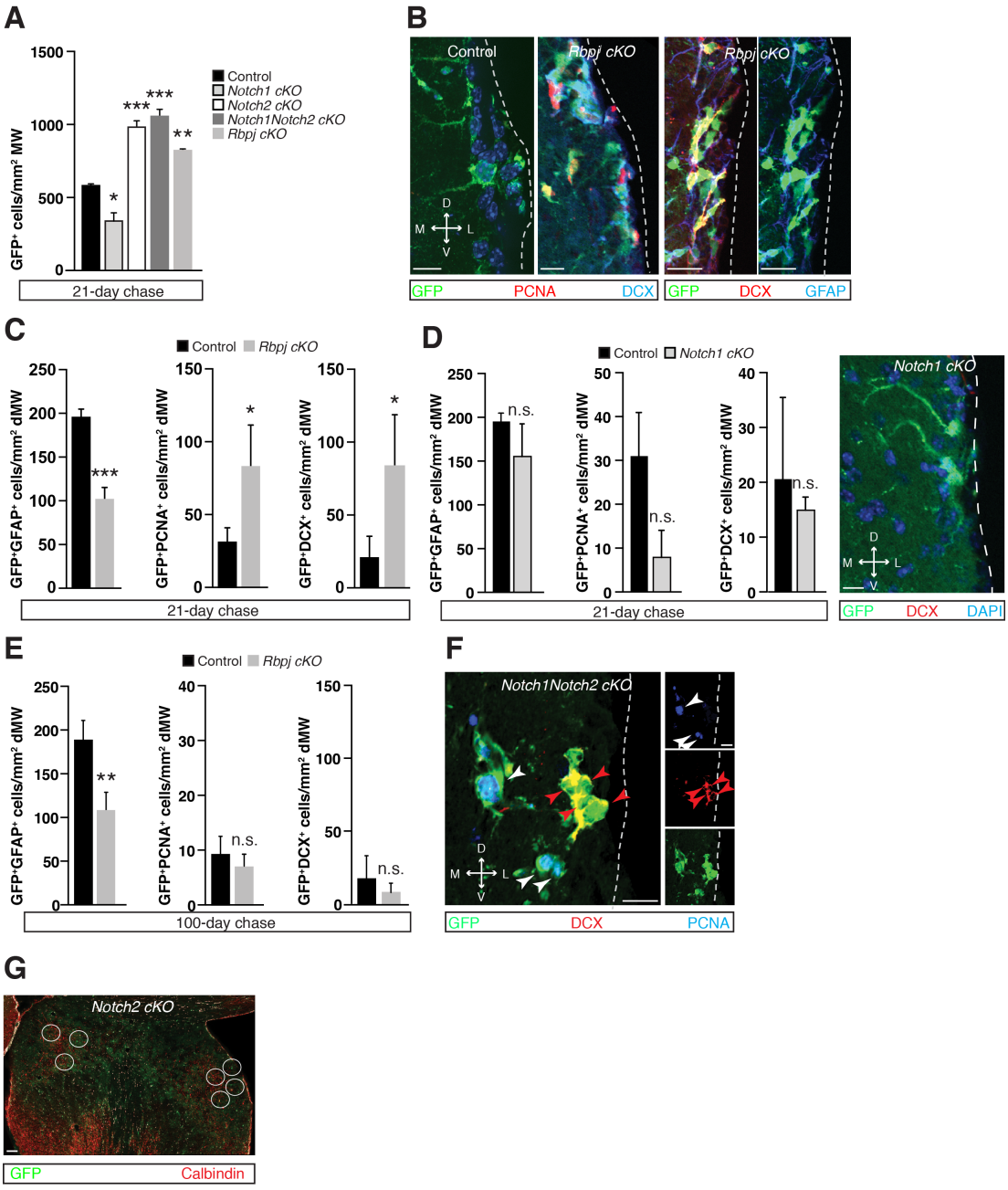

A
B
C
D
E
F
G
Control
Notch1 cKO
Notch2 cKO
Notch1Notch2 cKO
Rbpj cKO
GFP+ cells/mm² MW
21-day chase
Control
Rbpj cKO
Notch1 cKO
GFP PCNA DCX
GFP DCX GFAP
GFP DCX DAPI
GFP+GFAP+ cells/mm² dMW
GFP+PCNA+ cells/mm² dMW
GFP+DCX+ cells/mm² dMW
n.s.
100-day chase
GFP DCX PCNA
GFP Calbindin

Figure S6

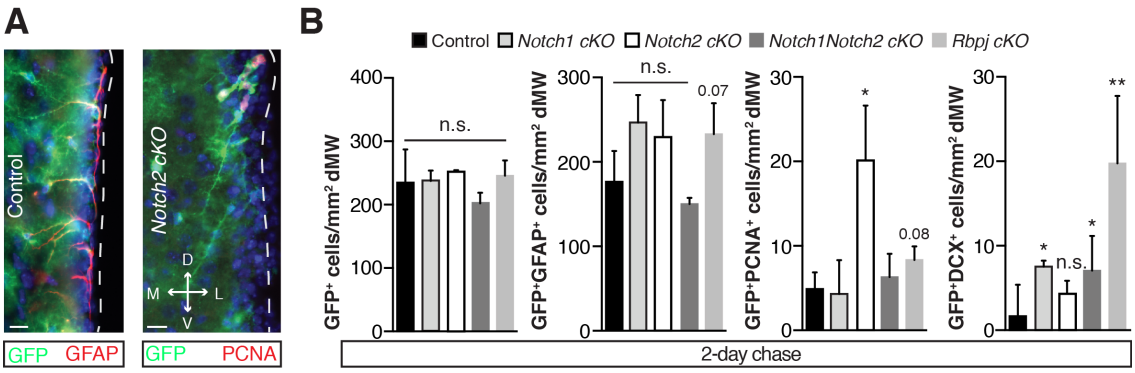
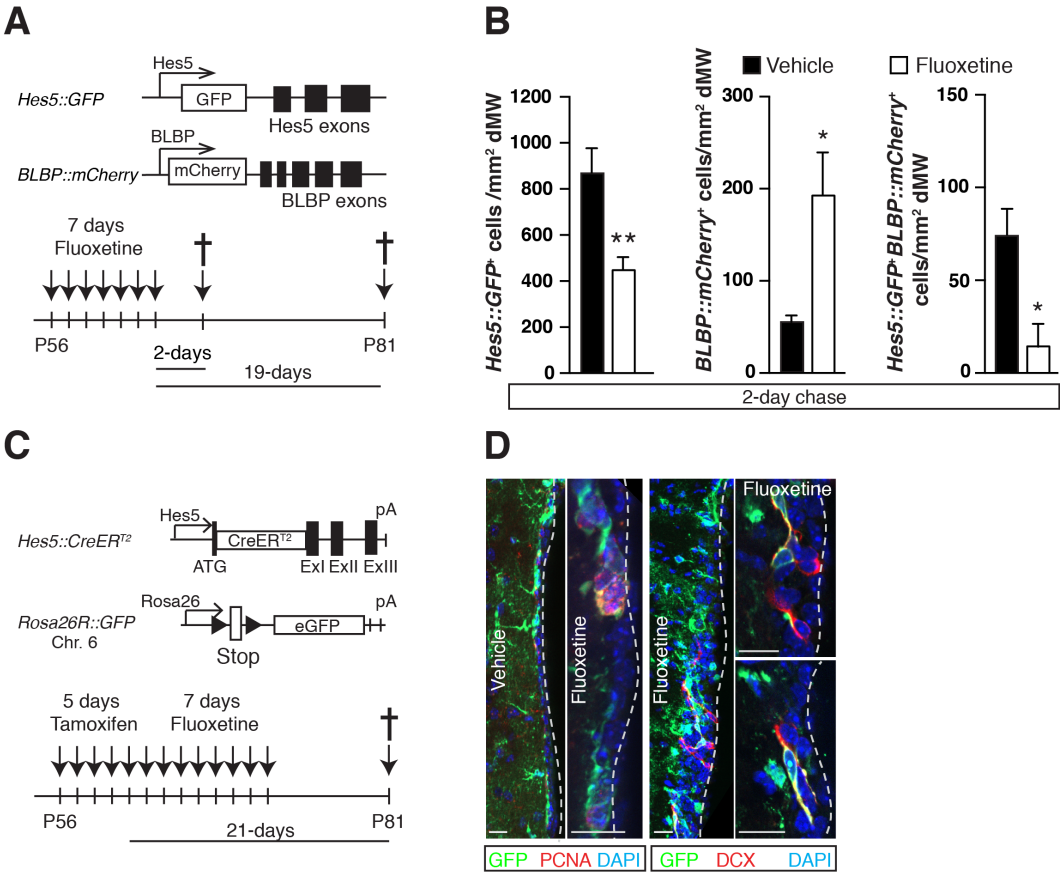


Figure S7



Supplemental Data Tables

Supplemental Data Tables refer to Figure 1-7 and Supplemantal Figures S1-S7 and Table S8, containing Top Ten GO Raw Data and Rbpj Trace, relating to Figurte 3 and S3 **can be found on the CD of this thesis.**

Supplemental Experimental Procedures

Animals and husbandry

Hes5::GFP, Hes5::CreER^{T2}, Notch2::CreER$^{T2\text{-}SAT}$, Rosa25R::GFP, Rosa25R::tdTomato, floxed *Notch1,* floxed *Notch2,* floxed *Rbpj* mice have been described elsewhere (Basak et al., 2012; Basak and Taylor, 2007; Besseyrias et al., 2007; Fre et al., 2011; Lugert et al., 2012; Schouwey et al., 2007). Mice were maintained on a C57Bl6 genetic background and kept on a 12-hour day/night cycle with food and water *ad libitum* under specified pathogen free conditions and according to Swiss Federal and Swiss Veterinary office regulations under license numbers 2537 and 2538 (Ethics commission Basel-Stadt, Basel Switzerland).

Administration of Tamoxifen and Fluoxetine and tissue preparation for immunochemical staining

Adult mice 8-10 weeks of age were used in the experiments. *Hes5::CreER^{T2}* mice carrying floxed *Rbpj*, floxed *Notch1* or floxed *Notch2* alleles were injected daily intraperitoneal (i.p.) with 2mg Tamoxifen in corn oil (100 µl of 20 mg/ml) for five consecutive days and killed 2, 21, 100 or 300 days after the end of the treatment. 8-10 weeks old *Hes5::GFP*, *BLBP::mCherry* animals were administered Fluoxetine (18 mg/kg) intraoral (i.o.) doses, for seven consecutive days and were killed 2 or 19 days after the treatment. Control animals received gelatin. A cohort of *Hes5::CreER^{T2}* animals underwent a double treatment of five days i.p injection of TAM and seven days i.o. treatment with Fluoxetine, respectively gelatine. Animals were injected i.p. with a lethal dose of Ketamin-xylazine and perfused with ice-cold phosphate buffered saline (PBS) followed by 4% PFA in PBS. Brains were excised, fixed overnight in 4% PFA in PBS, cryoprotected with 30% sucrose in PBS at 4°C 48 hours, embedded and frozen in OCT (TissueTEK), and 30 µm floating sections cut by cryostat (Leica). For whole-mount immunostaining of the dMW, brains of mice were excised and fixed overnight in 4% PFA in PBS, washed in PBS followed by micro-dissection under a binocular, and immunostained as described previously (Mirzadeh et al., 2008).

Ex vivo Microarray Analysis of Tamoxifen induced, recombined cells

Adult mice 8-10 weeks of age were used in the experiments. *Hes5::CreER^{T2}* mice carrying floxed *Notch2* alleles were injected daily intraperitoneal (i.p.) with 2mg Tamoxifen as stated previously. After five days consecutive administration animals were sacrificed 24 hours after the end of the treatment. Animals were euthanized in CO2, brains were dissected in L15 Medium (GIBCO) and cut into 0.55 mm thick sections using a McIllwains tissue chopper. The SVZ was microdissected under a binocular microscope avoiding contamination from the striatum, and digested using a Papain solution an mechanical dissociation (previously described – Lugert et al, 2010). Cells were resuspended in Leibovitz medium (Life Technologies), filtered through a 40 µm cell strainer (Miltenyi Biotec) and sorted on a BD FACS Aria III. Cells were discriminated by forward and side-scatter (for live cells – from the control) and gated for GFP-negative (wild-type levels) or GFP^{+} populations. Cells were directly sorted into cooled Trizol (Life Technologies). Appropriate amount of Chloroform was added and RNA extraction was performed using Isopropanol with LiCl (0.75M). RNA was immediately frozen to -80°C. RNA quality was tested on a Fragment Analyzer (Advanced Analytical) using a high sensitivity RNA

analysis kit (DNF-472). Samples were sent for Expression Profiling with Atlas Biolabs. Samples were subjected to a second quality control on an Agilent 2100 Bioanalyzer, small samples were amplified using the Ovation Picokit (NuGen) and then run on an Affymetric Biochip. GO analysis was done using DNAStar Lasergene Arraystar (DNAStar) software.

Predicting RBPJ binding sites in mouse promoter regions genome-wide

We curated a comprehensive set of mouse promoters (using the GRCm38/mm10 assembly) by combining data from CAGE experiments with Gencode annotations. In particular, we obtained a list of transcription start site (TSS) coordinates for mouse mRNA and lincRNA transcripts from Gencode (Harrow et al., 2006). We then complemented this set of putative TSSs with the robust CAGE peaks from (Consortium et al., 2014) which were converted to mm10 coordinates using liftOver (Hinrichs et al., 2006). We then created a set of promoter regions plus associated transcripts from this data using the following clustering procedure:

1. Initially, each CAGE peak and each TSS of a Gencode transcript were placed in a separate cluster.
2. At each iteration, the two nearest clusters were joined under the constraint that there can be no more than one CAGE peak per cluster. The distance between two clusters is defined as the distance of their nearest pair of TSSs.
3. Clustering stopped when there were no more clusters within 150 nucleotides of each other (i.e. roughly a single nucleosome).
4. All clusters that contained at least 1 transcript from Gencode were retained, i.e. CAGE peaks without associated transcripts were discarded.

Using this procedure, we obtained 30'114 mouse promoters, where each promoter corresponds to the genomic region spanned by the TSSs in the corresponding cluster.

A position specific weight matrix motif (WM) describing the binding specificity of the RBPJ transcription factor (TF) was obtained from the SwissRegulon collection of mammalian WMs (Pachkov et al., 2013). For each promoter, the promoter region was defined as the promoter plus 500 nucleotides upstream and 500 nucleotides downstream of the promoter. Binding sites for RBPJ were annotated in each promoter region as follows. For any potential binding segment of length l (where l=16 for RBPJ), a WM score was calculated as

$S_0=\sum_{i=1}^{\log((w_{s_i}^i)/b_{s_i})}$, where s_i is the nucleotide occurring at position i in the length-l segment, $w_{s_i}^i$ is the WM entry at position i for this nucleotide, and b_{s_i} is the background probability for the same nucleotide. Here we have simply set $b_s=1/4$ for all nucleotides. To account for the fact that TFs are also attracted to the DNA by an electrostatic binding force that is not sequence specific, we transformed this score as follows:

$S=\log(e^{S_0}+e^{E_0})$, where we have set the non-specific binding energy E_0 equal to zero. The posterior probability P for the segment corresponding to a binding site is then given in terms of the score S as

$P=e^{S_0+\tau_0}/(1+e^{S_0+\tau_0})$, where τ_0 is a parameter accounting for the (unknown) log-concentration of the TF. We set this concentration parameter τ_0 so as to maximize the

variance in the probability of binding site occurrence across all promoters. In particular, the probability Q_x for a given promoter x to have at least one binding site is given by:

$Q_x=1-\prod_{(s \in x)} P_s$, where the product is over all sequence segments s in promoter x, and P_s is the posterior probability that s is a binding site. We set τ_0 so as to maximize the variance of Q_x across the promoters. Finally, we discard all binding sites with a posterior less than 0.1. Using this procedure we obtained 21'087 RBPJ binding sites across the 30'114 mouse promoters.

