Notch Receptor Expression in Neurogenic Regions of the Adult Zebrafish Brain

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Abstract

The adult zebrafish brain has a remarkable constitutive neurogenic capacity. The regulation and maintenance of its adult neurogenic niches are poorly understood. In mammals, Notch signaling is involved in stem cell maintenance both in embryonic and adult CNS. To better understand how Notch signaling is involved in stem cell maintenance during adult neurogenesis in zebrafish we analysed Notch receptor expression in five neurogenic zones of the adult zebrafish brain. Combining proliferation and glial markers we identified several subsets of Notch receptor expressing cells. We found that 90\% of proliferating radial glia express notch1\textalpha, notch1\textbeta and notch3. In contrast, the proliferating non-glial populations of the dorsal telencephalon and hypothalamus rarely express notch3 and about half express notch1\textalpha/1\textbeta. In the non-proliferating radial glia notch3 is the predominant receptor throughout the brain. In the ventral telencephalon and in the mitotic area of the optic tectum, where cells have neuroepithelial properties, notch1\textalpha/1\textbeta/3 are expressed in most proliferating cells. However, in the cerebellar niche, although progenitors also have neuroepithelial properties, only notch1\textalpha/1\textbeta are expressed in a high number of PCNA\textsuperscript{+} cells. In this region notch3 expression is mostly in Bergmann glia and at low levels in few PCNA\textsuperscript{+} cells. Additionally, we found that in the proliferation zone of the ventral telencephalon, Notch receptors display an apical high to basal low gradient of expression. Notch receptors are also expressed in subpopulations of oligodendrocytes, neurons and endothelial cells. We suggest that the partial regional heterogeneity observed for Notch expression in progenitor cells might be related to the cellular diversity present in each of these neurogenic niches.

Introduction

Teleost fish, like many non-mammalian vertebrates, display widespread neurogenesis in adulthood (see review(s) [1–6]). Several proliferation zones were identified in distinct regions along the rostrocaudal axis, mainly located at the ventricular surfaces [7,8]. These zones contain precursor cells that actively cycle and generate offspring that migrates out to the mantle zone [8]. This is in contrast to neurogenesis in the adult murine brain, which is restricted to only two zones in the telencephalon – the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus (DG), in the hippocampus – and in the hypothalamus [9]. In mammalian models, these regions have been characterized at the ultrastructural level and their cellular composition and the molecular properties of the different cell types within these niches are known in detail (see review(s) [10–12]). Several lines of evidence suggest that some embryonic radial glia cells are neurogenic progenitors/neural stem cells (NSCs), that keep these properties throughout development and give rise to the SVZ cells (see review(s) [13,14]). However, few of the neurogenic niches have been analysed with respect to their cellular composition in the adult teleost brain [15–20]. In the zebrafish dorsal telencephalon, the cellular composition of progenitors is mixed, with a fraction of cells that do not display glia characteristics intermingled with others that show markers and morphology typical of radial glia [16,18]. In contrast, in the ventral part of the ventral telencephalon [18], optic tectum [17] and cerebellum [15], progenitor cells do not display radial glial properties but rather maintain neuroepithelial-like characteristics. It is still not understood how this divergence in the progenitor properties is achieved and what factors influence it.

The Notch pathway is a conserved pathway throughout the animal kingdom and has been intensely studied for its crucial role in cell fate decision, proliferation and cell death during embryonic neural development (for review see [21–26]). In the mammalian brain, both during development and in adulthood, active Notch signaling is required for NSCs maintenance [27–30] and self-renewal [29–31]. Studies have shown that Notch receptor activation suppresses neuronal [32–35] and oligodendrocyte differentiation [33,34,36–39] while promoting astrogliogenesis [33,34,40]. Expression studies in the marine embryonic telencephalon revealed that indeed several Notch receptors are present in the ventricular zone, where progenitors reside [40–42]. Also, in the postnatal and adult mouse brain, expression of Notch1

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receptor was detected in SVZ astrocytes, in migratory neuroblasts, in the ependymal layer, in a few cells of the olfactory bulb, in the subgranule cell layer of the dentate gyrus, and in Purkinje cells [29,30,34,43,44]. Some of the Notch expressing cells in the SVZ are proliferating and the expression levels decay with age [34]. In the same study it was observed that Notch activity in SVZ reactive astrocytes is increased upon cortical injury [34].

It has been recently proposed that, in the dorsal telencephalon of adult zebrafish, Notch signaling is responsible for turning neurogenic progenitors quiescent by blocking their proliferative capacity [45]. However, because the lineage relationships between cell types are not clear, the role of Notch signaling in the maintenance of the progenitor niche itself has not been clarified so far. It is not understood whether the different marker expression in the dorsal adult telencephalon [16,18] reveals distinct precursor populations, or if there is a common multipotent progenitor at the top of the hierarchy. The presence and requirement of Notch signaling at different steps of the progenitor hierarchy or in different progenitor subtypes has not been addressed so far in zebrafish.

Multiple Notch receptors are present in the zebrafish genome, namely notch1a, notch1b, notch2 (previously notch6) and notch3 (previously notch5) [46,47], and only notch2 expression is excluded from the nervous system during embryonic development [47]. Expression of these genes has been observed in the adult zebrafish telencephalon [45].

Because the cellular composition of progenitor cells in the adult zebrafish brain shows great diversity and different responses of progenitors to Notch signaling has been reported in mammals, we analysed in detail the expression of the four Notch receptors in five adult zebrafish neurogenic niches. To this end, we characterized the expression pattern of Notch receptors by in situ hybridization and co-stained them with glial, neuronal, oligodendrocytic, endothelial and proliferation markers. In the adult zebrafish brain, we find all known Notch receptors to be expressed, except notch6 which is not expressed in the nervous system during embryonic development [47].

In the dorsal telencephalon of adult zebrafish, notch1a, notch1b and notch3 expression is unevenly distributed, with a higher density in the ventricular zone of the dorsal-medial telencephalic area (Dm) [Fig. 1A, B] and in small clusters of cells in the ventricular zone of the lateral part of the dorsal-lateral telencephalic area (Dl) [Fig. 1A, B]. In the ventricular zone of the intermediate part of the dorso-lateral telencephalic area (Dil) a few scattered notch1a + and notch1b + cells are observed [Fig. 1A, B]. In contrast, notch3 + cells are present throughout the whole dT, uniformly distributed both in Dm and Dil (Fig. 1C–E). These expression patterns are found along the rostro-caudal axis of the adult telencephalon. Some scattered cells within the parenchyme are positive for notch1a, notch1b and notch3 both in the dT and vT [Fig. 1A, B, C]. To assess the identity of these notch receptor positive parenchymal cells, we performed double fluorescence in situ hybridization (FISH) for the receptors and alg2, a marker for oligodendrocytes. This revealed that all three Notch receptors are expressed in a subpopulation of alg2 + cells (Fig. S1). Using the reporter line Tg(fkh1:GFP) [48], that labels endothelial cells, we found that notch1a and notch3 localize in a subset of these GFP + cells (Fig. S2).