Quantitative PCR confirmation of Notch2 knockout

Ex vivo mRNA was prepared as described above. Isolated RNA was treated with DNaseI (Roche). cDNA was prepared using BioScript (Bioline) and random hexamer primers. qPCR was performed using SensiMix SYBR kit (Bioline). Primers for PCR reactions are as follows:

GAPDH	Fwd: CTCCCACTCTTCCACCTTCG
	Rev: CCACCACCCTGTTGCTGTAG
β−Actin	Fwd: AGGTGACAGCATTGCTTCTG
	Rev: GGGAGACCAAAGCCTTCATA
Notch2 (Exon 26/27)	Fwd: CAGGAGGTGATAGGCTCTAAG
	Rev: GAAGCACTGGTCTGAATCTTG

Immunofluorescence staining of floating sections and antibodies

Immunostaining on sections was performed as described previously (Giachino and Taylor, 2009; Lugert et al., 2010). Briefly, sections were blocked at room temperature for 30 minutes with 10% normal donkey serum or normal goat serum (Jackson Immunoresearch) in PBS containing 0.5% TritonX-100. Primary antibodies diluted in 2.5% donkey serum blocking solution were incubated overnight. Sections were washed with PBS and incubated at room temperature for 1-2 hours with the corresponding secondary antibodies in 5% donkey serum blocking solution and counter-stained with DAPI (1 μg/ml). Sections were mounted on glass slides (SuperFrost, Menzel) in DABCO mounting media and visualized using a Zeiss Observer with Apotome (Zeiss). For PCNA detection, the antigen was recovered at 80°C for 20 minutes in Sodium Citrate (10 mM, pH7.4).

Primary antibodies used were as follows: Anti-β-Catenin (rabbit, 1:1000, Sigma, C2206), anti-Calbindin D28k (rabbit, 1:5000, Swant, 300), anti-Calretinin (rabbit, 1:5000, Swant, 7609/4), anti-CD31 (Rat, 1:500, BD Pharmingen), anti-Doublecortin (goat, 1:500, Santa Cruz, sc-8066), anti-dsRed (rabbit, 1:500, CloneTech Takara, 632496), anti-Glial fibrillary acidic protein (mouse, 1:500, Sigma, G3893), anti-Glial fibrillary acidic protein (rabbit, 1:1000, Sigma, G9269), anti-Green fluorescent protein (chicken, 1:250, AvesLab, GFP-1020), anti-GFP (rabbit, 1:500, Invitrogen, A11122), anti-GFP, (sheep, 1:250, AbD Serotec, 4745-1051), anti-Neuronal nuclear antigen (mouse, 1:800, Millipore, MAB377), anti-Parvalbumin (mouse, 1:5000, Swant, Mc-AB235), anti-Proliferating cell nuclear antigen (mouse 1:1000, DAKO, M0879), anti-S100β

(rabbit, 1:1000, Swant, 37), anti-Sox2 (goat, 1:250, Santa Cruz, sc-17320), anti-Notch2 (rat, H. Robson Lausanne, 1:200).

Secondary antibodies used were as follows: Donkey anti-rabbit Ig Cy3 conjugated (1:500, Jackson Immunoresearch, 711165152), donkey anti-mouse Ig Cy3 conjugated (1:500, Jackson Immunoresearch, 715165151), donkey anti-rabbit Ig Cy5 conjugated (1:300, Jackson Immunoresearch, 711496152), donkey anti-mouse Ig Cy5 conjugated (1:300, Jackson Immunoresearch, 715175151), donkey anti-rabbit Ig 488 conjugated (1:500, Jackson Immunoresearch, 711545152), donkey anti-sheep Ig 488 conjugated (1:500, Jackson Immunoresearch, 713095147), donkey anti-goat Ig Cy3 conjugated (1:500, Jackson Immunoresearch, 705165147), and donkey anti-rat Ig Cy3 conjugated (1:500, Jackson Immunoresearch, 712160153).

Generation of adeno-*gfap*::Cre virus particles

Generation of adeno-*gfap*::Cre virus was described previously (Merkle et al., 2007). Briefly, Cre was placed under the control of the mouse *gfap* promoter (GFAPp) previously confirmed to be specifically active in $GFAP^+$ cells. The pAd/PLGFAPp- NLSCre-pA vector was transfected into HEK293 cells to produce replication-defective adenovirus, which was purified twice by cesium chloride banding. The titer was 1 x 10^{12} infectious particles/ml.

Stereotactic injection of adeno-*gfap*::Cre virus particles

Adult (8-10 week old) mice were anesthetized in a constant flow of Isoflurane (1-3%) in oxygen and immobilized on a stereotaxic apparatus (David Kopf instruments)(Giachino and Taylor, 2009). Mice were injected with Temgesic subcutaneous (0.05 mg/kg body weight). The skull was exposed by an incision in the scalp and a small hole (1 mm) drilled through the skull. Animals were stereotactically injected with 1 mL of titrated adeno-*gfap*::Cre virus (titer 1 x 1012 infection particles per ml) in saline, 0.1% bovine serum albumin using sharpened Borosilicate glass capillaries (Kwick-FilTM) at the coordinates: anterior/posterior 0 mm; medial/lateral 0 mm; dorsal/ventral 2.5 mm below the skull and relative to Bregma. Wounds were closed using surgical clips. One day after the surgery the animals received a second dose of Temgesic subcutaneous (0.05 mg/kg body weight) and were analyzed 21 days post-injection.

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Notch2 Maintains Adult Neural Stem Cell Quiescence in the Hippocampal Subgranular Zone

Authors: Runrui Zhang, **Anna Engler**, Claudio Giachino, Ichiko Saotome, Angeliki Louvi, Ursula Zimber-Strobl, Verdon Taylor; in preparation

Contribution: I planned, conducted and analyzed the Notch levels in the DG and prepared the corresponding figure. I planned and conducted Tamoxifen administrations for the conditional knock-out animals used for IHF and FACS experiments. I set and operated the FACS for the experiments.

Summary

We demonstrated, that the deletion of Notch2 from adult NSCs causes an activation of quiescent NSC population in the SVZ and an otherwise dormant niche the dMW. This was due to the activation of quiescent NSCs (Engler et al., in preparation). Based on these data we investigated the role of Notch2 in the neurogenic hippocampal DG. We were able to show that in the DG, as the SVZ, the expression of Notch1 and Notch2 overlap on Hes5 expressing cells. To date, the role of Notch2 has not been addressed in the DG. In order to analyze the role of Notch2 in the hippocampal DG, we deleted the *Notch2* gene from Hes5 expressing NSCs in the subgranular zone and traced their fate.

Notch2 deletion caused an activation of the quiescent NSCs in the DG of adult and geriatric mice. The activation of NSC proliferation led to the production of neuroblasts and a depletion of the quiescent NSC pool. In contrast, the overexpression of Notch2 intracellular domain led to an arrest of proliferation in the DG. Overall the results indicate that Notch2 is a key signal to maintain NSCs in a quiescent state in the adult brain.

Contribution

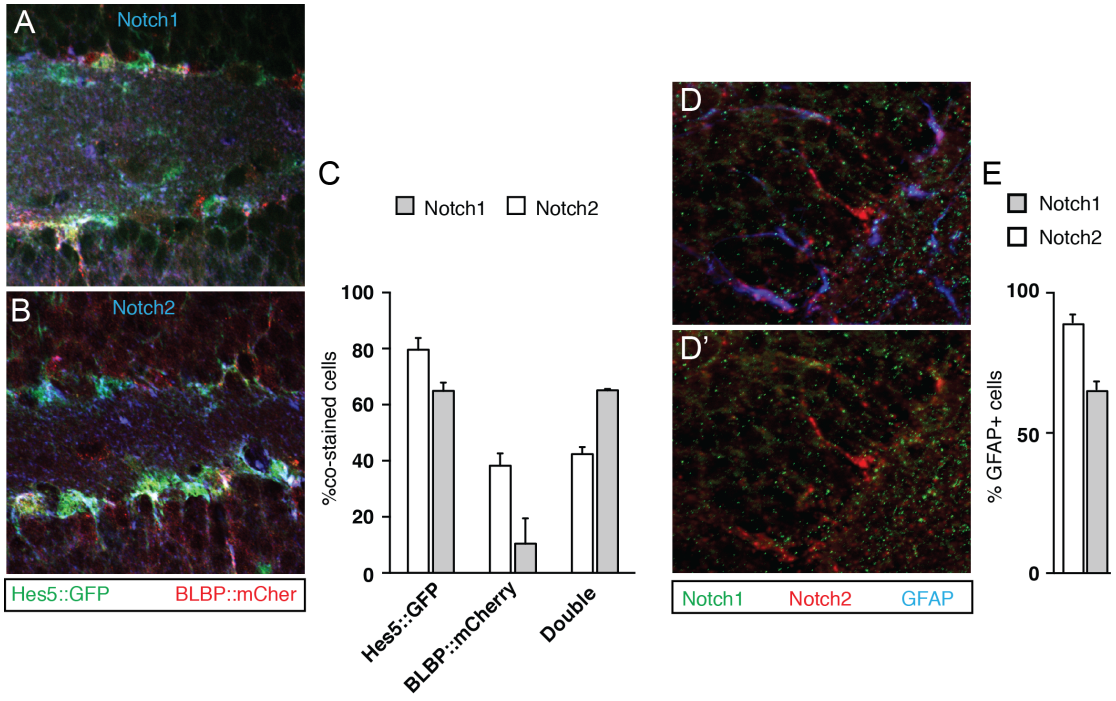

Figure: Notch1 and Notch2 are coexpressed by GFAP$^+$ cells in the DG SGZ

Immunohistochemistry of double transgenic *Hes5::GFP*, *BLBP::mCherry* animals co-stained with Notch1 (A) or Notch2 (B) antibodies. Quantification of the co-stained cells showed significant overlap of Notch1 and Notch2 with a slight preference for Notch2 expression by *Hes5::GFP*$^+$*BLBP::mCherry*$^-$ quiescent NSCs and *Hes5::GFP*$^-$ *BLBP::mCherry*$^+$ IPs and a slight increase in Notch1 expression by *Hes5::GFP*$^+$*BLBP::mCherry*$^+$ active NSCs relative to Notch2. Notch1 and Notch2 are prominently coexpressed by GFAP$^+$ cells (D) Notch1 and Notch2 overlap (D'). Almost all GFAP$^+$ radial Type-1 DG NSCs express Notch2 (E).

Adult Hippocampal Heterogeneity and its Modulation Under Physiological and Pathological Conditions

Authors: Anna Engler, Chiara Rolando, Claudio Giachino, Andrea Erni, Onur Basak, Verdon Taylor; in preparation for Glia

Contribution: I planned, conducted and analyzed the experiments, prepared all the figures and the manuscript. CR conducted the Fluoxetine experiments.

Summary

Recent works have highlighted the cellular heterogeneity within the SVZ (Codega et al., 2014; Giachino et al., 2014b). In the SGZ of the adult DG, NSCs (Type-1 cells) produce intermediate progenitor cells (IP, Type-2a), which retain expression of some stem and progenitor markers and therefore it is difficult to unequivocally distinguish them. In addition, two morphologically different types of NSCs, radial and horizontal exist in the DG (Lugert et al., 2010). Our previous results have shown that quiescent and active NSCs have distinct requirements for maintenance both in the SVZ (Engler et al., in preparation), and the DG (Zhang et al., in preparation). The detailed mechanisms of NSC maintenance in the DG are only poorly understood. We addressed the question whether distinct DG NSC subpopulations respond differently to external stimuli. We aimed to discriminate different NSC populations and we hypothesized that they might be reacting differently to aging, epilepsy and antidepressant.

The maintenance of quiescent and active NSCs is Notch signaling dependent. Thus, we used the Notch signaling reporters, *Hes5::GFP*, *BLBP::mCherry* double transgenic animals to analyze DG SGZ cellular composition in young and aged animals. We found a high level of NSC heterogeneity in the DG. Active NSCs, characterized by *Hes5::GFP*, *BLBP::mCherry* coexpression are lost upon aging. This active NSC pool can be replenished by induction of status epilepticus. The antidepressant and 5-HT uptake inhibitor Fluoxetine leads to the activation of *Hes5::GFP*$^{-}$ *BLBP::mCherry*$^{+}$ IPs, however, the NSCs remain unaffected.

We concluded that hippocampal NSCs exhibit a high level of heterogeneity. Quiescent and active NSCs respond differently to distinct pathophysiological stimuli. However, it remains unclear what are the molecular mechanisms behind NSC quiescence and activity and will be the scope of future investigation.

Manuscript

Heterogeneity of Adult Hippocampal Neurogenesis and its Modulation Under Physiological and Pathological Conditions

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Main Points:

- *Hes5::GFP*, *BLBP::mCherry* double transgenic animals highlight hippocampal heterogeneity, discriminating quiescent and active NSCs as well as IPs
- *Hes5::GFP*, *BLBP::mCherry* expression allow for direct ex vivo sorting of the three distinct progenitor populations
- *Hes5::GFP*, *BLBP::mCherry* double transgenic animals allow to analyze the reacting populations upon pathophysiological stimuli

TOCI: (Figure 0)

Key Words: Hes5, BLBP, ageing, seizures, kainic acid, antidepressant, fluoxetine

Abstract

OBJECTIVES: Adult neural stem cells (NSCs) are found in the adult hippocampal dentate gyrus (DG). We addressed the level of heterogeneity in the neurogenic DG and aimed to discriminate different stem cell populations and their responsiveness to ageing, epilepsy and antidepressant treatment.

EXPERIMENTAL DESIGN: We used *Hes5::GFP*, *BLBP::mCherry* double transgenic mice to analyze hippocampal heterogeneity in physiological conditions in young and aged animals, as well as in pathophysiological conditions such as seizure induction by kainic acid and manipulation of serotonin levels by administration of Fluoxetine.

PRINCIPAL OBSERVATIONS: We found a high level of heterogeneity in the DG niche. Active NSCs, characterized by *Hes5::GFP* and *BLBP::mCherry* coexpression, are lost upon ageing and can be induced by kainic acid (KA) induced seizures whereas quiescent *Hes5::GFP*$^{+}$*BLBP::mCherry*$^{-}$ NSCs remain. The antidepressant fluoxetine leads to the activation of *Hes5::GFP*$^{-}$*BLPB::mCherry*$^{+}$ transient amplifying progenitors most prominently in the young DG.

CONCLUSION: The hippocampal NSC pool exhibits a high level of heterogeneity. Quiescent and active NSCs respond to distinct (patho-) physiological stimuli in distinct manners. The here presented animals provide a tool for quick, direct screening of effects on hippocampal neurogenesis.

Text:

INTRODUCTION

The adult central nervous system contains neural stem cells (NSCs) that can replace postmitotic cells within restricted brain regions (Gage, 2000). Adult NSCs reside in the ventricular zone of the lateral ventricle wall (SVZ) and the subgranular zone (SGZ) in the hippocampal dentate gyrus (DG). Adult neurogenesis occurs throughout life in both regions and it is regulated by intrinsic and extrinsic mechanisms. In the SGZ new neurons are generated from NSCs throughout life in rodents (Kempermann et al., 2004) and also in humans (Eriksson et al., 1998; Spalding et al., 2013).

Neurogenesis is a dynamic process responsive to external stimuli, including epilepsy, ischemia, physical activity, learning, drug addiction stress, and depression (Abrous et al., 2005; Kempermann, 2015). Also, neurogenesis diminishes with age and it might be a result of decreased activity and/or depletion of NSCs (Encinas and Sierra, 2012; Lugert et al., 2010). Following injury or pathological challenge, NSCs can respond by increasing proliferation and differentiation, even in the aged brain. Seizures (SE) are associated with increased number of proliferating, neurogenic cells. Interestingly, seizures increase the activation of quiescent cells, recruiting them into an active state even in the aged DG (Lugert et al., 2010).

Prolonged seizures decrease adult neurogenesis possibly due to an exhaustion of NSCs. Seizures induce massive release of neurotransmitters (NT), neurotrophins and small signaling molecules, which are known to modulate neurogenesis (Sierra et al., 2015). Besides pathological stimuli, the administration of drugs including the 5-HT uptake inhibitor Fluoxetine, has also been shown to have an effect on the SGZ. NSCs seem to be in close proximity to serotonergic axons. The administration of antidepressants leads to an increase in symmetric divisions of early progenitor cells (Encinas et al., 2006).

The adult NSCs in the SGZ responsible for the changes observed in neurogenesis are defined as type-1 cells and are subdivided into radial (Kempermann et al., 2004) and horizontal (Lugert et al., 2010; Steiner et al., 2006; Suh et al., 2007) populations. The radial type NSCs display characteristics of quiescent NSCs, whereas the horizontal NSCs are more proliferative and therefore more frequently express the cell cycle marker PCNA. NSCs are able to self-renew and give rise to differentiated progeny. Clonal analysis showed that single NSCs have the potential to activate, return to quiescence and reenter the cell cycle (Bonaguidi et al., 2011; Encinas et al., 2011). These complex dynamics in the DG NSC population make it critical to develop specific tools to examine individual stem cell subpopulations and states

(Bond et al., 2015). Type-1 NSCs produce intermediate Type-2a progenitor cells. Upon neuronal determination, Type-2b cells express NeuroD1 and Doublecortin (Dcx) (Steiner et al., 2006). Type-2b cells generate Type-3 neuroblasts, which exit cell cycle before fully maturing into granule neurons.

In the SGZ the NSCs and progeny are found in direct cell-cell contact within their niche. In this context active Notch signaling promotes NSC maintenance (Ables et al., 2011). Canonical Notch signaling leads to the transcription of Notch target genes of the Hes/Hey family. Among these, expression of Hes5 is relatively restricted to NSCs. Hes5 expressing NSCs in the DG can be subdivided into radial, quiescent NSCs and horizontal more active NSCs (Lugert et al., 2010). Activated NSCs in the SVZ express brain lipid binding protein (BLBP) (Giachino et al., 2014b), another direct Notch signaling target (Anthony et al., 2005). In order to determine the level of heterogeneity in the DG and discriminate quiescent and active NSCs within their niche we analyzed *Hes5::GFP BLBP::mCherry* double-transgenic animals.

MATERIALS AND METHODS

Animals

Transgenic mice with a C57BL/6 background expressing GFP under the Hes5 promoter (Basak and Taylor, 2007) and mCherry under the BLBP promoter (Giachino et al, 2013) were used at 8 weeks and 52 weeks. The genotypes of the mutants were confirmed by PCR analysis of genomic DNA. All experiments were performed in accordance with the guidelines of the Swiss Veterinary office and approved by the Canton council (2538 and 2537).

Tissue Generation

Animals were euthanized with Ketamin-Xylazine and intracardial perfusion was performed using PBS and 4% freshly prepared PFA in PBS. Perfused animals were decapitated and brains were isolated. The tissue was cryoprotected in 30% Sucrose in PBS. Tissue was cut in 30 μm thick coronal sections and used for immunohistochemistry.

Immunohistochemistry

Immunostaining on sections was performed as described previously(Giachino and Taylor, 2009; Lugert et al., 2010). Briefly, sections were washed thoroughly and blocked at room temperature for 30 minutes (with 10% normal donkey serum (Jackson Immunoresearch) in PBS containing 0.5% TritonX-100. Primary antibodies diluted in 2.5% normal donkey serum blocking solution were incubated overnight. Sections were washed with PBS and incubated at room temperature for 1-2 hours with the corresponding secondary antibodies in 5% normal donkey serum blocking solution and counter-stained with DAPI (1 μg/ml (roughly)). Sections were mounted on glass slides (SuperFrost, Menzel) in DABCO mounting media and visualized using a Zeiss Observer with Apotome (Zeiss),. For PCNA detection, the antigen was recovered at 80°C for 20 minutes followed by 25°C for 45 minutes in Sodium Citrate (10 mM, pH7.4).

Primary antibodies used were as follows: anti-BrdU (rat, 1:1000, AbSerotec, OPT0030), anti-Doublecortin (goat, 1:500, Santa Cruz, sc-8066), anti–dsRed (rabbit, 1:500, CloneTech Takara, 632496), anti-Glial fibrillary acidic protein (mouse, 1:500, Sigma, G3893), anti-Glial fibrillary acidic protein (rabbit, 1:1000, Sigma, G9269), anti-Green fluorescent protein (chicken, 1:250, AvesLab, GFP-1020), anti-GFP (rabbit, 1:500, Invitrogen, A11122), anti-GFP, (sheep, 1:250, AbD Serotec, 4745-1051), anti-Proliferating cell nuclear antigen (mouse 1:1000, DAKO, M0879), anti-S100b (rabbit, 1:1000, Swant, 37), anti-Sox2 (goat, 1:250, Santa Cruz, sc-17320), anti-Tbr2 (rabbit, 1:500, Abcam, AB23345). Secondary antibodies

used were as follows: Donkey anti-rabbit Ig Cy3 conjugated (1:500, Jackson Immunoresearch, 711165152), donkey anti-mouse Ig Cy3 conjugated (1:500, Jackson Immunoresearch, 715165151), donkey anti-rabbit Ig Cy5 conjugated (1:300, Jackson Immunoresearch, 711496152), donkey anti-mouse Ig Cy5 conjugated (1:300, Jackson Immunoresearch, 715175151), donkey anti-rabbit Ig 488 conjugated (1:500, Jackson Immunoresearch, 711545152), donkey anti-sheep Ig 488 conjugated (1:500, Jackson Immunoresearch, 713095147), donkey anti-goat Ig Cy3 conjugated (1:500, Jackson Immunoresearch, 705165147), and donkey anti-rat Ig Cy3 conjugated (1:500, Jackson Immunoresearch, 712160153).

Quantification and statistical analysis

Stained sections were analyzed with fixed photomultiplier settings on a Zeiss Observer with Apotome (Zeiss). Images were processed with Photoshop or ImageJ. Data are presented as averages of a minimum of three sections per region and multiple animals (n in figure legends). Statistical significance was determined by Student's T-test on mean values per animal, and two-way ANOVA for cross-comparison of 3 and more data sets. Significance was determined at * - $P<0.05$, ** - $P<0.01$, *** - $P< 0.001$ or P values are given in the graphs. Deviance from mean is displayed as standard deviation if not otherwise indicated.

FACS

Adult Hes5::GFP, BLBP::mCherry animals (8 weeks or 52 weeks) were killed in a CO_2 atmosphere and decapitated. The brain was isolated and DG was microdissected from 0.5 mm vibratome sections The tissue was dissociated in Papain:Ovomucoid at 37ºC. After dissociation Ovomucoid was additionally added, the sample was filtered through a 30µm filter and centrifuged (5min, 1000 rpm). The supernatant was removed and cells were resuspended in Leibowitz Medium without Phenolred. Single cells were analyzed and 3 populations, GFP high, BLBP high and GFP and BLBP high all from endogenous fluorescent protein expression discriminated. We would like to stress the importance of the appropriate age-matched negative controls. Autofluorescence of isolated cells is increased in aged animal.

Seizure Induction

Animals obtained a single dose i.p. injection with Kainic Acid (10mM) (ToCris, Cat.No° 0222/65). Young animals obtained a 20mg/kg dose, aged animals 15mg/kg and were monitored for 2 hours. Seizure severity was determined according to previously set standards in which 1 represented an injected mouse without phenotype and 6 a mouse with severe seizures. Animals in analyses were required to reach seizure level 4, which was identified by

prolonged freezing and uncontrolled, seated seizing. Animals were sacrificed after 4 days and tissue was used for immunohistochemical analysis.

Fluoxetine Treatment

8-10 week old and 52 week old Hes5::GFP, BLBP::mCherry animals obtained daily intraoral (i.o.) doses of 18mg/kg Fluoxetine (Gelatine in Vehicle controls), for seven consecutive days and were killed 2 days after the end of treatment. A cohort of 8-10 week old *Hes5::GFP*, *BLBP::mCherry* animals underwent a 19-day chase experiment.

RESULTS

Comparative analysis of adult hippocampal dentate gyrus heterogeneity of young and aged animals

We analyzed the DGs of adult, 8-week old *Hes5::GFP BLBP::mCherry* double positive animals (GFP^+ $mCherry^+$). We observed GFP^+ $mCherry^+$, GFP^+ $mCherry^-$ and GFP^- $mCherry^+$ cell subpopulations (Figure 1A, Figure 1E), similar to what we observed previously in the SVZ (Giachino et al., 2014b). GFP^+ $mCherry^-$ cells had a radial morphology and expressed the astrocytic marker GFAP whereas GFP^+ $mCherry^+$ cells had a horizontal morphology and did not express GFAP (Figure 1B). GFP^+ $mCherry^+$ cells and GFP^- $mCherry^+$ cells were more frequently positive for the proliferative marker PCNA than GFP^- $mCherry^+$ cells (Figure 1C).

Of the GFP^- $mCherry^+$ population, a large proportion was $Tbr2^+$, characterizing them as Type-2b cells and some of expressed Dcx indicating that they were Type-3 neuroblasts. These $GFP^-mCherry^+Dcx^+$cells were morphologically distinguishable from $GFP^-mCherry^-Dcx^+$ only, type-3 neuroblasts (Figure 1D) 90% of the total GFP^- $mCherry^+$ cells were co-stained for BLBP protein validating the transgene expression. The few $mCherry^+$ cells that were negative for BLBP immunostaining were all Dcx^+ with a typical morphology of newly generated neuroblasts suggesting perdurance of the mCherry protein or lower sensitivity of the antibody staining (data not shown). Thus, using *Hes5::GFP, BLBP::mCherry* animals we could subdivide NSCs (GFP^+) and their immediate progeny GFP^- $mCherry^+$ into subpopulation with distinct antigenic and proliferative properties.

Upon advanced aging neurogenesis decreases in the DG (Jessberger et al., 2007b). We compared the DG of 8-week old young adult *Hes5::GFP BLBP::mCherry* animals with aged (52-week old) and geriatric (78/102-week old) animals (Supplementary Figure 1A, B). The

number of GFP$^+$ mCherry$^+$ and GFP$^-$ mCherry$^+$ cells was drastically reduced in the aged animals. In contrast, the number of GFP$^+$ mCherry$^-$ cells was only slightly reduced (Figure 1E). The remaining GFP$^+$ mCherry$^-$ cells frequently showed a radial rather than horizontal morphology, suggesting maintenance of the quiescent NSCs and a potential transition from an active to a more quiescent state within the NSC pool (Supplementary Figure 1B).

The decrease of cell proliferation in aged DG prompted the question how proliferation is changing in the fluorescently labeled cell populations. We examined cell proliferation with proliferating cell nuclear antigen (PCNA) as a marker of proliferating cells (Figure 1F) and short BrdU pulse analysis. In young animals, the GFP$^+$ mCherry$^+$ NSCs were actively dividing, 23.2% incorporated BrdU in a 2-hours pulse and 78.1% were positive for PCNA (Supplementary Figure 1F, G). Most GFP$^+$ mCherry$^-$ NSCs have an astrocytic character (84.2%), expressing glial fibrillary acidic protein (GFAP) (Supplementary Figure 1F). Of these GFP$^+$mCherry$^-$GFAP$^+$ cells only a fraction (16.8%) co-stained for the astrocyte marker S100β in young mice. The number of S100β$^+$ cells in the DG was slightly increased with age suggesting astrocytosis (Supplementary Figure 1E). Although the number of proliferating cells was significantly reduced with age and the number of GFP$^+$ mCherry$^+$ cells drastically reduced (Figure 1E, F, G), a fraction of the remaining double positive cells was still proliferating albeit to a lower extent (Supplementary Figure 1F, G). All classes of cells, except radial type-1 cells, were significantly reduced in the aged animals (Figure 1 E-I, K Supplementary Figure S1A-C).

We further validated these data by *ex vivo* FACS analysis using the transgenic animals expressing fluorescent proteins GFP and mCherry (Figure 1J). GFP$^+$ mCherry$^-$ quiescent NSCs were the largest population of transgene expressing cells in the SGZ, followed by GFP$^-$ mCherry$^+$ IPs and GFP$^+$ mCherry$^+$ active NSCs. In aged animals these ratios were drastically changed. While the GFP$^+$ mCherry$^-$ population was not reduced, the GFP$^-$ mCherry$^+$ and GFP$^+$ mCherry$^+$ cells were barely detectable (Figure 1K), supporting our conclusions from the histological analysis that quiescent NSCs remain in the aged DG but active NSCs and IP are lost (Figure 1E). Thus, active NSCs (GFP$^+$ mCherry$^+$) and IPs (GFP$^-$ mCherry$^+$) are lost during aging, but the quiescent NSCs (GFP$^+$ mCherry$^-$) remain largely unaffected.

Change in Hippocampal Composition upon Seizures Induction by Kainic Acid Treatment

Epileptic seizures are associated with a loss of hippocampal neurons and increased proliferation of SGZ NSCs (Parent, 2007). In the adult mouse, models of temporal lobe epilepsy, seizures (SE) increases progenitor proliferation in the DG (Lugert et al., 2010;

Parent et al., 1997; Sierra et al., 2015). We addressed whether the fluorescently labeled, distinct DG populations respond differently to pathological activating stimuli and administered epileptogenic kainic acid (KA) systemically to stimulate seizures in young and aged mice (Figure 2A). Seizures started 20 minutes after administration and lasted on average for 2 hours. Animals were sacrificed 5 days after treatment.

We observed changes in GFP and mCherry expressing populations both in young and aged animals. The GFP^+ $mCherry^-$, quiescent NSCs were significantly increased in young animals after KA, and a tendency for increase was observed in the GFP^+ $mCherry^+$ active NSCs (Supplementary Figure 2A). We observed a significant increase in actively dividing $PCNA^+$ cells (Supplementary Figure 2B). The NSCs that stayed proliferating were GFP^+ $mCherry^-$ and GFP^+ $mCherry^+$ (Figure 2B, C) Interestingly, we saw a decrease in the GFP^- $mCherry^+Tbr2^+$ type-2 cell pool (Figure 2D), accompanied by an increase in Dcx^+ newborn neurons (Fig2 E, F) after seizures. This increase in Dcx^+ was accounted for by an increase in $GFP^-mCherry^+Dcx^+$ (Supplementary Figure 2C) likely as a result of accelerated progenitor differentiation.

Change in Hippocampal Composition upon Administration of Antidepressant Fluoxetine

After looking at the behavior of NSC subpopulations in a pathological situation in which many NTs are released, we looked at a more physiological stimulus. We administered animals with the Serotonin (5-HT) reuptake inhibitor Fluoxetine to modulate the levels of 5-HT specifically. In order to address which cells in the hippocampus respond following Fluoxetine administration, we treated animals for 7 days and analyzed the short term effects on the NSC populations in young and aged animals (Figure 3A).