### Results

We focused our analysis on five neurogenic zones: the dorsal and ventral telencephalon, the hypothalamus, the optic tectum, and the cerebellum. It has been shown that proliferating cells in these areas are periventricularly located [10] or maintain contact with the ventricle by an apical process [13]. Some of these cells are radial-glia [7,16,18,20] but others display a more neuroepithelial-like morphology [15,17]. We examined if Notch receptors expression differs among niches or if it is conserved and relates with the cellular proliferative status. Expression of notch1a, notch1b and notch3 but not notch2 was detected in the adult brain. Thus notch2 is not further mentioned.

#### notch1a, 1b and notch3 expression in the adult telencephalon

In the adult zebrafish telencephalon, notch1a, notch1b and notch3 are mostly expressed in cells lining the ventricular surface (Fig. 1A–C). In the ventricular zone of the ventral telencephalon (vT) all three are strongly expressed (Fig. 1A–C). In the dorsal telencephalon (dT), notch1a + and notch1b + cells are unevenly distributed, with a higher density in the ventricular zone of the dorsal-medial telencephalic area (Dm) [Fig. 1A, B] and in small clusters of cells in the ventricular zone of the lateral part of the dorsal-lateral telencephalic area (Dl) [Fig. 1A, B].
proliferating cells are notch1a−/3− or notch1a−/1b−. Within the PCNA− population, notch1a+ cells are always notch3+, but not vice versa (Fig. 4A, B). Occasionally, PCNA−/notch1a−/notch1b− cells can be found (Fig. 4C). This means that only a very small fraction of proliferating cells are negative for Notch receptors.

All together, these results show that i) all three Notch receptors are expressed in approximately the same number of proliferating glial cells, ii) notch3 is expressed in the majority of S100β+ / PCNA− glial cells, whereas notch1a and notch1b are found just in half of this population, iii) notch1a and notch1b are often co-expressed in proliferating cells, iv) notch3 is expressed in very few S100β− / PCNA+ cells in contrast to notch1a and notch1b which are present in roughly more than half of this population.

**notch1a, 1b and notch3 show an apical to basal gradient of expression in proliferating cells of the ventral telencephalon**

The ventral telencephalic neurogenic niche is subdivided into the dorsal nucleus of the ventral telencephalon (Vd) and the ventral nucleus of the ventral telencephalon (Vv) [49]. In the...
Figure 2. **notch1a**, **notch1b** and **notch3** expression in radial glia and proliferating cells of the dorso-medial ventricular zone of the telencephalon. Confocal images showing localization of Notch receptors by FISH (white), radial glia labelled with S100β (red), and PCNA⁺ proliferating cells (green); DAPI (blue) is used as nuclear counterstaining. Schematics in A indicate the cross-section levels through the telencephalon and the dorsal telencephalic area represented in the micrographs. A–C, **notch1a** and D–E, **notch1b** expressing cells are scattered throughout the dorso-medial ventricular zone of the DT and co-localize with both PCNA⁻/S100β⁺ cells (filled arrowheads) and a subpopulation of PCNA⁻/S100β⁺ cells (white arrows); yellow arrows indicate Notch receptor⁻/PCNA⁻/S100β⁻ cells; unfilled yellow arrowheads indicate **notch1b**⁻/PCNA⁺ cells. F–H, **notch3** expressing cells localize to a great extent with the S100β marker, including both PCNA⁻ (white arrows) and PCNA⁺ (white arrowheads) cells; unfilled yellow arrowheads indicate **notch3**⁻/PCNA⁺ cells. I, Schematics indicating the cross-sections levels through the telencephalon, along the rostro-caudal axis, and examples of Dm areas used for marker co-localization analysis in J. J, Quantification on the co-localization of Notch receptor by FISH with PCNA and S100β, for the Dm region; all ventricularly located cells of the indicated area were counted; cells were distinguished based on their glia character (S100β⁺). Notch receptor expression and proliferative status (PCNA⁺). n = 9 (fish), 4–6 tissue sections per fish, at the rostro-caudal levels indicated in I; total number of cells counted = 7245. Values represented as mean percentage ± SEM. Significance levels: \( p < 0.001(\ast\ast\ast) \), \( p < 0.05(\ast) \). Abbreviations: Dm, dorso-medial telencephalic area; DT, dorsal telencephalic area; vT, ventral telencephalic area; Scale bars = 50 μm in A, in D and in F; 10 μm in C (applies to B), in E and in H (applies to G). doi:10.1371/journal.pone.0073384.g002
Figure 3. *notch1a*, *notch1b* and *notch3* expression in radial glia and proliferating cells of the dorso-lateral telencephalic ventricular zone. Confocal images showing localization of Notch receptors by FISH (white), radial glia labelled with S100*β* (red), and PCNA*+* proliferating cells (green); DAPI (blue) is used as nuclear counterstaining. Schematics in A indicate the cross-section levels through the telencephalon and the dorsal telencephalic area represented in the micrographs. A–B, *notch1a* and C–D, *notch1b* expressing cells are scattered throughout the dorso-lateral ventricular zone of the dT and localize with both PCNA*−/S100*β*+* cells (arrowheads) and a subpopulation of PCNA*−/S100*β*−* cells (white arrows); yellow arrows indicate Notch receptor*−/PCNA*−/S100*β*−* cells; unfilled yellow arrowheads indicate Notch receptor*−/PCNA*−/S100*β*−* cells. E–F, *notch3* expressing cells localize to a great extent with S100*β*, including both PCNA*−* (white arrows) and PCNA*−* (white arrowheads) cells; unfilled yellow arrowheads indicate *notch3*−/PCNA*−* cells. G, Quantification on the co-localization of Notch receptor by FISH with PCNA and S100*β*, for the DI region; schematics on the left indicate the cross-section levels through the telencephalon, along the rostro-caudal axis, and examples of DI areas used for marker co-localization analysis; all ventricularly located cells of the indicated area were counted; cells were distinguished based on their glia character (S100*β*+*), notch expression and proliferative status (PCNA*+*). n = 9 (fish), 4–6 tissue sections per fish, at different rostro-caudal levels; total number of cells counted = 6433. Values represented as mean percentage ± SEM. Significance levels: *p* < 0.001(***), *p* < 0.01(**), *p* < 0.05(*). Abbreviations: DI, dorso-lateral telencephalic area; dT, dorsal telencephalic area; vT, ventral telencephalic area; Scale bars = 50 μm in C (applies to A) and in E; 10 μm in B, D and F. doi:10.1371/journal.pone.0073384.g003
proliferation zone of Vd, glial markers are present whereas the proliferation zone of Vv is S100β− and shows only weak expression of other glial markers [18]. Moreover, the proliferation zone of Vv is positive for the intermediate filament marker Nestin, cells display neuroepithelial-like characteristics and show interkinetic nuclear migration [18]. In Vd, notch1a and notch1b are expressed in some, but not all of the S100β+ glial cells (Fig. 5A, B), while notch3 is found throughout the ventricular zone of Vd (Fig. 5C).

In Vv, we observe that Notch receptor expression overlaps with the majority of proliferating cells (Fig. 5), with only very few Notch receptor negative (more obvious caudally) (Fig. 6). Proliferating cells with nuclei either in an apical or basal position show Notch receptor expression. However, expression intensity differs along the apical-basal axis of the proliferation zone. To assess whether there is a differential expression between proliferating cells with distinct apical or basal nuclei positions we divided proliferating cells into two areas (apical and basal) and measured the mean grey value (MGV) for the corresponding areas (for details see Materials and Methods). We found a clear decrease of the fluorescence signal intensity in basally compared to apically located nuclei of proliferating cells. The maximum MGV was always obtained in apical nuclei for all three receptors (Fig. 5D). Moreover, fluorescence profile analysis shows that this gradient can be steeper for notch3 than for notch1a/1b (Fig. -S3). These results suggest that Notch receptor expression levels vary during cell cycle.

In contrast to dT, expression of notch1a, notch1b and notch3 is very similar in the proliferation zone of vT. To test the overlap of receptor expression in the vT proliferation zone, we performed double FISH for notch1a and notch3 and analysed their expression along the rostral-caudal axis (Fig. 6). At more rostral levels, the majority of proliferating cells express both receptors (Fig. 6A). We also observed that PCNA+ /Notch receptor− cells are usually basally located and in the most ventral part of Vv (Fig. 6A, S3B). More caudally, where the telencephalic ventricle closes, the vT

Figure 4. Overlapping and complementary Notch receptor expression in the dorsal telencephalon. Confocal images showing localization of Notch receptors pairs by double FISH (white and red) and PCNA+ proliferating cells (green); DAPI (blue) is used as nuclear counterstaining. Cross-sections at the indicated level through the telencephalon. Corresponding dorsal telencephalic areas represented in the micrographs are indicated in the cross section schematics of each panel. A, B, notch1a/3 co-expression in Dm and Dl, respectively. C, notch1a/b co-expression in Dm. These receptors are co-expressed both in PCNA+ (filled white arrowheads) and PCNA− (unfilled white arrowheads) cells; within the PCNA+ population, few cells are notch1a−/notch3+ (filled yellow arrowheads) while others are negative for both receptors (filled yellow arrows); within the PCNA− population, some cells express either notch3 or notch1b but not notch1a alone (unfilled yellow arrowheads). Scale bars = 50 μm.

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proliferation zone is reduced in size [16,18]. We observed that Notch receptor expression follows this pattern (Fig. 6B–D). Here, we also found PCNA + that were either positive or negative for Notch receptor expression. notch1a and notch3 partially overlap in PCNA + cells that flank the proliferating zone (Fig. 6B–D). However, in this case notch3 expression is clearly broader than notch1a. Occasionally, we found PCNA + cells expressing only notch1a (Fig. 6B).

In summary, notch3 shows an overall broad expression compared to notch1a and notch1b expression, that is present in clusters within the notch3 domain (Fig. 7A, B). Notch receptor expressing cells are mainly located along the ventricular zone of the ventral and dorsal telencephalon in PCNA + and PCNA − radial glia (Fig. 7). In the rostral vT proliferation zone Notch receptors are very similarly expressed with a stronger signal in cells with apically located nuclei (Fig. 7C).

**notch1a, 1b and notch3 expression in proliferative areas and radial glia of the adult hypothalamus**

The zebrafish hypothalamus is the largest diencephalic area and is subdivided in ventral, dorsal and caudal parts [50]. The hypothalamic proliferation zone is contiguous along these areas and proliferating cells are generally ventricularly located [8]. At the posterior recess in the caudal hypothalampus, proliferation is found periventricularly [8]. S100β cells are also present in these hypothalamic areas, similar to what has been observed for other teleosts [51], and they are arranged in several adjacent cell layers, extending processes to the ventricular surface (Fig. S4).

**notch1a and notch3 expression** is found along the periventricular ventral hypothalamic (Hv) in groups of cells with different expression intensities (Fig. 8A, B). In Hv, notch3 expression is continuous and sometimes present a few cells away from the ventricular surface (Fig. 8C).

In the dorsal periventricular hypothalampus (Hd), we detected single scattered notch1a + (Fig. 8A’, A”) and notch1b + (Fig. 8B’, B”) cells at the lateral recess (LR), with different signal intensities. In the LR, most cells did not show notch1a or notch1b expression (asterisk in Fig. 8A’ ’B”, S5B). At the junction between Hv and Hd, additional rows of notch3 expressing cells are found away from the ventricle (Fig. 8C). Moreover, in Hd/LR notch3 is strongly expressed in the cells lining the ventricle with a ventral gap in expression that becomes more evident caudally (Fig. 8C–C’).

In the caudal hypothalampus (Hc), at the level of the posterior recess, positive cells for notch1a and notch1b are arranged in a pattern that resembles the distribution of proliferating cells in this zone as previously shown [8]. In Hc, notch3 is expressed in a very similar fashion to notch1b (Fig. 8C–C’).

Co-labelling with the glial marker S100β and proliferation marker PCNA revealed that notch3 covers most of the S100β + domain and localizes with the majority of S100β + /PCNA + cells (Fig. S4). notch3 is also expressed in most of the S100β − /PCNA + cells, mainly in Hv (Fig. S4). In Hd, PCNA + cells are often notch3 − (Fig. S1C, F). Double FISH for notch1a/3 shows that notch1a is expressed in part of the notch3 domain (Fig. S5). We observed that few proliferating cells are negative for both receptors (Fig. S5).