The number of GFP^+ $mCherry^-$ cells was not changed either in young or aged animals (Supplementary Figure 3A) and actively dividing stem cells (GFP^+ $mCherry^+$) were not affected (Figure 3B). The number of dividing NSCs was not changed after Fluoxetine treatment (Supplementary Figure 3C, D). Interestingly, we observed a substantial increase in the GFP^- $mCherry^+$ cell population after Fluoxetine treatment (Figure 3B, C). We also observed a significant increase in total $PCNA^+$ dividing cells (Supplementary Figure 3B). This proliferative response to Fluoxetine treatment was mainly due to Type-2, GFP^- $mCherry^+$ IPs (Figure 3D). The dividing Type-2a cells were most likely the origin of the increase in Type-2b, $GFP^-mCherry^+Dcx^+$ cells (Figure 3E). These Type-2b cells also accounted for the increased Dcx^+ cells in the SGZ of young animals we observed after Fluoxetine treatment

(Supplementary Figure 3E). Therefore, the increase in proliferating cells observed in the DG is mainly due to the increased number of dividing GFP$^-$ mCherry$^+$ IPs.

Our detailed analysis of *Hes5::GFP, BLBP::mCherry* double transgenic animals revealed that hippocampal progenitor heterogeneity is comparable to the heterogeneity within the SVZ. Here, we showed that hippocampal NSCs/progenitors have a high heterogeneity. Our results indicate that the different populations of NSCs and progenitors are not only part of a lineage but on its own have crucial functions in responds to pathophysiological stimuli.

DISCUSSION

In this study we were identified distinct NSC and progenitor populations that were at the base of ageing, epilepsy and antidepressant administration in adult neurogenesis. We showed that young *Hes5::GFP BLBP::mCherry* transgenic mice have a high level of neurogenic progenitor heterogeneity in the hippocampal DG SGZ and can be used to discriminate quiescent and active cells. GFP$^+$ mCherry$^-$ cells are infrequently dividing and they are characterized as quiescent NSCs. GFP$^+$ mCherry$^+$ cells are frequently dividing and a high percentage are in S-Phase, but do not express IP markers and are therefore characterized as active NSCs. GFP$^-$ mCherry$^+$ cells are frequently dividing and frequently found in S-Phase and they represent IPs. These mice will allow for molecular analysis of the distinct NSC populations in future experiments.

Our findings substantiate the current knowledge regarding DG (Giachino et al., 2014b; Lugert et al., 2010) and its activation following seizures and Fluoxetine treatment (Encinas et al., 2006; Jessberger et al., 2005). We were able to localize the responsive cell populations underlying the observed effects in response to KA and Fluoxetine. Especially in the case of Fluoxetine this gave a more detailed insight into the cellular population activated in response to increase Serotinin levels. It was previously shown that Fluoxetine treatment leads to a proliferative activation of early progenitors (Encinas et al., 2006), here we identified the activated population to be GFP$^-$ mCherry$^+$ IPs. Furthermore, our results combined with recent publications in the SVZ (Llorens-Bobadilla et al., 2015) make it worth to consider that quiescent and active cells might have further intrinsic increments of complexity.

Quiescent and active NSCs respond in distinct manners to different stimuli. Aging, epilepsy and increase of NTs reportedly affect adult neurogenesis. Upon SE, which causes a massive release of NTs, neurotrophins and ions, the NSCs are increased due to an increase in proliferation but the IPs are decreased due to enhanced differentiation. Analyzing newly generated Dcx$^+$ cells we assume that the decrease of the IP pool is due to a direct transition of

the cells to early neuroblasts without undergoing extensive expansion. Whether the observed activation of NSCs is an independent effect or whether the decrease of IPs contributed to this effect by an unknown feedback mechanism, or a combination of both, remains to be investigated.

Interestingly, increased Serotonin induced by the addition of the 5-HT uptake inhibitor Fluoxetine, does not affect NSC proliferation. The Hes5::GFP$^-$BLBP::mCherry$^+$ IPs are activated in the young animals in response to Fluoxetine. In the aged animals this activation was not observed, presumably due to a lack of this GFP$^-$ mCherry$^+$ population. These results indicate that the increased number of GFP$^-$ mCherry$^+$ was due to a direct activation of this population rather than a transition of GFP$^+$ mCherry$^+$ active NSCs to IPs. In addition to the activation of IPs, quiescent NSCs transitioned from a radial to a horizontal morphology, however, they did not up regulate mCherry, indicating a transition state between quiescence and active. We predict that this is a feedback mechanism due to the increased IP pool on the NSCs.

Our study highlights the differences between young and aged adult neurogenesis in the rodent. We underlined the potential of reactivation of quiescent NSCs in the aged DG, which might be beneficial for healthy ageing in humans. It might be of interest for future studies to analyse if there is a single released factor by seizures that could induce quiescent NSC maintenance for future clinical applications. Indeed, recent results from studies carried out in humans (Ngandu et al., 2015) indicate that cognitive functions of the brain by physical exercise and proper cognitive training can be jumpstarted. The prospect for enhancing regenerative potential in the brain with oral medication is tantalizing. The here present mice will allow a simple approach to discriminate quiescent and active NSCs from TAPs by means of fluorescent protein expression and thus are a beneficial tool for drug screenings for rejuvenating drugs.

AUTHOR CONTRIBUTIONS

A.E., C.R., and V.T., conceived experiments, analyzed and interpreted the data, and wrote the manuscript. C.G. and A.E. conceived experiments and edited the manuscript. O.B. generated transgenic animals and edited the manuscript. All authors approved the final manuscript.

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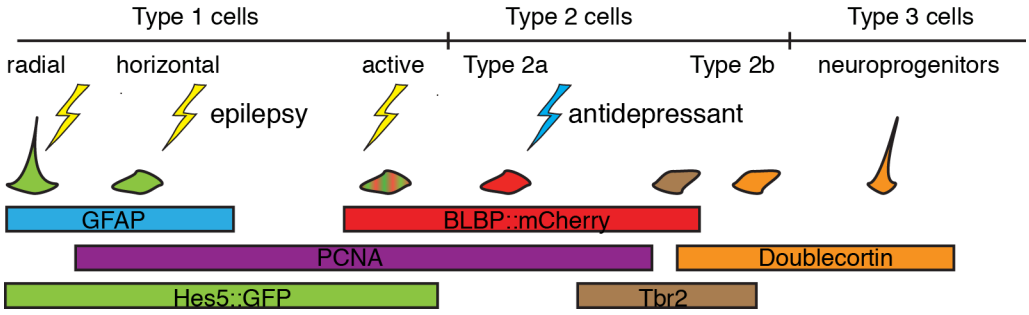
FIGURE LEGENDS

Figure 0: **TOCI; Hippocampal Heterogeneity and Responds to Pathological Stimuli**



The hippocampus is a highly heterogeneous structure. *Hes5::GFP, BLBP::mCherry* double transgenic animals allow discrimination of NSCs subpopulations. Radial GFP^+ cells represent quiescent NSCs and express the astrocytic marker GFAP. Horizontal GFP^+ cells represent a morphologically distinct SC population, which will becomes $mCherry^+$ upon activation and express the proliferation marker PCNA. As they progress in the lineage NSC lose the expression of GFP^+ and become IPs. Early IPs express mCherry but lack Tbr2 expression and late IPs are defined as $mCher^+Tbr2^+$ Type-2b cells. Only the earliest Dcx^+ cells will still express mCherry; although they will be $BLBP^-$. This residual mCherry allows for discrimination of newly generated Dcx^+ cells and older Dcx^+ cells in the DG. Type-1 cells respond to epileptic seizures and $mCherry^+$ only, Type-2a cells that respond to Fluoxetine treatment with increased proliferation.

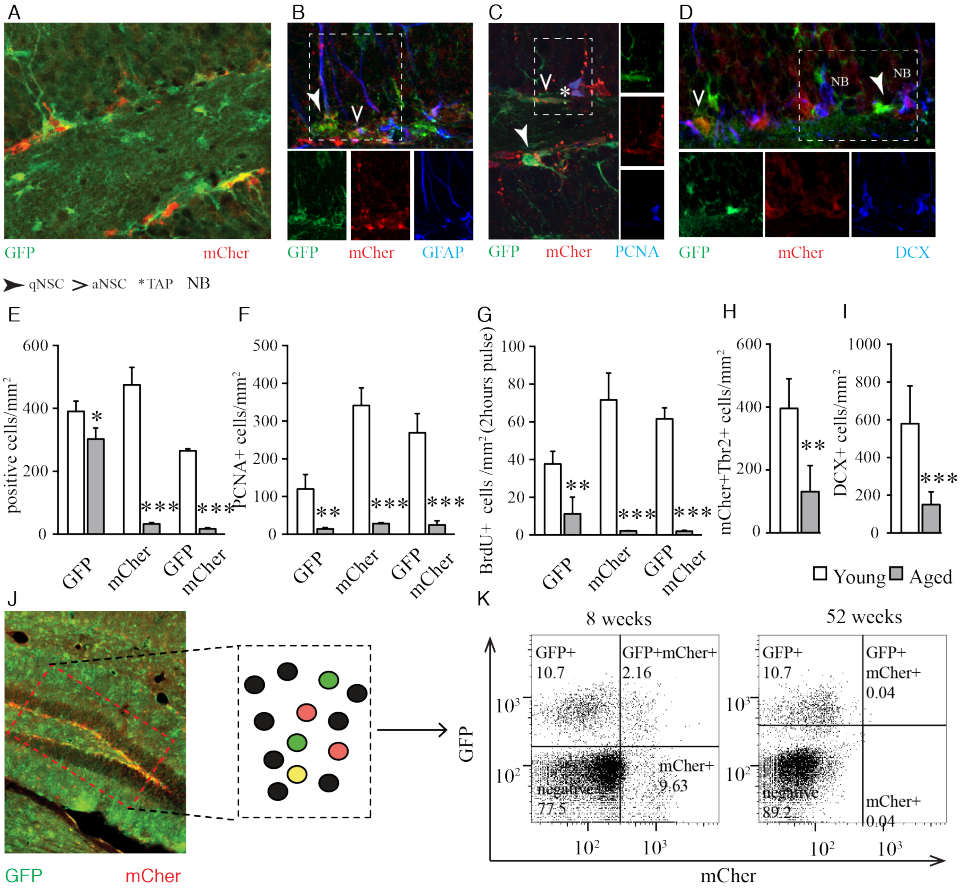


Figure 1: Comparative analysis of adult hippocampal dentate gyrus heterogeneity of young and aged animals

Immunohistochemistry of young (8-week old animals) dentate gyri, stained with GFP, mCherry (A) and astrocytic marker GFAP (B) or PCNA (C) or Dcx (D). Quantification of composition of GFP^+ and $mCherry^+$ cells in young and aged animals (E); heterogeneity is decreasing with progressive age; Quantification of dividing cells ($PCNA^+$) (F) and cells in S-Phase (G) in the dentate gyrus of young and aged animals. Proliferation is decreasing with age; $GFP^+mCherry^+$ double positive and $mCherry^+$ cells are the major dividing populations. Quantification of $mCher^+Tbr2^+$ cells (H) and Dcx^+ cells (I) in the SGZ of the DG. Experimental setup for ex vivo sorting of GFP^+ and $mCherry^+$ cells from the DG (J) and FACS analysis (K). Full arrow = quiescent NSCs; Empty arrow = active NSCs; Asterisk = transient amplifying progenitor cells; NB = neuroblast; Scale bars indicate 100 µm in A, B; 20 µm in E; 10 µm in F, G, H, I; Significances: Values are means ± stdev; * - $P<0.05$, ** - $P<0.01$, *** - $P<0.001$,

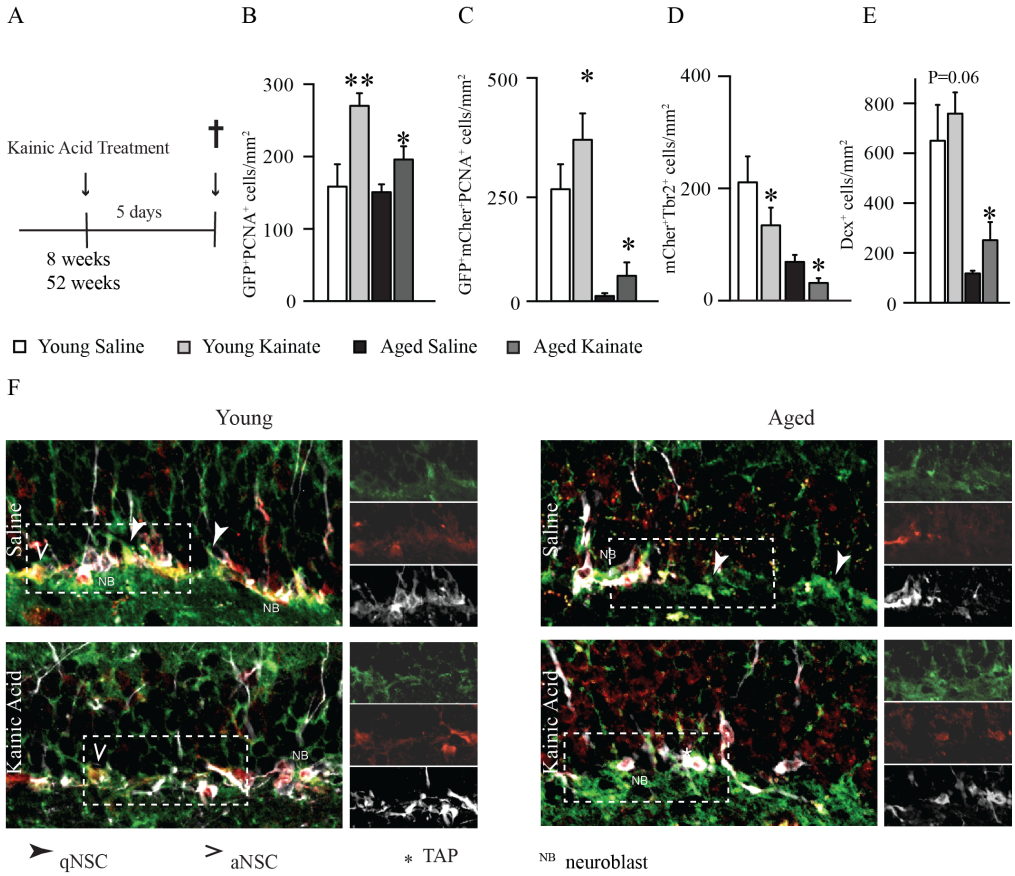


Figure 2: Change in Hippocampal Composition upon Seizure Induction by Kainic Acid Treatment

Schematic representation of experimental setup: Animals were injected at 8 weeks or 52 weeks of age with Kainic Acid or Saline respectively. Seizures were achieved within half an hour and lasted for about 2 hours; animals were chased for 5 days (A). Quantification of GFP^+PCNA^+ dividing quiescent stem cells (B); ($GFP^+mCherry^+PCNA^+$ dividing active stem cells (C) and $mCherry^+Tbr2^+$ cells (D) as well as Dcx^+ cells (E). The number of dividing, stem cells is significantly increased in both young and aged animals after kainic acid administration. The number of Type-2 cells was reduced upon seizure. A slight, albeit non-significant increase was observed in young animals; the responds of aged animals reached significant levels. Immunohistochemistry of young (8 weeks) and aged (52 weeks) animals, analyzed post-seizure (F); Control animals were treated with Saline; Young animals showed an increase in GFP^+ Type-1 cells and a slight decrease of $mCherry^+$ cells Type-2 cells post-seizure. Aged animals showed and increase in DCX^+ cells and a significant increase in $GFP^+mCherry^+$ active Type-1 cells, albeit at low levels; Full arrow = quiescent NSCs; Empty arrow = active NSCs; Asterisk = transient amplifying progenitor cells; NB = neuroblast; Scale bars indicate 25 µm; Significances: Values are means ± stdev; * - $P<0.05$, ** - $P<0.01$, *** - $P<0.001$,