In summary, Notch receptors are expressed in the hypothalamic periventricular areas, partially co-localizing with proliferating cells (Fig. 9). notch3 expression is continuous throughout the ventricular zone, and along the rostro-caudal axis, with the exception of the LR, where it partially excludes proliferating cells.
notch1a and notch1b expression domains are very similar and more restricted than notch3, specially in Hc and Hd (Fig. 9).

notch1a, 1b and notch3 expression in the proliferative areas and radial glia of the adult optic tectum

It has been previously described that, in the optic tectum (TeO) of adult teleost fish, active proliferating cells are located in the dorsomedial area of the periventricular gray zone (PGZ) [8,17,19]. These cells give rise to both neurons and glia that are later integrated into the mature tectal layers. This progenitor zone does not display typical radial glia properties as observed in the dorsal telencephalon [17,19]. Instead, radial glia cells are located in the deep layer of PGZ (dPGZ), lining the ventricle.

Another proliferation zone in the mesencephalon is the posterior mesencephalic lamina (PML), that seals the tectal ventricle and extends until the cerebellum [8,52]. In this study, we refer to the active proliferation areas as the mitotic region of the dorsomedial area of PGZ (mPGZ) and the posterior mesencephalic lamina (PML).

We observed expression of notch1a, notch1b and notch3 in dPGZ, mPGZ and PML (Fig. 10). Both notch1a (Fig. 10A) and notch1b (Fig. 10B) exhibit a stronger signal in mPGZ and PML than in dPGZ. A few cells in the neuronal layer of the TeO expressed notch1a and notch1b, the latter having a stronger signal and expression in a higher number of cells (Fig. 10). Similarly to what we observed in the telencephalon, these scattered notch1a and notch1b cells also express olig2 (Fig. S6). However, a subpopulation
of the notch1b + cells in the TeO does not express olig2 but is positive for the neuronal marker HuC/D (Fig. S6B). notch3 is strongly and homogeneously expressed in dPGZ, mPGZ and PML (Fig. 10C). Co-localization with the proliferation marker PCNA and the glial marker S100b shows that Notch receptors are present in both glia and proliferating cells (Fig. 11). notch3 is strongly expressed in the majority of S100b+ cells (Fig. 11J–M) whereas notch1a and notch1b expression is scattered in a subset of these cells (Fig. 11B, F). Most proliferating cells in mPGZ and PML express all three Notch receptors (Fig. 11A, E, J). To address if in fact they are co-expressed in the same proliferating cells, or if their expression is complementary, we performed double FISH. Indeed, only few proliferating cells lack expression of any of the receptors (yellow arrows in Fig. 12A, B). Furthermore, very few PCNA+ cells expressed either notch1a or notch1b, but none only notch3 (white arrows in Fig. 12A, B). In the PGZ S100b+ glia, notch1a is expressed in a subpopulation of notch3-expressing cells (Fig. 12A) while notch1a/1b are mostly complementary (Fig. 12B).

In summary, Notch receptors are expressed in the majority of mPGZ and PML cells, notch3 is expressed in all S100b+ glia of dPGZ, whereas notch1a and notch1b are in a subset of these cells (Fig. 12C). notch1a and notch1b are also present in olig2+ cells of the PGZ and more superficial tectal layers and notch1b in some HuC/D + neurons (Fig. 12C). These expression patterns are found throughout the whole rostrocaudal extent of PGZ and PML.

**notch1a, 1b and notch3 expression in the adult cerebellar niche**

Similarly to the TeO progenitor niche, proliferating cells in the adult cerebellar progenitor niche do not exhibit glial characteristics [15]. Here, progenitors and glia represent two spatially distinct populations. Progenitor cells reside in a cap-like structure of the dorsomedial corpus cerebelli and valvula cerebelli, at the interface between the molecular (ML) and granular cell layer (GL) [15]. We detected notch1a and notch1b expression in the cap-like structure (Fig. 13A, D). A few notch1b+ cells are also present in the intermediate molecular layer (IML) (Fig. 13D). notch3 expressing cells are scattered in the IML, more abundantly than notch1b, with a few positive cells in the ML and GL as well (Fig. 14A). We then asked if all three Notch receptors co-localize with PCNA+ progenitors and S100b+ Bergman glia, the latter located in the IML. We observed that notch1a and notch1b localize with a large portion of PCNA+ proliferating cells in the cerebellar niche and in a few S100b+ cells, ventrally adjacent to the progenitors (Fig. 13B–F). A few cells positive for notch1a or notch1b but negative for either PCNA or S100b are also detected in the ML, IML and GL (Fig. 13B, E). Co-staining of notch3 with PCNA and S100b reveals that most proliferating cells lack notch3 expression while most of the S100b+ Bergman glia cells express notch3 (Fig. 14B–D). Also notch3 expressing cells, negative for either PCNA or S100b are observed in the ML, IML and GL. We have not observed co-
localization of any of the Notch receptors with HuC/D neurons in this region (data not shown).

In summary, notch1a is present predominantly in the progenitor niche, whereas notch1b is also strongly expressed in a few Bergman glia cells. notch3 is mostly excluded from the niche but expressed in a high proportion of the Bergman glia (Fig. 14E).

Discussion

The study of adult neurogenesis and neuronal regeneration in zebrafish is fairly recent, thus an understanding of how important developmental pathways are involved in these processes is still missing. The Notch signaling pathway is important both in neural development and in adult neurogenesis, where it has mainly been studied in mammals. In the adult zebrafish brain, the cellular composition within a neurogenic zone and between neurogenic regions is similarly diverse as in mammals. So far, the presence and requirement of Notch signaling in distinct progenitor subtypes or in different steps of the progenitor hierarchy has not been addressed in the adult zebrafish. Thus, a detailed expression analysis of Notch pathway components in this model is important for proper interpretation of further functional studies of this signaling pathway in the context of adult neurogenesis and neuronal regeneration. It has been previously reported that notch1a/1b/2/3 are expressed in the ventricular zone of the adult zebrafish telencephalon [45], however, no systematic and comparative expression analysis in several neurogenic zones has been done.

Here we described in detail the expression pattern of three Notch receptors in five neurogenic regions of the adult zebrafish brain, and characterize Notch receptor expressing cells with respect to their proliferative status and glial-type precursor cell identity. Our analysis suggests that the adult zebrafish neurogenic niches have common properties, but also display local and regional heterogeneity regarding Notch receptor expression (Fig. 15). This heterogeneous distribution of Notch receptors could be related to a differential requirement for Notch signaling either in different progenitor types or at different stages/states of progenitor activity.