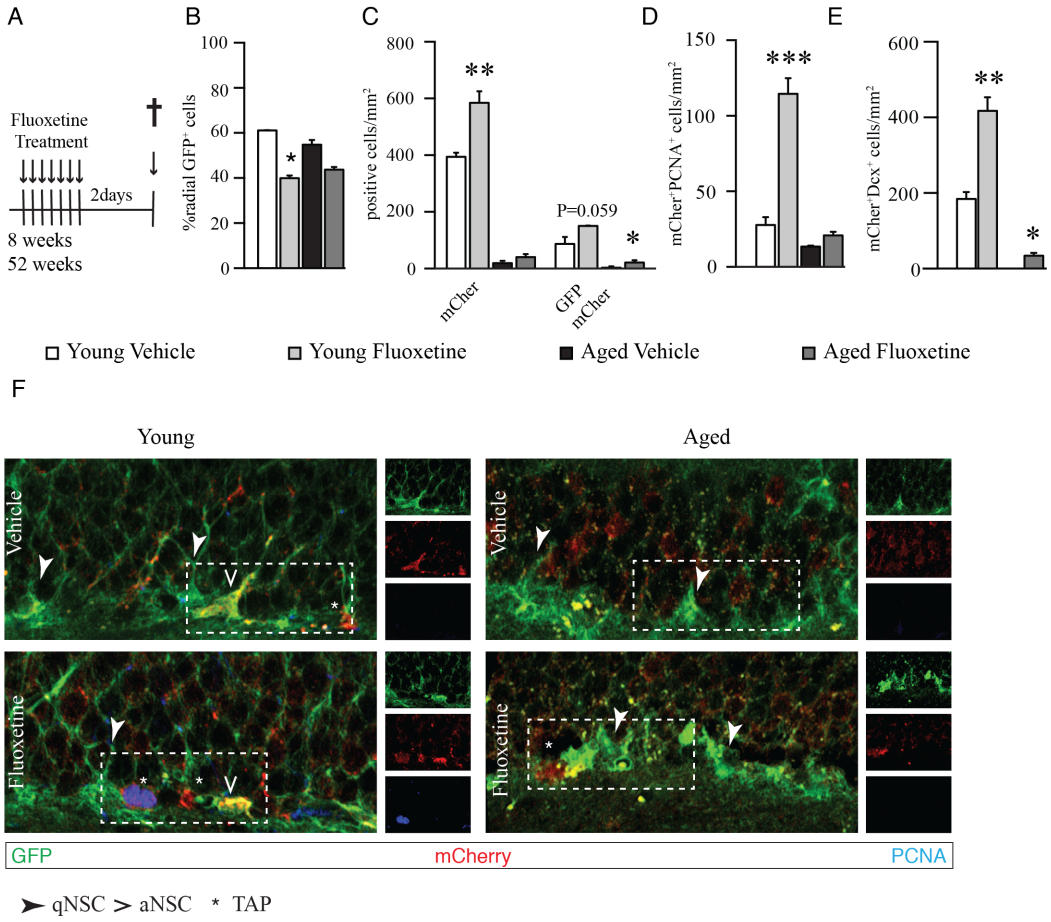


Figure 3: Change in Hippocampal Composition upon Administration of Antidepressant Fluoxetine

Schematic representation of experimental setup: Animals were administered with Fluoxetine or Gelatine at 8 weeks or 52 weeks of age for 7 consecutive days. Animals were sacrificed 2 days after last treatment (A). Quantification of radial GFP^+ only cells in the SGZ after Fluoxetine treatment (B), $mCherry^+$ TAPs and $GFP^+mCherry^+$ active NSCs (C), $mCherry^+PCNA^+$ proliferating cells (D) and $mCherry^+Dcx^+$ newborn early neuroblasts (E). Number of radial GFP^+ cells was significantly reduced in young animals and displayed a tendency of reduction in aged animals. We saw a significant increase of $mCherry^+$ cells in both young and aged animals. Double positive, active NSCs only showed a significant increase in the aged animals. The major dividing population in the DG after Fluoxetine administration is the TAPs in young animals. This diminished population in the aged could not respond. Immunohistochemistry of young (8 weeks) and aged (52 weeks) animals, analyzed post Fluoxetine treatment (F); Control animals were treated with a Gelatine Vehicle; Full arrow = quiescent NSCs; Empty arrow = active NSCs; Asterisk = transient amplifying progenitor cells; NB = neuroblast; Scale bars indicate 25 µm; Significances: Values are means ± stdev; * - $P<0.05$, ** - $P<0.01$, *** - $P<0.001$

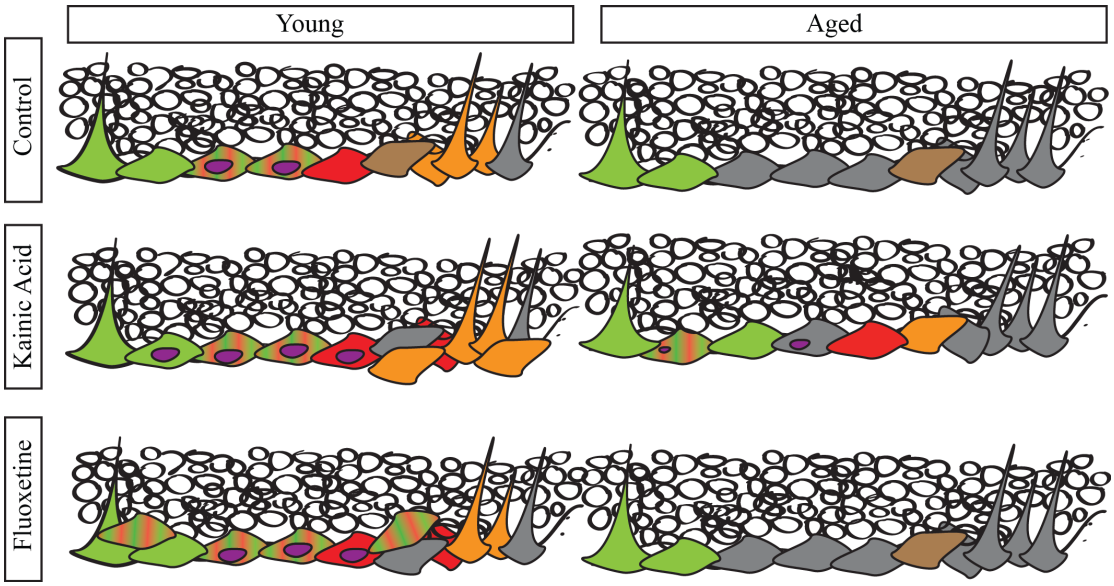
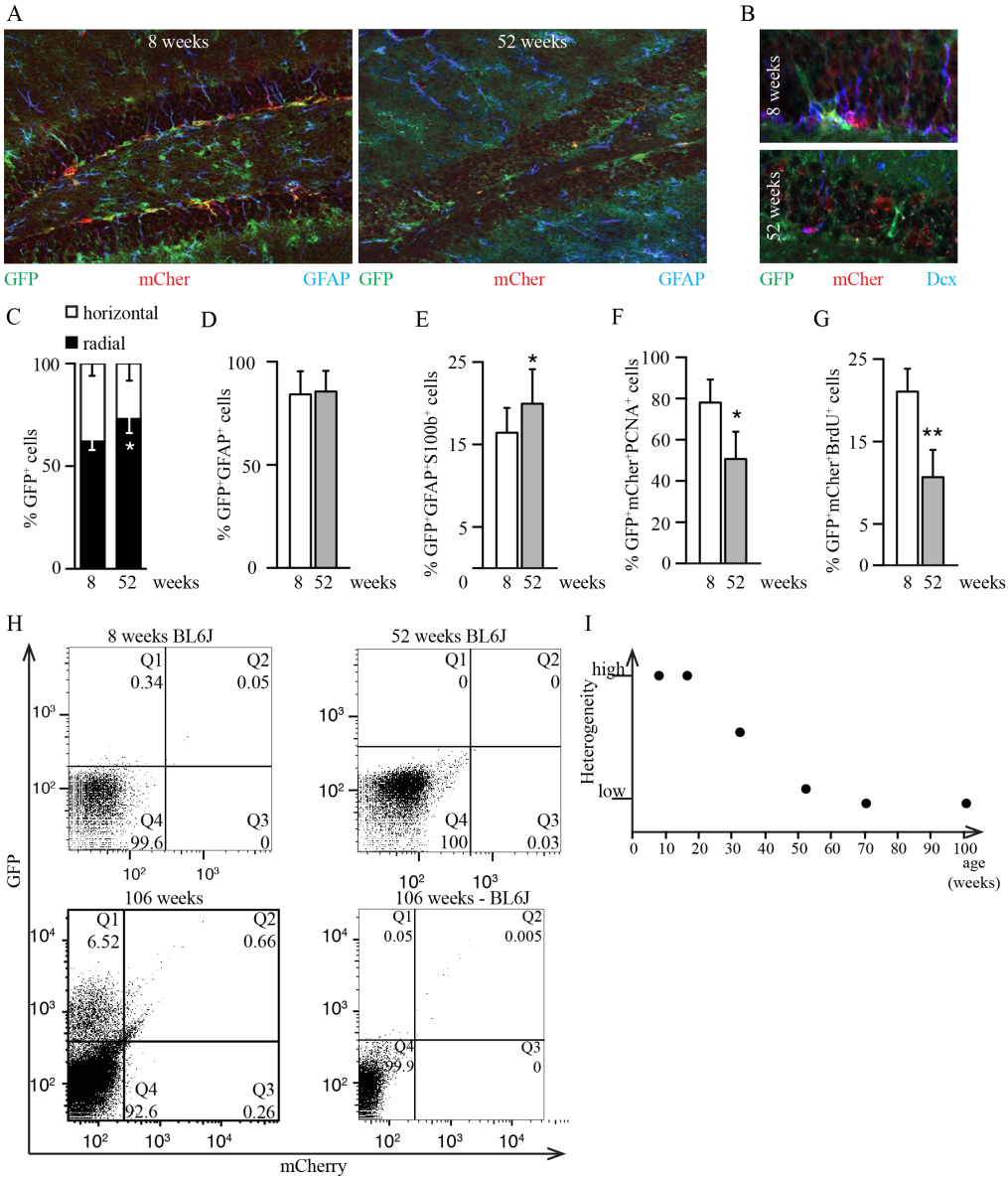


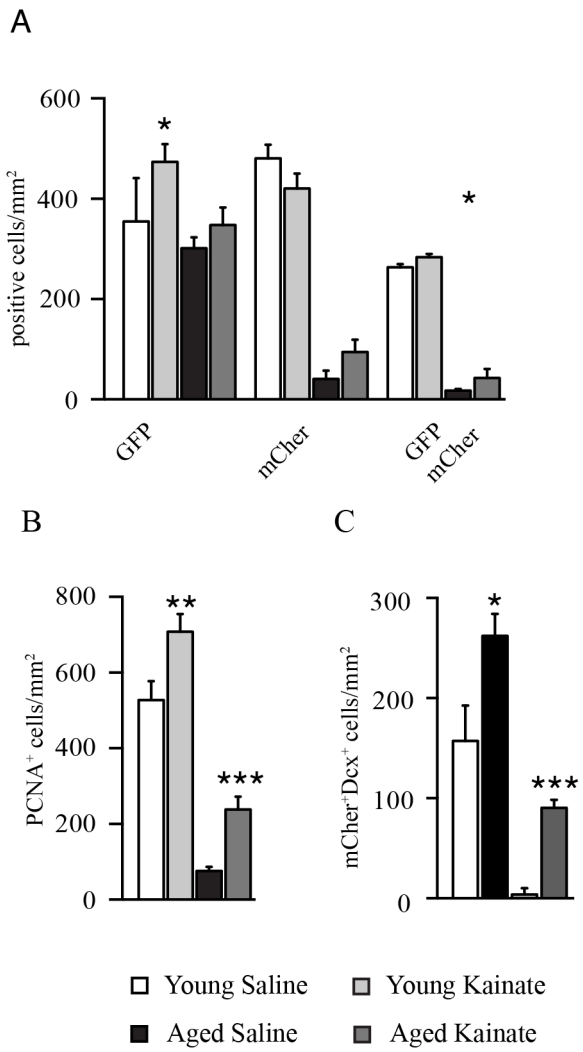
Figure 4: Graphical Summary of Hippocampal Heterogeneity in Physiological and Pathological Conditions

Summary of the temporary and pathological changes occurring in the adult hippocampal dentate gyrus. Under control conditions young DG displays a high heterogeneity and this diversity is dramatically reduced in the aged brain. Upon administration of epileptogenic Kainic Acid, the young DG becomes proliferative and the aged DG is regaining a certain level of heterogeneity, indicated by the appearance of active Type-1 cells and an increased number of late Type-2b cells. Administration of Fluoxetine has no effect on the aged DG, but it induces an increased number of proliferating Type-2a cells in the young DG.



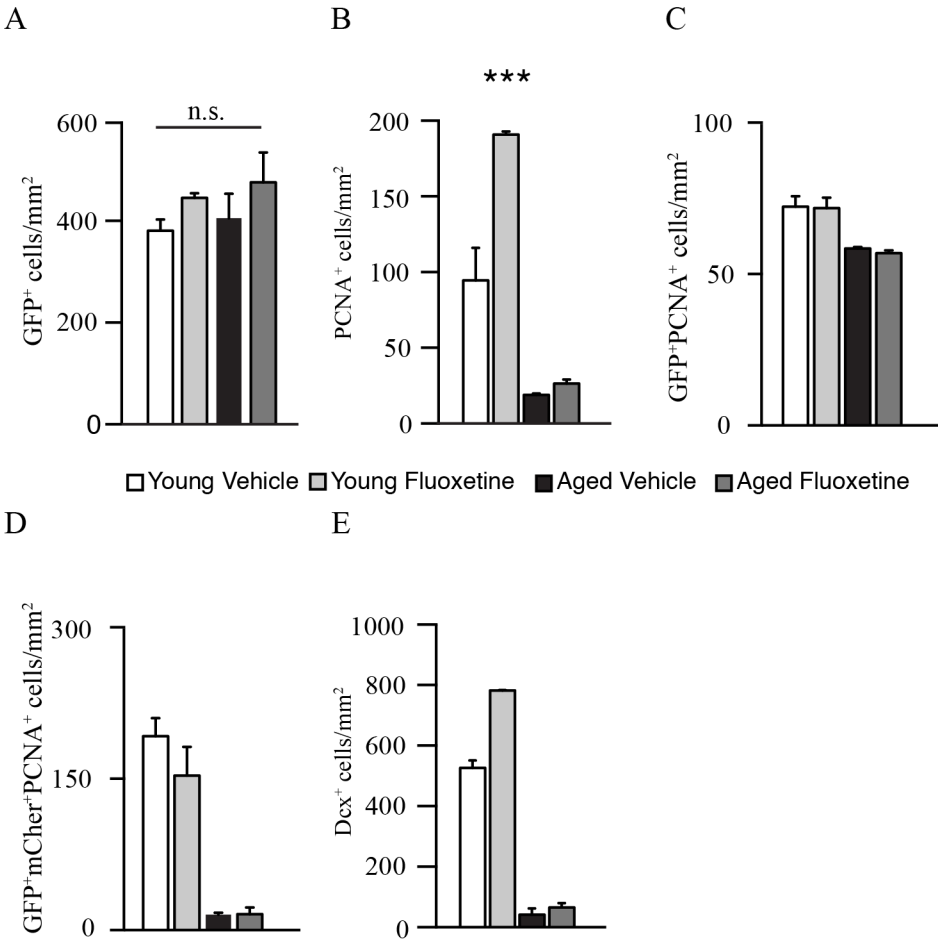
Supplementary Figure 1: Comparative analysis of adult hippocampal dentate gyrus heterogeneity of young and aged animals;

Comparison of GFP$^+$ and mCherry$^+$ cells in young (8 weeks), and aged (52 weeks) animals analyzing GFP$^+$, mCherry$^+$ cells and GFAP$^+$ (A) and Dcx$^+$ (B) cells. Quantification of percentage GFP$^+$ cells in regards to their radial or horizontal properties (C), the coexpression with GFAP (D) and the triple expression of GFAP and S100β (E). Quantification of dividing, PCNA$^+$ cells (F) and BrdU$^+$ cells in S-Phase (G) positive for GFP and mCherry. Proliferation is significantly decreasing with age however the few remaining GFP$^+$ mCherry$^+$ cells maintain at large their proliferative property. Control FACS analysis of young, aged and geriatric animals (H); Note: auto-fluorescence is slightly increased in the aged tissue; Hippocampal heterogeneity is drastically reduced with age (I). Significances: Values are means ± stdev; * - P<0.05, ** - P<0.01, *** - P<0.001



Supplementary Figure 2: Change in Hippocampal Composition upon Seizures Induction by Kainic Acid Treatment

(A) Quantification of *Hes5::GFP; BLBP::mCherry* animals in young and aged conditions 5 days after saline or kainic acid administration. GFP^+ only cells were slightly increased in the young, whereas the composition of $mCherry^+$ only cells did not significantly change; a slight increase of GFP^+ $mCherry^+$ active stem cells was observed in aged animals after kainic acid administration. Quantification of dividing $PCNA^+$ cells (B) and $mCherry^+$ Dcx^+ early neuroblasts after seizure (C); Significances: Values are means ± stdev; * - $P<0.05$, ** - $P<0.01$, *** - $P<0.001$,



Supplementary Figure 3: Change in Hippocampal Composition upon Administration of Antidepressant Fluoxetine

The total number of GFP^+ cells in the DG is not changed significantly after Fluoxetine treatment (A). Quantification of total number of proliferating $PCNA^+$ cells (B) quiescent GFP^+PCNA^+ cells (C) and active GFP^+ $mCherry^+$ $PCNA^+$ cells (D) as well as the total number of Dcx^+ neuroblasts (E) in the SGZ; Significances: Values are means ± stdev; * - $P<0.05$, ** - $P<0.01$, *** - $P<0.001$

Thesis Discussion

The main focus of my PhD thesis was the study of Notch signaling and the role of individual Notch receptors in the regulation of adult neurogenesis and NSC quiescence. In my work I demonstrated that Notch signaling is essential for adult NSC maintenance. Importantly, I identified for the first time a specific role for Notch2 that is not compensated by the other Notch receptors. Notch2 functions as a key signal in the maintenance of quiescent NSCs. I provided the first evidence that a vestigial quiescent population of NSCs reside in the dorsal medial wall (dMW) of the SVZ. This newly discovered population of cells is capable of forming new neurons upon Notch2 inhibition. Furthermore, these quiescent dMW NSCs are responsive to antidepressants and can be activated by Fluoxetine treatment.

Notch2 promotes quiescence of both SVZ and SGZ NSCs. Interestingly, Notch1 and Notch2 are coexpressed in the stem cells in both adult neurogenic regions, despite their non-redundant roles in the regulation of NSC behavior. To get a better understanding of NSC heterogeneity and Notch dependence we characterized the composition of the SGZ niche in great detail. Using transgenic reporter mice for Notch signaling effectors Hes5 and BLBP, namely *Hes5::GFP, BLBP::mCherry* (Giachino et al., 2014b) we identified distinct subpopulations of quiescent and active NSCs and progenitors in the adult hippocampus. Furthermore, we took advantage of these transgenic tools to better characterize the responses of NSCs subpopulations to physiological and pathological stimuli *in vivo*.

Neurogenic Stem Cells in a Dormant Niche are activated by antidepressant Fluoxetine and suppressed by Notch2 signaling

Notch2 signaling keeps quiescent NSCs in check

Our combinatorial analysis of conditional knockouts for Notch signaling components represents an unprecedented study of Notch signaling in NSCs of the adult murine brain. Notch is a key pathway that controls NSC activity and differentiation in the adult neurogenic niches. Canonical Notch signaling downstream of the four Notch paralogues is mediated by the transcription factor Rbpj. Previous data indicate that loss of canonical Notch signals disturbs adult neurogenesis and the production of new neurons (Basak et al., 2012; Imayoshi et al., 2010). However, in the SVZ of the lateral ventricular wall, Notch1 and Rbpj cKO experiments

demonstrate a central difference in phenotype. Whereas loss of Rbpj lead to activation of quiescent NSCs, a wave of enhanced neurogenesis and depletion of both the active and quiescent NSC pools, loss of Notch1 impaired the self-renewing and neurogenic potential of activated NSCs without affecting the quiescent NSC pool (Basak et al., 2012; Imayoshi et al., 2010).

The work presented here leads to three main conclusions. Firstly, the Rbpj cKO phenotype was by-end-large phenocopied by combined loss of Notch1 and Notch2 thus indicating that these two receptors are the major players in activating the canonical Notch signal in the murine SVZ. Secondly, although coexpressed, Notch1 and Notch2 have distinct functions depending on the NSC activation state. We confirmed previous results showing that Notch1 is important for activated NSCs and Rbpj is essential for the maintenance of both active and quiescent NSCs (Basak et al., 2012; Imayoshi et al., 2010) whereas, Notch2 is required by mitotically inactive, dormant NSCs. Thirdly, we showed that this function of Notch2 is conserved between stem cells in the neurogenic but also non-neurogenic regions, and that in the latter neurogenesis can be re-activated upon Notch2 inhibition.

Progenitors in non-neurogenic regions of the adult brain may be controlled *in vivo* by Notch2 signals, which mask their neurogenic potential *in situ*. For example, astrocytes that retain the ability to divide in non-neurogenic brain regions may be restricted from adopting a neuronal fate through lateral activation of Notch signaling in local niches. Intriguingly, recent data indicate that mutations in Notch receptors, including Notch2, are found in human glioma subtypes suggesting that impaired Notch signaling in stem cells and/or parenchymal progenitors could be involved in brain tumor formation (Cancer Genome Atlas Research et al., 2015; Giachino et al., 2015; Suzuki et al., 2015).

While the specific role for Notch2 in NSC quiescence was not described previously, there were indications of Notch dependent regulation of quiescence in other models. Notch3 is essential for NSC quiescence in fish (Chapouton et al., 2010b). Loss of canonical Notch signaling in astrocytes within the mouse striatum after stroke results in increased neurogenesis and ablation of Rbpj in striatal astrocytes initiates neuronal production (Magnusson et al., 2014) although the receptor involved remains unknown. These findings lend direct support to our results showing that dMW B1-cells are repressed by Notch2, which prevents both entry into cell cycle and the generation of neurons even outside the classical neurogenic regions.

The dorsal medial wall is a vestigial niche of the SVZ

Previous works reported the presence of neural progenitors outside the classic neurogenic niches including the forth ventricle, the optic nerve, the cerebral cortex, the striatum and the hypothalamus of the adult brain (Luo et al., 2015; Luzzati et al., 2006; Magnusson et al., 2014; Nato et al., 2015; Palmer et al., 1999; Robins et al., 2013). In all of these reports the crucial role of the niche is highlighted. The homeostatic balance between NSC maintenance and differentiation is determined by the microenvironment and the intrinsic determinants. Upon local damage or by forced expression of pro-neurogenic transcription factors including Ascl1, Neurog2 and NeuroD1 *in vitro* and *in vivo* dormant progenitors and astrocytes are activated (Berninger et al., 2007; Guo et al., 2014; Heinrich et al., 2010; Liu et al., 2015; Masserdotti et al., 2015). Expression of the proneural transcription factors is repressed by Notch signaling thereby preventing NSCs adopting a neuronal fate (Kageyama et al., 2005). This partially explains how Notch signaling controls the developmental switch of NSC fate during differentiation, inhibiting neurogenesis whilst favoring astroglial fate (Gaiano et al., 2000).

The dMW seems to promote NSC maintenance and neurogenesis over gliogenesis, but the dormant stem cells do not generate neurons unless they enter the cell cycle. Although the complete molecular and cellular structure of the dMW niche has not been defined we were able to show that the NSCs present in the dorsal septal wall are embedded in pinwheel structures of ependymal cells. They bi-directionally contact the lateral ventricle with protrusion through the ventricular lining and blood vessels in the subependymal zone. In the lateral wall it has been proposed that direct contact with the vascular niche can promote NSCs maintenance (Shen et al., 2008) and even quiescence (Ottone et al., 2014). Besides the contacts to blood vessels, NSCs are in close proximity to axons. In the classic neurogenic niches neurogenesis is modulated by several neurotransmitters for instance via GABA receptors (Giachino et al., 2014a; Song et al., 2012) glutamate receptors (Nochi et al., 2012), Serotonin (Encinas et al., 2006; Tong et al., 2014b) and Acetylcholine (Paez-Gonzalez et al., 2014).

The quiescent NSCs in our study are able to respond to environmental cues (loss of Notch signaling, increase of 5-HT) to generate new neurons. The septal nuclei in the brain of humans receive input from many brain regions including the olfactory bulb, hippocampus, hypothalamus and thalamus and they are part of the pleasure zone of the brain with a role in reward and reinforcement. Whether neurogenesis in

the dMW is linked to pathophysiological stimuli that modulate neurogenesis in the classic neurogenic brain regions remains to be determined (Anthony et al., 2014).

The NSCs in the dorsal septal wall are in contact with a plexus of serotonergic axons. SVZ NSCs rapidly divide and generate newborn neuroblasts in response to the antidepressant and serotonin-uptake inhibitor Fluoxetine (Tong et al., 2014a; Tong et al., 2014b). dMW mB1-cells respond similarly to the antidepressant treatment with an increase in progenitor production potentially due to a direct signal from Serotonin.

Stem cells of the adult SVZ are set-aside during embryonic development. During the peak of neurogenesis in the developing forebrain, some NSCs in the lateral ganglionic eminence stop dividing (Fuentealba et al., 2015; Furutachi et al., 2015). These NSCs become incorporated into the primordial of the postnatal lateral ventricle wall and originate the neurogenic stem cells of the SVZ in the adult. It is unclear whether dMW NSCs are also set-aside during brain development. It would be of major interest to understand whether dMW NSCs are remnant from development.

Notch2 Maintains Adult Neural Stem Cell Quiescence in the Hippocampal Subgranular Zone

Our recent findings (Engler et al., in preparation) establish that even in non-neurogenic regions of the adult mammalian brain, NSCs may be present but remain in a Notch2-repressed dormant state and these can be reactivated to form new neurons. The SGZ of the DG is one of the two major neurogenic niches of the murine brain that contains quiescent and active NSC subpopulations. Also in the SGZ Notch1 and Notch2 protein are coexpressed on the quiescent and active NSCs (Zhang et al., in preparation). We could show that in the SGZ, loss of Notch2 leads to activation of the quiescent NSCs and increased production of neuroblasts, similar to what was observed in the SVZ (Engler et al., in preparation; Zhang et al., in preparation). Thus, the role of Notch2 in maintaining quiescence of adult NSCs is conserved in both canonical adult neurogenic niches and also in dormant stem cells residing in non-neurogenic regions. Although Notch2 appears to be essential for quiescent NSC maintenance throughout the brain, the molecular mechanism remains unknown.

Upon loss of Notch2 the quiescent NSC are activated and lost in the long run, illustrating the essential role of this receptor. The DNA binding motif of Rbpj (Engler

et al., in preparation), the canonical Notch signaling mediator, can be found in close proximity to various known quiescence genes such the bHLH gene Id1 (Rodriguez Viales et al., 2015), Nfix (Martynoga et al., 2013) and various Forkhead box O gene members (FoxO) (Renault et al., 2009) suggesting a direct regulation of these factors by Notch family members. On the other hand it is possible, that the same gene families (Id, NFI, FoxO) are needed for a proper feedback loop for the maintenance of quiescent stem cells. NFI binding motifs are an indication for quiescence specific enhancers. Interestingly, NFI binding motifs are found amongst others in the Notch2, Foxo3, Id1 and Ascl1 locus (Martynoga et al., 2013).

It is possible that these feedback loops are broken by the loss of one member. Besides the regulation on the direct transcriptional level, Notch target genes might be an additional measure to keep quiescence in check. Although there are no putative Rbpj binding sites in Ascl1, Notch regulates the expression of Ascl1 indirectly via Hes1 and Hes5. The proneural factor Ascl1 has been shown not only to be involved in differentiation but also more recently stem cell proliferation (Castro et al., 2011). *Hes* transcription factors are known to repress the expression of various proneural genes, such as *Ascl1* during embryogenesis (Kageyama et al., 2007). A similar mechanism might occur in the adult, especially due to the recently described role of Ascl1 in transition from quiescence to active NSCs (Andersen et al., 2014) while the degradation of Ascl1 by Huwe1 causes a return to quiescence (Urban et al., 2016). Interestingly, there are four putative Rbpj binding sites in close proximity to the Huwe1 gene start site.

The maintenance of quiescent NSCs is a delicate interplay of different transcription factors up- and downstream of Notch signaling. Understanding the role of quiescent NSCs and the mechanisms underlying their long-term maintenance will be of importance for future studies.

Adult Hippocampal Heterogeneity and its Modulation Under Physiological and Pathological Conditions

Notch receptors and Rbpj are present throughout the neurogenic lineage, but only quiescent and active NSCs and their early progeny express Notch signaling mediators Hes5 (Lugert et al., 2010; Ohtsuka et al., 1999) and BLBP (Anthony et al., 2005; Giachino et al., 2014b). Interestingly, quiescent NSCs only express Hes5, active NSCs express Hes5 and BLBP and early progenitors (TAPs) express BLBP but not Hes5. Thus, to easily distinguish quiescent and active NSCs from their early

progeny in the SVZ we have recently developed double transgenics with GFP and mCherry driven by the Hes5 (*Hes5::GFP*) and BLBP (*BLBP::mCherry*) promoters and regulatory elements, respectively (Giachino et al., 2014b).

We extended our previous work and exploited *Hes5::GFP, BLBP::mCherry* double transgenic animals to examine distinct NSC and progenitor populations in the SGZ of the DG. We addressed the effects of ageing, epilepsy and antidepressant administration on NSCs and progenitors. We showed that young adult *Hes5::GFP*, *BLBP::mCherry* animals display a high level of heterogeneity in the hippocampal DG. We found that quiescent and active NSCs can be discriminated in the DG on the basis of Hes5 and BLBP expression. Moreover, we identified the cell population that are responsive to Fluoxetine (Encinas et al., 2006) and seizures (Jessberger et al., 2005). These findings demonstrate how Hes5 and BLBP expression could be used to precisely define the identity of cell populations in the SGZ that respond to drugs, neurotransmitters, hormones and other stimuli that can modulate neurogenesis.

At the current point in time the mechanisms underlying ageing of NSCs are only poorly understood. In geriatric animals it is observed that the NSCs left are radial type quiescent NSCs. Most likely a combination of intrinsic and extrinsic factors lead to the depletion of active NSCs and the maintenance of quiescent NSCs in the adult. The loss of active NSCs can be explained in two ways.

1. There are no more stem cells provided from the quiescent pool and the actively dividing cells eventually exhaust.
2. Active NSCs go back to a quiescent state if Ascl1 is down regulated upon the up regulation of Huwe1 (Urban et al., 2016). Whether Ascl1 is down regulated physiologically upon ageing is not known.