Notch signaling in regions of active neurogenesis

In rodents, Notch receptors are expressed in germinatal zones where active neurogenesis occurs both in the embryo [40–42,53] and the adult [42,43], with different receptors showing overlapping and complementary expression patterns [42,53]. Similarly, we found an extensive overlap in receptor expression patterns in the neurogenic zones of the adult zebrafish brain. In the dorsal

Figure 8. Notch receptor expression in the adult zebrafish hypothalamus. Notch receptors are differentially expressed throughout the hypothalamus. Cross-sections at the indicated level through the diencephalon. Brightfield images show the expression of A–A’, notch1a, B–B’, notch1b and C–C’, notch3 at different rostro-caudal levels of the hypothalamus. Strong (filled arrowheads) and weak (unfilled arrowheads) expressing cells are detected. Asterisks (★) indicate areas of no or very weak expression in the Hd. Abbreviations: DIV, diencephalic ventricle; Hc, periventricular caudal hypothalamus; Hd, periventricular dorsal hypothalamus; Hv, periventricular ventral hypothalamus; LR, lateral recess of the DIV; PR, posterior recess of the DIV; PTN, posterior tuberal nucleus. Scale bar = 100 µm in A (applies to all).

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zebrafish telencephalon and hypothalamus, proliferating cells negative for the marker S100β are rarely expressing notch3 and only half of them are notch1a/1b-positive (Fig. 15A). Moreover, in the ventral telencephalon and the mPGZ of the optic tectum, where progenitors have been described to have a neuroepithelial morphology [17,18], notch1a, notch1b and notch3 are present in the majority of proliferating cells and are mostly co-expressed (Fig. 15B). However in the cerebellum, where the neural stem cells also possess neuroepithelial properties [15], notch3 expression is very weak and observed in very few cells in contrast to notch1a

**Figure 9. Summary of Notch receptor expression pattern and cellular characteristics in the adult zebrafish hypothalamus.** Illustration shows the overall expression of the analysed Notch receptors at different rostro-caudal levels of the hypothalamus; they are mainly present inventricular zone cells of Hv, Hd and Hc and localize with glial and proliferation markers. Abbreviations: DIV, diencephalic ventricle; Hc, periventricular caudal hypothalamus; Hd, periventricular dorsal hypothalamus; Hv, periventricular ventral hypothalamus; LR, lateral recess of the DIV; PR, posterior recess of the DIV; PTN, posterior tuberal nucleus.

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**Figure 10. Notch receptor expression in the adult zebrafish optic tectum.** Cross-sections at the indicated level through the mesencephalon: optic tectum area shown in the micrographs is indicated in the cross section schematic in A. Brightfield images show the expression (by ISH) of A, notch1a, B, notch1b and C, notch3 along the PML and mPGZ (filled arrowheads) and in the dPGZ (filled arrows). A few notch1a + and notch1b + cells are also found in more superficial layers of the PGZ and TeO (unfilled arrows). Abbreviations: PGZ, periventricular gray zone of the optic tectum; dPGZ, deep layer of the PGZ; mPGZ, mitotic region of the PGZ; PML, posterior mesencephalic lamina; TeO, optic tectum. Scale bars = 100 μm in A (applies to all).

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Figure 11. Notch receptor expression in radial glia and proliferating cells of the adult zebrafish optic tectum. Cross-sections at the indicated level through the mesencephalon; optic tectum area shown in the micrographs is indicated in the cross section schematic in A. Confocal
and notch1b that are expressed in many proliferating cells (Fig. 15C).

Our expression pattern analysis is consistent with what has been observed in zebrafish larval brains, where notch1 expression was found to be located ventricularly, together with proliferative cells [54]. In another teleost species, Carassius auratus (goldfish), a notch homologue has been identified, and the expression of this receptor was also found in ventricularly located cells of the adult brain [53]. In contrast to what has been described by Chapouton et al. [45] we not only observed that notch3 is expressed in most of the radial glia population of the dorsal telencephalon and hypothalamic neurogenic niches, but also that it is associated, together with notch1a and notch1b, with proliferating cells throughout the adult brain. A possible explanation for these differences might be that we have performed the in situ hybridization on 12 μm thin sections instead of whole brains [45] which allows for better probe penetration and less probe trapping in ventricular areas. Moreover, we were unable to detect notch2 expression in the adult brain, opposed to what has been previously reported [45].

In the mammalian brain several studies support the idea that NSCs present high Notch activity, essential for their maintenance and proliferation [26,27,56–59], whereas neuronal committed progenitors or differentiating neurons display reduced or no Notch activity [26,59,60]. Nevertheless, effects on proliferation seem to be dichotomous, tissue- or cell dependent, dose dependent, and receptor dependent [61–67]. It is as yet not clear if progenitors in the adult zebrafish brain have distinct developmental origins and which might constitute different progenitor pools, or if there is a common progenitor from which all cell types are derived. It will therefore be interesting to analyze the modification of Notch signaling activity in the adult zebrafish brain on different cell types and diverse differentiation processes. Currently this aspect is difficult to study due to the lack of specific NSC markers and tools to reliably monitor Notch signaling activity in different cell types or at distinct steps of differentiation.

Notch in different progenitors of the adult zebrafish CNS

In the dorsal telencephalon we observed that approximately 20% of the radial glia cells are proliferating, in homeostatic conditions. In the adult zebrafish telencephalon at least part of the radial glia population shows high Notch activity [18,45]. These cells are neurogenic progenitors and increase their proliferative activity upon stab-lesioning to the dorsal telencephalon [68]. In the adult rodent brain, upon injury or stroke, Notch expression in reactive astrocytes increases [34] whereas it decreases in ependymal cells [69]. In ependymal cells, high Notch activity prevents them to re-enter cell cycle in response to injury, limiting their neurogenic potential [69]. In the adult zebrafish, the role of Notch signaling in progenitor proliferation during constitutive neurogenesis [45,70,71] and after injury [71,72] is still unclear, as it might depend on the cellular context.