With age NSCs most likely accumulate DNA damage, undergo epigenetic changes and accumulate damaged cellular components. In the young NSCs, a diffusion barrier leads to the asymmetric segregation of cellular components and damaged proteins, thus the daughter cells do not inherit damaged cellular compounds. With age, this barrier is weakened and damaged proteins will be distributed more symmetrically (Moore et al., 2015). Potentially this accumulation of damage will force the cells towards quiescence.

Besides the intrinsic factors, extrinsic supporting factors and signals are changed in the geriatric niche. One of the known properties changing with age is the

permeability of the BBB leading to decreased levels of glucose influx (Mooradian, 1988). Additionally, the increase of quiescent NSCs will automatically lead to a decrease in neurogenesis and thus a loss of daughter cells within close proximity to the NSCs. A loss of the feedback mechanism from the progenitor cells might add to the quiescent phenotype observed in the aged animals.

Adult NSCs in a quiescent state are irreversibly dormant. They can be reactivated by genetic and pathological means. The loss of Notch2 leads to the activation of quiescent NSCs both in the young animals in the LW and dMW of the SVZ as well as the SGZ. In aged animals the loss of Notch2 lead to the activation of quiescent NSCs in the LW of the SVZ and the SGZ, however not the dMW (Zhang et al., in preparation). The thought of pharmacologically stimulating neurogenesis in the aged brain can have broad applications. However, forced reactivation of quiescent SCs might have long-term side effects. Recent studies conducted on HSCs in aged mice have shown a forced exit from quiescence, and thus the reentry into mitosis, leads to an exhaustion of the HSC pool (Walter et al., 2015). Similar observations were also made in the murine brain after continuous induction of seizures, which ultimately resulted in the terminal differentiation of the NSCs into astrocytes and the depletion of the NSC pool (Sierra et al., 2015). In a more extreme situation the loss of dormant SCs might be the induction of a continuous, uncontrolled proliferative state, potentially leading to cancer development (Ignatova et al., 2002).

Outlook

We have studied the contribution of the individual Notch receptor paralogues Notch1 and Notch2 to adult neurogenesis in detail with a combinatorial conditional gene knockout approach (Engler et al., in preparation). Our analysis and the exciting results underlined the complexity of NSC maintenance. Notch2 regulates activation of quiescent NSCs in the SVZ, maintaining them in a mitotically inactive state, whereas Notch1 is essential for active NSC maintenance (Basak et al., 2012). These findings suggested that Notch1 and Notch2 signaling are both required for different aspects of NSC biology. In support of this, the Rbpj phenotype was phenocopied by the combined deletion of both Notch1 and Notch2 indicating that, although coexpressed by quiescent and active NSCs, they play non-compensatory roles as regulators of adult NSCs (Engler et al., in preparation). Based on their coexpression and lack of compensatory function, these results strongly suggest that Notch1 and Notch2 have distinct downstream pathways and gene targets.

Identification of distinct molecular targets of Notch signaling

To identify the distinct targets of the individual Notch paralogues Notch1 and Notch2. For this reason we have established two novel mouse lines, containing flag-tags in the endogenous 3'-end of the Notch locus. We are expecting to gain insight into distinct Notch1 and Notch2 target genes from these animals and to explain the distinct roles of the Notch receptors in maintenance of quiescent and active NSC states.

Using a CRISPR-Cas9 based approach we generated animals where the endogenous Notch1 and Notch2 proteins have been C-terminally tagged with a Flag epitope. These mice allow for the analysis of the endogenous Notch1 and Notch2 signaling. We hypothesize that the two paralogues have distinct gene targets. We will examine the Notch1 and Notch2 endogenous gene targets in NSCs and their distinct functions in active and quiescent NSCs. We hope to obtain genome wide traces of Notch1 versus Notch2-ChiP-Seq, which in combination with the available Rbpj trace will allow the identification of canonical Notch signal targets of the distinct paralogue.

Conclusion

Since the early 1990s, adult neurogenesis and NSCs have evolved from a topic of interest of a few scientists to an established field that has made tremendous progress impacting our perspective of brain plasticity (Bond et al., 2015). Initially the process of adult neurogenesis was characterized, followed by efforts to identify underlying mechanisms and its functional significance. Though our understanding of adult NSCs has come a long way, there are still challenges for future research.

The here presented work continued to examine and manipulate NSC regulatory mechanisms. We underlined that adult NSCs are a very heterogeneous population of cells. Using lineage analysis, cell tracing and genetic manipulation we distinguished between different temporal states and distinct populations *in vivo*. Future studies will benefit from our and others' identification of sub-populations and distinct responsive capacities. Furthermore, we have taken a closer look at NSC quiescence. We were able to show that Notch2 mediates the choice between quiescence and activation. Our future research will focus on Notch molecular targets and potential interactions between signaling pathways to comprehend the molecular hierarchy in NSCs.

Additionally, our work has demonstrated that NSCs are found outside of the classical neurogenic regions, in more dormant states. One of the many differences between rodent and human is the lesser extend of active adult neurogenesis. Potentially in humans, adult neurogenesis is less defined by the classical neurogenic regions, but more by the dormant astrocyte like cells. These dormant cells might be reactivated upon injury by endogenous manipulation of the Notch signaling pathway. It is the ultimate goal of adult neurogenesis research to manipulate NSCs to improve human brain health. The contribution provided in this work and by others will hopefully give new insights into the mechanisms of neurogenesis mediated plasticity and brain repair. It is up to future studies to explore the potential and limits of NSCs in human health.

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Materials and Methods

Animals and Husbandry

Hes5::GFP, *Hes5::CreER^{T2},* *Notch2::CreER$^{T2\text{-}SAT}$,* *Rosa25R::GFP, Rosa25R::tdTomato,* floxed *Notch1,* floxed *Notch2,* floxed *Rbpj* mice have been described elsewhere (Basak et al., 2012; Basak and Taylor, 2007; Besseyrias et al., 2007; Fre et al., 2011; Lugert et al., 2012; Schouwey et al., 2007). Notch1-flag and Notch2-flag lines were generated using Crispr-Cas9 as described below. Mice were kept according to Swiss Federal and Swiss Veterinary office regulations under license numbers 2537 and 2538 (Ethics commission Basel-Stadt, Basel Switzerland). Mice were maintained on a C57Bl6 genetic background and kept on a 12-hour day/night cycle with food and water *ad libitum* under specified pathogen free conditions. The genotypes of the mutants were confirmed by PCR analysis of genomic DNA. Transgenic mice were used at distinct time points – 8 weeks, 52 weeks, 74 weeks, and 106 weeks – for analysis.

Table 1: List of Genotyping primers with conditions and expected band sizes.

Genotype	Primer Sequence	Annealing	Band size
Hes5::GFP	**Fwd:** TCCGCTCCGCTCGCTAATC **Rev:** AGCTCGCCGCCACTACCAG **Rev:** TCCCGACGCATCTTCTCCAC	58°C 1min	wt: 210 bp tg: 360 bp
BLBP::mCherry	**Fwd:** AGGCCCCGCTGACTTCC **Rev:** TCGGGGGTTTCTAAGGAT **Rev:** CACGCGCTCCCACTTGA	54°C 45 s	wt: 500 bp tg: 650 bp
Hes5::CreER^{T2}	**Fwd:** ACCAGGTTCGTTCACTCATGG **Rev:** AGGCTAAGTGCCTTTCTACAC	53°C 1min	tg: 300bp
CAG-Stop-GFP	**Fwd:** CTTCAGCCGCTACCCCGACCACA **Rev:** ATCGCGCTTCTCGTTGGGGTCTTT	58°C 1 min	tg: 500bp
Notch1flox/flox	**Fwd:** CTGACTTAGTAGGGGGAAAAC **Rev:** AGTGGTCCAGGGTGTGAGTGT	58°C 1.5 min	wt: 300 bp tg: 380 bp
Notch2flox/flox	**Fwd:** GTGAGATGTGACACTTCTGAGC **Rev:** GAGAAGCAGAGATGAGCAGATG	58°C 1min	wt: 230 bp tg: 300 bp
Rbpj flox/flox	**Fwd:** GAAGGTCGGTTGACCCAGATAGC **Rev:** GCAATCCATCTTGTTCAATGGCC	58°C 1min	tg: 600bp
Notch1-flag	**Fwd:** CTGAAGCACTGGAAAGGACTC **Rev:** GCCCTGCCCACATCACTGC	58°C 1 min	wt: 320 bp tg: 420 bp
Notch2-flag	**Fwd:** ATAACCTTCACTCGCCCCTCAGC **Rev:** GTGCCAACCTATCATCCTTTCC	58°C 1min	wt: 350 bp tg: 450 bp

Administration of Chemicals

Tamoxifen Administration

Adult mice 8-10 weeks of age were injected daily intraperitoneal (i.p.) with 2 mg TAM in sunflower oil (100 µl of 20 mg/ml) for five consecutive days and killed 2, 21, 100 or 300-days after the end of the treatment.

Kainic Acid Administration

Adult mice 8-10 weeks and aged 52-weeks of age were injected intraperitoneal (i.p.) with Kainic Acid and sacrificed 4-days post induction. Animals obtained a single dose Kainic Acid (10mM) (ToCris, Cat.No° 0222/65). Young animals obtained a 20mg/kg dose, aged animals 15mg/kg and were monitored for 2 hours. Seizure severity was determined according to set standards (listed below) in which 1 represented an injected mouse without phenotype and 6 a mouse with severe seizures. Animals in analyses were required to reach seizure level 4, which was identified by prolonged freezing and uncontrolled, seated seizing. Animals were sacrificed after 4 days and tissue was used for immunohistochemical analysis.

Fluoxetine Treatment

Adult mice 8-10 weeks and aged 52-weeks of age were injected intraoral (i.o.) with Fluoxetine and sacrificed 2-days or 19-days after the last treatment. Animals obtained Fluoxetine (1.8 mg/kg) for seven consecutive days. Vehicle control animals obtained i.o. gelatin solution.

Tissue Generation

Animals were euthanized i.p. with a lethal dose of Ketamin-Rompun and perfused with ice-cold phosphate buffered saline (PBS) followed by 4% PFA in PBS. Brains were excised, fixed overnight in 4% PFA in PBS, cryoprotected with 30% sucrose in PBS at 4°C 48 hours, embedded and frozen in OCT (TissueTEK). Tissue was sectioned into 30 µm floating sections cut by cryostat (Leica). Tissue was stored at -20°C in Anti-freeze solution.

For whole-mount tissue preparation, the dMW of the SVZ was dissected. Brains of mice were excised, cut in half and fixed overnight in 4% PFA in PBS, washed in PBS followed by micro-dissection under a binocular of the medial wall and the lateral

wall, as previously described (Mirzadeh et al., 2008). Stainings were done after dissection was finished.

Immunohistochemistry

Immunostainings were performed as follows: Sections were washed thoroughly and blocked at room temperature for 30 minutes with 10% normal donkey serum (Jackson Immunoresearch) in PBS containing 0.5% TritonX-100. Primary antibodies diluted in 2.5% blocking solution were incubated overnight. Sections were washed with PBS and incubated at room temperature for 1-2 hours with the corresponding secondary antibodies in 5% blocking solution and counter-stained with DAPI (1 µg/ml). Sections were mounted on glass slides (SuperFrost, Menzel) in DABCO mounting media and visualized using a Zeiss Observer with Apotome (Zeiss). For PCNA detection, the antigen was recovered at 80°C for 20 minutes and 25°C for 45 minutes in Sodium Citrate (10 mM, pH7.4).

Westernblot

Cultured cells were collected and lysed in Ripa. Whole cell extracts were fractionated by SDS-Page and transferred to a polyvinylidene difluoride (PVDF) membrane using Transblot Turbo (BioRad) according to manufacturer's protocol. After transfer membrane was blocked with 5% non-fat milk in TBST (10mM Tris, pH 8.0, 0.5% Tween 20) for 60 min, at room temperature. Primary antibodies were added in fresh, 5% milk and incubated over night, at 4°C with light agitation. After primary antibody incubation, membranes were washed three times, 10 minutes with TBST and incubated with secondary antibody in 5% milk, for 1-2 hours at room temperature, with light sample agitation. Blots were washed three times, 10 minutes with TBST. Blots were rinsed in PBS and developed with ECL system (Amersham Biosciences) according to manufacturer's protocol for 5 minutes. Blots were developed on ChemiDoc MP Imaging System (BioRad). Imaging took place every 5 minutes for 1 hour.

Table 2: Primary antibody list: Antibodies were used for indicated purposes according to dilution provided.

Antigen	Species	Use	Dilution	Provider/Cat.No.
Acetylated-Tubulin	mouse	IHC❖	1:700	Sigma/ T6793
β–Catenin	rabbit	IHC❖	1:1000	Sigma/ C2206
BLBP	rabbit	IHC IF	1:300 1:500	Millipore/ ABN14
BrdU*	rat	IHC	1:1000 1:2000	AbSerotec/ OPT0030
Calbindin D28K	rabbit	IHC	1:5000	Swant/ 300
Calretinin	rabbit	IHC	1:5000	Swant/ 7609/4
CD31	rat	IHC	1:500	BD Pharmingen/ 550274
Cleaved Caspase 3 (Casp3)	rabbit	IHC	1:1000	Cell Signaling/ 9664S
Doublecortin (Dcx)	goat	IHC IF	1:500 1:750	Santa Cruz/ sc-8066
Ds-Red	rabbit	IHC IF	1:500 1:700	CloneTech Takara/ 632496
Flag	mouse	WB	1:2000	Sigma/ F3165
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	mouse	WB	1:1500	Calbiochem7 CB1001
Glial Fibrillary Acidic Protein (GFAP)	mouse	IHC	1:500	Sigma/ G3893
	rabbit	IHC IF	1:1000 1:1000	Sigma/ G9269
	chicken	IHC IF	1:500 1:700	Abcam/ ab4674
Green Fluorescent Protein (GFP)	rabbit	IHC IF	1:500 1:750	Invitrogen/ A11122
	chicken	IHC IF	1:250 1:300	AvesLab/ GFP-1020
		WB	1:1000	Millipore/ 06-896
	sheep	IHC IF	1:250 1:300	AbD Serotec/ 4745-1051
Neuronal nuclear antigen (NeuN)	mouse	IHC	1:800	Millipore/ MAB377
Notch1	rabbit	IHC IF	1:700 1:1000	Animal 3, D120 (Nyfeler et al., 2005)
		WB	1:1000	Cell Signaling/ 3608S
	rat	IF	1:500	Gift H. Robson
Notch2	rat	IHC	1:200	Gift H Robson
	rabbit	IF WB	1:2000 1:1000	Cell Signaling/ 5732P

Olig2**	rabbit	IHC	1:1000	Chemicon/ AB9610
Parvalbumin	mouse	IHC	1:5000	Swant/ Mc-AB235
Proliferating cell nuclear antigen (PCNA)**	mouse	IHC IF	1:700 1:1000	DAKO/ M0879
S100β	Rabbit	IHC	1:1000	Swant/ 37♦
	mouse	IHC	1:700	Sigma/ S2532
Sox2	goat	IHC	1:250	Santa Cruz/ sc-17320
Tbr2	rabbit	IHC	1:500	Abcam/ AB23345♦

*with HCl retrieval; **with Cytrate Retrieval; ❖whole mount stainings; ♦discontinued;

Table 3: Secondary antibody list; Antibodies were used for indicated purposes according to dilution provided. All secondary antibodies in use were raised in donkey and purchased from Jackson Immunoresearch

Fluorochrome	Species	Use	Dilution	Cat.No
Alexa488	rabbit mouse sheep goat rat chicken Streptavidin	IHC IF	1:500 1:700	711-545-152 715-546-151 713-545-147 705-545-147 712-546-153 703-545-155 016-540-084
Cyanine 3 (Cy3)	rabbit mouse sheep goat rat Streptavidin	IHC IF	1:500 1:700	711-165-152 715-165-151 713-165-147 705-165-147 712-166-153 016-160-084
Cyanine 5 (Cy5)	rabbit mouse goat rat Streptavidin	IHC IF	1:300 1:500	711-496-152♦ 715-175-151 705-176-147♦ 712-175-153 016-170-084
DyLight 649	rabbit mouse goat Streptavidin	IHC IF	1:500 1:700	711-496-152♦ 715-495-151♦ 705-495-147♦ 016-490-084♦
DyLight 647	rabbit mouse goat chicken	IHC IF	1:500 1:700	711-605-152 715-605-150 705-605-147 703-605-155

Horse Radish Peroxidase (HRP)	rabbit mouse goat chicken Streptavidin	TSA WB	1:500 1:10000	711-035-152 715-035-151 705-035-147 703-035-155 016-030-084
Fluorescein isothiocyanate (FITC)	sheep goat	IHC IF	1:300 1:500	713-095-147 705-093-147
Biotin	rabbit mouse sheep rat chicken	IHC	1:300	711-065-152 715-065-151 713-066-147 712-065-153 703-065-155
♦discontinued;				

Fluorescent Activated Cell Sorting (FACS)

Animals were euthanized in CO2, brains were dissected in L15 Medium (GIBCO) and cut into 0.55 mm thick sections using a McIllwains tissue chopper. The region of interest was microdissected under a binocular microscope avoiding contamination from other tissues, and digested using a Papain solution an mechanical dissociation (previously described – Lugert et al, 2010). The tissue was dissociated in Papain (7min), 0.5 volumes of Ovomucoid were added and dissociation continued (12 min) at 37ºC. After dissociation Ovomucoid was additionally added (2 volumes) and dissociated manually by gentle up and down pipetting. The sample was filtered through parachute (30 µm) and centrifuged (5min, 1000 rpm). The supernatant was removed and cells were resuspended in Leibowitz medium (Life Technologies) and sorted on a BD FACS Aria III.