Here we show that notch1a, notch1b and notch3 are expressed in proliferating glial cells, which form the majority of the constitutively proliferating neural progenitor population (Fig. 15A). However, in the glial marker-negative proliferating cells notch1a and notch1b are found in roughly half of this population, whereas notch3 expression is rare (Fig. 15A). As the majority of label retaining cells (slow cycling) in the telencephalon are glial marker negative [18], we hypothesize that notch1a/1b but not notch3 might be expressed in a fraction of the slow cycling population (putative NSCs). Considering that the cellular composition of the ventricular zone cells is very diverse, different cell types could be more or less responsive to variations in Notch signaling activity, and the outcome of such variations may be very different depending on the cell type/state and neurogenic niche. It could also well be that combinatorial signaling mediated by the activation of different Notch receptors triggers distinct responses in different cells at different stages.

Notch and glial identity

The role of Notch signaling in glial cell fate determination seems to be time and context dependent [27,33,73]. During retina development in zebrafish, overexpression of NICD promotes the gliogenic fate at the expense of neurogenesis [74]. In mouse cerebellar development, Notch receptors are expressed in Bergman glia and are important for normal development and maturation of this brain region [75]. This is consistent with our observations that notch3 is strongly expressed in the majority of S100β+ cells, throughout the adult zebrafish brain, with notch1a and notch1b expressed in a subset of notch3+ S100β+ cells. Based on our expression data, we hypothesize that notch3 might have a more prominent role in the glial lineage, whereas notch1a/1b expression might be related to their progenitor character (Fig. 15A). In mammals, both receptor redundancy and receptor sub-functionalization have been proposed [40,65,76], however this remains controversial. In zebrafish also little is known in this regard thus functional studies are needed to test whether Notch receptors have a redundant role in adult neurogenesis or not. Based on the diversity of cells and combination of Notch receptors expression found we can speculate that, in the adult zebrafish neurogenic niches, a certain “notch code” might exist resulting in different outcomes for cell fate, stem cell maintenance, cell survival and proliferative capacity.

Notch receptors and the cell cycle

Progenitors in the ventral part of the ventral telencephalon (Vv) of the adult zebrafish brain show a pseudostratified neuroepithelial-like morphology and interkinetic nuclear migration [18]. In this region, we observed that Notch receptor expression is stronger in cells closer to the ventricle (apically), where mitosis occurs and decreases away from the ventricle, similar to chick and zebrafish embryonic neuroepithelium [77,78]. Disruption of the interkinetic nuclear movement resulted in more cells in S-phase and lead to increased neurogenesis, possibly due to a reduced exposure to Notch signaling [77,79]. In contrast, during mouse brain development, Notch signaling is activated in ventricular zone
neuroepithelial cells undergoing S-phase, but not in M-phase cells at the lumenal surface of the ventricular zone [59].

In chick spinal cord, a study reports that Notch activity increases at a higher frequency prior to mitosis [80]. However, there are also cells that activate Notch signaling after mitosis, while they move basally, either in only one sibling or in both, suggesting that Notch activation at different cell cycle phases might be related with the cell fate of daughter cells [80]. Conceivably, the situation...

**Figure 12. Overlapping and complementary notch1a/3 expression in the adult zebrafish optic tectum.** Confocal images showing localization of Notch receptor pairs by double FISH (white and red) and PCNA + proliferating cells (green). Cross-sections at the indicated level through the mesencephalon; tectal area shown in the micrographs is indicated in the cross section schematics. A, notch1a/3 and B, notch1a/1b expression domains in the dPGZ and mPGZ layers. Co-expression of these receptors both in PCNA + (filled white arrowheads) and PCNA − cells (unfilled white arrowheads); notch3 + -only cells in A and notch1b + -only cells in B are indicated by unfilled yellow arrowheads; white arrows indicate notch1a + /notch3 − /PCNA + cells in A; a few PCNA − cells are Notch receptor − (filled yellow arrows); unfilled white arrows indicate cells positive for notch1a alone. C, Summary of Notch receptor expression pattern and cellular characteristics in the TeO. Abbreviations: Cce, corpus cerebelli; PGZ, periventricular gray zone of the optic tectum; dPGZ, deep layer of the PGZ; mPGZ, mitotic region of the PGZ; PML, posterior mesencephalic lamina; TeO, optic tectum. Scale bars = 50 μm. doi:10.1371/journal.pone.0073384.g012
in Vv may be similar to the chick neural tube, where divisions resulting in two daughter cells with similar Notch activity stay progenitors while siblings with an asymmetric Notch activity adopt different fates (Fig. 15B). However, additional studies looking at Notch signaling activity in the ventricular zone of Vv will be necessary.

**Notch receptor expression outside proliferation zones**

Notch signaling is not only active in germinal zones but also in non-proliferative areas. Similarly to the adult mouse cerebellum [43], notch3, and partially notch1b, are expressed in Bergmann glia in the adult zebrafish. Additionally, Notch1 receptor is expressed in mature neurons in the rodent cortex [35,42,81] where it is necessary for proper neocortical neuronal migration [81]. Moreover, it has been shown that Notch signaling regulates dendrite growth [82–85] and morphology [35,81]. Interestingly, during zebrafish spinal cord neurogenesis, notch1a is expressed both in neural precursors and in differentiating neurons whereas notch1b and notch3 are found only in neural precursors [86]. In contrast, in the adult zebrafish brain we observe expression of all three

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**Figure 13. notch1a and notch1b expression in the adult cerebellar niche.** Cross-sections at the indicated level through the mesencephalon; cerebellar area shown in the micrographs is indicated in the cross-section schematic in A. Brightfield images show expression of A, notch1a and D, notch1b in the cerebellum (arrowheads). Confocal images showing localization of the glial marker S100β (red) and PCNA (green), with B–C, notch1a and E–F, notch1b by FISH (white). B–C, Expression of notch1a localizes with a large fraction of PCNA + cells in the niche (unfilled arrowheads); weak (white arrow) or no expression of notch1a is detected in the Bergmann glia (S100β +). E–F, notch1b is expressed in a subpopulation of PCNA + cells (unfilled arrowheads) and in a few S100β + cells (white arrows). A few scattered notch1a + and notch1b + cells in the ML, IML and GL do not localize with the analysed markers (unfilled arrows). Abbreviations: GL, granule cell layer; IML, intermediate layer; ML, molecular layer. Scale bars = 50 μm in A, B, D and E; 20 μm in C and F.

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receptors in putative neuronal precursors but only notch1b in a HuC/D + neuronal population. However, we did not detect Notch receptor expression in newborn neurons adjacent to germinat zones. The notch1b expression that we observed in neurons could indicate that also in the adult zebrafish brain Notch signaling might serve later stages of neuronal differentiation and/or neuronal activity.