FACS Analysis from Hes5::GFP, BLBP::mCherry

Hes5::GFP, BLBP::mCherry animals were sacrificed at 8, 26, 52, 76 and 106 weeks of age. Hippocampal DG were microdissected and processed as described above. FACS gates were set with age-matched controls, BL6J, Hes5::GFP single, BLBP::mCherry single animals. Analysis was done using FACS Aria III. Subsequent evaluation using FlowJo

FACS Sorting from Hes5-CreER^{T2}, CAG-GFP

Transgenic animals containing *Notch2$^{flox/flox}$, Hes5-CreER^{T2}, CAG-GFP* (Control without Notch allele) animals were injected for 5 consecutive days with Tamoxifen, as described above and sacrificed 24 hours after the last treatment. Hippocampal

DG and SVZ were microdissected and processed as described above. FACS gates were set with age matched BL6J animals. Sorting was done using FACS Aria III. Cells were discriminated by forward and side-scatter (for live cells – from the control) and gated for GFP-negative (wild-type levels) or GFP^+ populations. Cells were directly sorted into cooled Trizol (Life Technologies) and RNA isolation performed as described below. Separate samples were collected for Reanalysis. Subsequent evaluation was performed using FlowJo.

Microarray analysis

Animals were sacrificed 24 hours after TAM treatment. Tissue was prepared for FACS sorting as described above and GFP^+ cells sorted directly into Trizol reagent (Thermo Fisher Scientific) and RNA extracted according to manufacturers recommendations. RNA quality was tested using a Fragment Analyzer (Advanced Analytical). Samples were sent for Expression Profiling with Atlas Biolabs. Samples were subjected to a second quality control on an Agilent 2100 Bioanalyzer, small samples were amplified using the Ovation Picokit (NuGen) and then run on an Affymetric Biochip. Affymetrix expression profiling was performed on Affymetrix GeneChip Mouse Gene 1.0 ST arrays (ATLAS Biolabs). GO analysis was performed using DNASTAR Microarray software (DNASTAR).

RNA extraction

Cell samples were collected in Trizol (Life Technologies). Appropriate amount of Chloroform (1:4) was added to Trizol. Samples were shaken vigorously and centrifuged for 30 minutes at 13'000 rpm. Aqueous phase RNA extraction was performed using Isopropanol with LiCl (0.75M) and Glycoblue. RNA was immediately frozen to -80°C. RNA quality was tested on a Fragment Analyzer (Advanced Analytical) using a high sensitivity RNA analysis kit (DNF-472).

Quantitative PCR

Animals were sacrificed 24 hours after TAM treatment. Tissue was prepared for FACS sorting as described above and GFP^+ cells sorted directly into Trizol reagent (Thermo Fisher Scientific) and RNA extracted according to manufacturers recommendations. RNA quality was tested using a Fragment Analyzer (Advanced Analytical). cDNA was prepared using BioScript (Bioline). qRT-PCR was performed using SensiMix SYBR kit (Bioline).

Ex vivo mRNA was prepared as described above. Isolated RNA was treated with DNaseI (Roche). cDNA was prepared using BioScript (Bioline) and random hexamer primers. qPCR was performed using SensiMix SYBR kit (Bioline). Primers for PCR reactions are as follows:

Table 4: Quantitative PCR primers

Gene	Primer Sequence
GAPDH*	**Fwd:** CTCCCACTCTTCCACCTTCG **Rev:** CCACCACCCTGTTGCTGTAG
β–Actin*	**Fwd:** AGGTGACAGCATTGCTTCTG **Rev:** GGGAGACCAAAGCCTTCATA
Rpl29*	**Fwd:** ACAGAAATGGCATCAAGAAACCC **Rev:** TCTTGTTGTGCTTCTTGGCAAA
Notch2 (Exon 26/27)	**Fwd:** CAGGAGGTGATAGGCTCTAAG **Rev:** GAAGCACTGGTCTGAATCTTG
Cdk1	**Fwd:** AAATTGGAGAAGGTACTTACGG **Rev:** CTCCTTCTTCCTCGCTTTC
Foxo3	**Fwd:** CTGCGGGCTGGAAGAACTC **Rev:** TTGCCCGTGCCTTCATTC
CCNE1	**Fwd:** CTAATGGAGGTGTGCGAAG **Rev:** AAGAAGTCCTGTGCCAAGTAG
* normalizing genes	

Generation of adeno-gfap::Cre virus particles

Generation of adeno-*gfap*::Cre virus was described previously (Merkle et al., 2007). Briefly, Cre was placed under the control of the mouse *gfap* promoter (GFAPp) previously confirmed to be specifically active in $GFAP^+$ cells. The pAd/PLGFAPp- NLSCre-pA vector was transfected into HEK293 cells to produce replication-defective adenovirus, which was purified twice by cesium chloride banding. adeno-*gfap*::Cre virus (titer 1 x 10^{12} infection particles per ml) in saline containing 0.1% bovine serum albumin.

Stereotactic injection of adeno-gfap::Cre virus particles

Adult (8-10 week old) mice were anesthetized in a constant flow of Isoflurane (1-3%) in oxygen and immobilized on a stereotaxic apparatus (David Kopf instruments)(Giachino and Taylor, 2009). Mice were injected with Temgesic subcutaneous (0.05 mg/kg body weight). The skull was exposed by an incision in the scalp and a small hole (1 mm) drilled through the skull. Animals were stereotactically injected with 1 µL of titrated adeno-*gfap*::Cre virus (titer 1 x 10^{12} infection particles per ml) in saline, 0.1% bovine serum albumin using sharpened Borosilicate glass

capillaries (Kwick-FilTM) at the coordinates: anterior/posterior 0 mm; medial/lateral 0 mm; dorsal/ventral 2.5 mm below the skull and relative to Bregma. Wounds were closed using surgical clips. One day after the surgery the animals received a second dose of Temgesic subcutaneous (0.05 mg/kg body weight) and were analyzed 21 days post-injection.

Quantification and statistical analysis

Stained sections were analyzed with fixed photomultiplier settings on a Zeiss Observer with Apotome (Zeiss). Images were processed with Photoshop or ImageJ. Data are presented as averages of a minimum of three sections per region and multiple animals. Statistical significance was determined by Student's T-test on mean values per animal, Whitney-Mann U-test was used for distributions and two way ANOVA for cross-comparison of 3 and more data sets. Significance was determined at * - $P<0.05$, ** - $P<0.01$, *** - $P< 0.001$ or P values are given in the graphs. Deviance from mean is displayed as standard deviation if not otherwise indicated.

Abbreviations

SVZ	Subventricular zone	E8	Embryonic day (8)
SGZ	Subgranular zone	P19	Postnatal day (19)
LW	Lateral wall	NCC	Neural crest cells
DG	Dentate gyrus	NEP	Neuroepithelial progenitors
NSC	Neural stem cells	PNS	Peripheral nervous system
dMW	Dorsal medial wall	SVZ	Subventricular zone
aSC	Adult stem cells	RGC	Radial glia cells
eSC	Embryonic stem cells	VZ	Ventricular zone
CNS	Central nervous system	IPC	Intermediate progenitor cells
BV	Blood vessel	BBB	Blood brain barrier
TAP	Transient amplifying progenitor	SE	seizure
IP	Intermediate precursor	SSRI	Selective Serotonin reuptake inhibitor

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Education

2012-2016 **Doctorate studies**
Notch Signaling Balances Adult Neural Stem Cell Quiescence and Heterogeneity
Supervisor: Prof. Dr. Verdon Taylor, University of Basel
Summary: In the adult, neural stem cells reside in specific brain regions. The main focus of my PhD studies is to address the contribution of Notch signaling in the regulation of neural stem cell maintenance and activation during physiological and pathological conditions.

2011-2012 **Master of Science in Molecular Biology; Grade: 5.5 / 6.0**
MSK1 as mediator of BDNF in GABAergic neurons
Supervisor: Prof. Dr. Yves Alain Barde; University of Basel
Summary: We found that BDNF signaling via TrkB and MAPK pathway, specifically via MSK1, induces morphological changes in neurons and affect GABAergic neurons differentiation.

2007-2011 **Bachelor of Science in Molecular Biology**
University of Basel

Peer-reviewed publications

Engler A., Rolando C., Giachino C., Erni A., Zhang R., Saotome I., Berninger P., van Nimwegen E., Zimber-Strobl U., Radtke F., Artavanis-Tsakonas S., Louvi A., Taylor V. Fluoxetine activates dormant neurogenic stem cells in a vestigial forebrain niche, planned submission August 2016, Cell Stem Cell;

Engler A.*, Rolando C.*, Giachino C., Erni A., Lugert S., Taylor V., Modulation of Adult Hippocampal Neurogenesis in Physiological and Pathological Conditions; planned submission, September 2016; Glia

Zhang R., **Engler A.**, Giachino C., Saotome I., Zimber-Strobl U., Louvi A., Taylor V., Notch2 Maintains Adult Neural Stem Cell Quiescence in the Hippocampal Subgranular Zone; in preparation

Rolando C.*, Erni A.*, Beattie R., **Engler A.**, Grison A., Taylor V. Regulation of multi-lineage potential of hippocampal stem cells by Drosha and NFIB keeps oligodendrocytic differentiation in check, Cell Stem Cell, 2016;

Grants and Awards

2016-07-22 Swiss Society Neuroscience, Travel Fellowship for the Notch Gordon Conference at Bates College; CHF 1500

2016-04-26 Nachwuchsförderung Klinische Forschung (Promotion of Young Scientists); "Distinct Targets of Notch Signaling in Active and Quiescent Neural Stem Cells"; 6-month salary + consumables; CHF 49'487

2015-05-29 Scientific PhD Retreat - Presentation Award, 2nd place; "Neurogenic stem cells in a dormant vestigial niche are suppressed by Notch2 signaling"

Technical Knowledge and Additional Trainings

2014 – present **Licensed BD FACS Aria III Operator,** including maintenance, experimental assistance and troubleshooting

Jun. 2014 **BD FACSAria III Cell Sorter**, Operator Course, European Training Center, Erembodegem, Belgium, June 16th – June 20th 2014

Oct. 2012 Introductory course in **laboratory animal scienc**e, LTK Module 1E; 22nd – 26th October 2012

Abstracts

Engler A., Rolando C., Giachino C, Taylor V., Differential roles of Notch1 and Notch2 signaling in the adult murine brain, Gordon Research Seminar & Conference – Notch Signaling in Development, Regeneration & Disease, 30th July – 5th August 2016, Bates College, USA; *Oral Presentation & Poster*

Irkhof P., **Engler A.**, Spalinger M., Richter H., Grüniger S., Warum wir mit Tieren forschen – "Why we do animal experimentation"; reatch ETH Zürich, 21st April 2016, Basel, Switzerland, *Selected Speaker* at Podiums Conversation

Engler A., Rolando C., Giachino C, Junghans D., Taylor V., Untangling Adult Neurogenesis, Notch by Notch; Diss:kurs University of Basel, 3rd February 2016, Basel, Switzerland, *Selected oral presentation*

Engler A., Rolando C., Giachino C, Junghans D., Taylor V., Differential roles of Notch1 or Notch2 signaling in the adult murine brain, The Notch Meeting, 4th – 8th of October 2015, Athens, Greece, *Oral presentation*

Engler A., Rolando C., Taylor V., Identification of a remnant niche in the adult brain, PhD Retreat 2015, 28th – 30th May 2015, Schwarzsee, Switzerland, *Oral presentation*

Engler A., Rolando C., Giachino C., Taylor V., Heterogeneity of adult murine hippocampal neurogenesis in physiological and pathological conditions, Stem cells in development and disease, 9th -10th September 2014, Basel, Switzerland, *Poster*

Engler A., Giachino C., Rolando C., Taylor V., Modulation of Adult Hippocampal Neurogenesis in Physiological and Pathological Conditions, Keystone Symposia, Adult Neurogenesis, 12th – 17th May 2014, Stockholm, Sweden, *Poster*

Engler A., Rolando C., Giachino C., Taylor V., Adult hippocampal neurogenesis - in good times and bad times, PhD Retreat 2014, 16th – 18th January, 2014, Hasliberg, Switzerland, *Poster*

Engler A., Giachino C., Rolando C., Taylor V., Notch Signaling and mammalian neurogenesis, PhD retreat, 2013, 27th – 29th March, 2013, Hasliberg, Switzerland, *Poster*

Teaching Activity

Fall 2013 **Teaching assistance "Einführung in die Biologie",** Semester Course at the University of Basel, 12 teaching units for Bachelor Students

May 2012 **Laboratory assistance block course "Cell and Neurobiology"**at the Universtiy of Basel, 5 days practical teaching for Bachelor Students

Working Experience

Aug. 2010 Internship at Biozentrum with Dr. C.A. Schöneberger; Actin Assembly

Jul. 2008 Internship at Oerlikon Balzers, R & D Semiconductor Wafers

Management Skills

May. 2015 **Organization committee** for the PhD Retreat in Schwarzsee, CH, 3-day conference for 52 PhD Students of the University of Basel

Jan. 2014 **Organization committee** for the PhD Winter Retreat in Hasliberg, CH, 3-day conference for 45 PhD Students of the University of Basel

2009 – 2011 **Students' representative** for the students house Mittlere Strasse 33, Basel, for 103 students

Languages and Additional Skills

German native **French** good command

English fluent

Jan. 2016 **diss:kurs Training "Mündliche Präsentationen";** Presentation Training, course for selected participants, University of Basel, 27th/28th January 2016

Dec. 2015 **Proposal Writing**; Scientific Writing, Advanced Courses University of Basel, 26th November/03rd December 2015

Sept. 2015 **Academic Writing Conventions and Styles**: Writing to be Published; Scientific Writing, Advanced Courses University of Basel, 14th -16th September 2015

Nov. 2014 **Articles in the Life Sciences**: Structure and Clarity, Scientific Writing, Advanced Courses University of Basel, 14th November 2014