Figure 14. notch3 expression in the adult cerebellar niche. Cross-sections at the indicated level through the mesencephalon; cerebellar area shown in the micrographs is indicated in the cross-section schematic. A, Brightfield image shows notch3 expressing cells in the cerebellum (black arrowheads). B–D, Confocal images showing localization of the glial marker S100 (red) and PCNA (green), with notch3 by FISH (white). B–C, notch3 is weakly expressed in a small subset of PCNA + cells in the stem cell niche (unfilled arrowhead). B–D, Strong notch3 expression is detected in S100 + cells indicated by the white arrows; notch3 expression is also detected in some scattered cells of the ML, IML and GL that do not localize with the analysed markers (unfilled arrows); E, Summary of the expression pattern and cellular characteristics of Notch receptor expressing cells in the adult cerebellum. Abbreviations: GL, granule cell layer; IML, intermediate layer; ML, molecular layer. Scale bars = 50 μm in A and B; 20 μm in C and D. doi:10.1371/journal.pone.0073384.g014

PNRG - Proliferating non-RG (S100B+/PCNA+)
BG - Bergmann glia (S100B+/PCNA-) N - Neurons (HuC/D+)

Notch receptor expression

n1α+/β+/3+
n1α+/β+/3'
n1α+/β+/3' +
n1α'/β+/3'

ML IML GL

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Oligodendrocytes are another cell type produced in neurogenic regions of the adult zebrafish brain [17,18,68,87]. *olig2* is a marker for both oligodendrocyte precursor cells (OPCs) and, in combination with other markers, for more mature oligodendrocytes [18,87–90]. Interestingly, we also found Notch receptor expression in a subpopulation of *olig2* + cells in the telencephalon parenchyma and optic tectum. We also detected Notch receptor expression in *olig2* + proliferating cells in the ventricular zone (not shown), but the parenchyma subpopulation of Notch receptor + / *olig2* + cells were not proliferating. It has been shown that Notch signaling, together with *olig2*, promotes the specification of OPCs [38] but inhibits their differentiation [36,37]. Assuming that Notch signaling has a similar role in adult oligodendrogenesis, this parenchymal Notch receptor + / *olig2* + subpopulation could represent immature oligodendrocytes.

Notch signaling is also involved in the development of the vascular system (see review(s) [91,92]). Notch1 has been implicated in vascular homeostasis [91], and in zebrafish, *notch3* expression is associated with the arterial fate during arterial-venous differentiation [93]. In the present study, we observed a subset of endothelial cells in the telencephalon that express *notch1a* and *notch3*. This suggests that also in the adult zebrafish brain, Notch signaling might be important for vessel homeostasis or for the maintenance of arterial phenotype in some cells.

**Conclusion**

Our results showed that Notch receptor expression domains overlap to a great extent in the neurogenic areas, but also that regional and cellular heterogeneity in the expression of different receptors exists in the adult zebrafish brain. The differences observed between receptor expression in certain niches could indicate that they are involved in distinct cellular processes. Therefore, we hypothesise that: i) predominantly *notch3* might be important for the glial properties of progenitors as well as other glial cells; ii) *notch1a/1b* might modulate progenitors proliferative status in a level-dependent manner [67]; iii) combinatorial Notch receptor expression could be necessary to achieve a certain basal level of Notch activity, required for NSC maintenance; iv) also in the adult brain, Notch signalling might be involved in multiple processes including neuronal maturation, oligodendrogenesis and blood vessel homeostasis. All these hypothesis remain to be tested functionally in the context of adult neurogenesis in the zebrafish brain. Only cell type specific modulation of the pathway will allow to unravel the role of Notch signalling in these processes. Moreover, labelling and lineage tracing of different progenitor cells are needed to achieve a better understanding of the ontogeny, properties and persistence of neurogenic niches in adulthood.

**Materials and Methods**

**Ethics statement**

All procedures were in accordance with the live animal handling and research regulations of the University and State of Saxony, Germany, review boards, the Regierungspräsidium Dresden (permit AZ 24D-9168.11-1/2008–1 and –4). This institutional review board specifically approved this study.

**Animals and tissue preparation**

Fish were kept under standard conditions as previously described [94,95]. Wildtype experimental animals were adult fish from the *gol-b1* line in the AB genetic background [96]. Adult fish were 6 months old and both sexes were used in this study. Transgenic animals Tg(/fli:GFP) [48] were 1.5 years old.

Whole heads with the brain exposed were fixed in 4% PFA/0.1 M phosphate buffer pH 7.4, at 4°C overnight. After fixation they were washed twice with 0.1 M phosphate buffer, pH 7.4 and transferred into 20% sucrose/0.2% EDTA in 0.1 M phosphate buffer, pH 7.4, at 4°C overnight for decalcification and cryoprotection. Heads were embedded and frozen in 7.5% gelatine/20% sucrose. For chromogenic in situ hybridization experiments, brains were cryosectioned in 14 μm sections. For fluorescent in situ hybridization (FISH) stainings, brains were cryosectioned in 12 μm sections. Sections were collected on superfrost slides. After sectioning the slides were stored at –20°C.
**In situ hybridization and FISH**

Riboprobe preparation was done as previously described [97], using the NTP labelling mixture (Roche). In this study we used the following probes: notch1a [46] (2.7 kb, nucleic position 3027–5012 of NM 131441.1), notch1b [47] (1.3 kb, nucleic acid position 4738–6060 of NM_131302.2), notch2 [47] (3.2 kb of NM_00115094.1), notch3 [47] (1.3 kb, nucleic acid position 4724–5803 of NM_131549.2) and olig2 [96]. The *in situ* hybridization was done as previously described [97] with some modifications. Hybridization was done at 60°C followed by stringent washes at 62°C. Sections were incubated with either anti-digoxigenin-AP (#1109274910, Roche) or anti-fluorescein-AP (#1142638910, Roche) Fab fragment diluted 1:4000 and subsequently stained with the substrate NBT/BCIP (Roche). In the case of FISH, a similar protocol was followed but with some modifications. Briefly, after thawing the sections, they were rehydrated in PBS, and then incubated in 0.3% H2O2 for 30 minutes. After quenching, the tissue was post-fixed in 4% PFA for 20 minutes. Prior to hybridization, sections were washed in PBS and then in PBS-Tx. For the blocking step, sections were incubated for 1 hour at RT in 1% of blocking reagent (Perkin-Elmer) in MABT. After blocking, sections were incubated overnight at 4°C in anti-digoxigenin-POD, or anti-fluorescein-POD Fab fragment (Roche), diluted 1:500. For detection we used the TSA Plus Cy3/Cy5 kit (NEL744001KT/ NEL745001KT, Perkin-Elmer). The FISH shows the same expression pattern as in the chromogenic *in situ* reaction, with better sensitivity for detecting cells expressing lower levels of notch transcripts. All *in situ* hybridizations were done on at least five individuals. All probes were tested on embryos to confirm probe specificity. We have also performed negative controls: 1) with least five individuals. All probes were tested on embryos to confirm tissue was post-fixed in 4% PFA for 20 minutes. Prior to hybridization, sections were washed in PBS and then in PBS-Tx. For the blocking step, sections were incubated for 1 hour at RT in 1% of blocking reagent (Perkin-Elmer) in MABT. After blocking, sections were incubated overnight at 4°C in anti-digoxigenin-POD, or anti-fluorescein-POD Fab fragment (Roche), diluted 1:500. For detection we used the TSA Plus Cy3/Cy5 kit (NEL744001KT/ NEL745001KT, Perkin-Elmer). The FISH shows the same expression pattern as in the chromogenic *in situ* reaction, with better sensitivity for detecting cells expressing lower levels of notch transcripts. All *in situ* hybridizations were done on at least five individuals. All probes were tested on embryos to confirm probe specificity. We have also performed negative controls: 1) with probe and without anti-DIG antibody, and 2) without probe and without anti-DIG antibody to assess for unspecified signal (antibody or tyramide trapping). In these tests no signal was detected under the fluorescence microscope.

**Immunohistochemistry**

Immunostainings for S100β (polyclonal rabbit, Dako Cyto., 1:500), PCNA (monoclonal mouse, molecular probes, 1:1000) and HuC/D (monoclonal mouse, molecular probes, 1:200) were done after completion of the FISH protocol. For PCNA and HuC/D stainings we retrieved the antigen in 50 mM Tris Buffer, pH 8, for 8 minutes at 95°C and 15 minutes at RT, prior to primary antibody incubation. As previously described [8], we performed antibody incubation we washed the sections 3 times in PBSTx, and incubated in species-specific Alexa 488-, 555-, and 633-fluorophores. To minimize cross talk between the channels, sequential image acquisition was performed. Images were processed using Fiji (http://fiji.sc), CombineZP v7.0 (http://www.hadleyweb.pwp.blueyonder.co.uk/GZIP/News.htm) and Adobe Photoshop CS4. Figures were assembled using Adobe Illustrator CS4. Confocal images shown are single planes or maximum projections of up to 3 planes (maximum 5 μm thick, corresponding to only 1 cell layer). Co-localization analysis of the different markers was done in the Z-stack using the orthogonal view tool in Fiji. We considered a positive cell for Notch receptor expression when at least 2 dots of tyramid precipitate where observed in more than one plane of the cell.

**Cell counting and statistical analysis**

The quantifications of co-localization between the FISH, S100β and PCNA markers were performed in a total of 9 individuals, using 6 sections (12 μm) per fish, representative of main telencephalon parts along the rostro-caudal axis. In these quantifications we subdivided the dorsal telencephalon into dorsal-medial and dorsal-lateral areas. Co-localization of the markers was analysed through each z-stack using the cell counter plugin and the orthogonal view tool in Fiji, with the aid of DAPI nuclear counterstaining and S100β, glial marker that labels cells cytoplasm. Statistical significance analysis was done in the statistical computing software R (http://www.r-project.org/) using ANOVA followed by a post hoc Tukey's HSD test.

**Fluorescence measurements and statistical analysis**

To analyse Notch receptor expression levels in proliferating cells of the ventral telencephalon we measured the mean grey value (MGV) of the FISH signal for each receptor. We selected the regions of interest based on the position of the PCNA + nucleus (apical or basal). A nucleus was considered apical if positioned immediately adjacent to the ventricular surface; as basal if the nucleus was between 2nd–4th rows of nuclei away from the ventricle. We measured the MGV in selected apical- or basal-area of 2–6 tissue sections of the rostro-ventral telencephalon. To each measurement we subtracted the background signal within the tissue. After background subtraction, MGVs were normalized against the maximum MGV of each experiment. In total, 28 apical-area and 28 basal-area MGVs were obtained for notch1a (n = 4), 24 for notch1b (n = 4) and 26 for notch3 (n = 5). For data visualization and median comparison we used notched boxplots in R and modified them with Adobe Illustrator CS4. To confirm statistically significant differences in expression of each gene between apical and basal or between genes in the same nuclei position we applied the Wilcoxon rank-sum test or ANOVA followed by a post-hoc Tukey’s HSD test, respectively. Fluorescence profile analysis was done using Fiji Plot Profile. Fluorescence values for each gene expression were normalized for background signal in the tissue and their corresponding maximum. Plots were done in Microsoft Office Excel 2007 and modified in Adobe Illustrator CS4.

**Supporting Information**

**Figure S1 Notch receptor expression in telencephalic oligodendrocytes**. Confocal images of double FISH showing the localization of Notch receptor (white) and olig2 (green) in the dorsal telencephalon parenchyme. A–C, notch1a, notch1b and notch3 are expressed in a subpopulation of parenchymal olig2 + cells (white arrows). Yellow arrows indicate Notch receptor + / olig2 + cells; unfilled white arrows in B indicate notch1b + / olig2 + cells. Scale bar = 50 μm in A (applies to all). (TIF)

**Figure S2 Notch receptor expression in fltl1:gfp endothelial cells in the telencephalic parenchyme**. Confocal images showing localization of notch1a and notch3 and gfp + endothelial cells in the dorsal telencephalon parenchyme. A–B notch1a and notch3 expression in a few endothelial cells (white...
arrows); unfilled arrow in B indicates a notch3+/gfp− cell adjacent to the blood vessel. Scale bar = 20 μm in A (applies to B).

**Figure S3** Apical to basal gradient of Notch receptor expression in the ventral telencephalic niche. Fluorescence profile measurements of Notch receptor expression in the two indicated areas (dark grey rectangle in schematics) of the Vv proliferation zone. Stronger fluorescence signals are detected in proliferating cells (PCNA+/+) with a more apical nucleus. A, Where the proliferation zone is thinner (only 2 PCNA+/+ nuclei), all three receptors show a steep profile with comparatively low levels of expression in the more basal proliferating cell. B, Where the proliferation zone is thicker (3 PCNA+/+ nuclei), notch3 is mostly expressed in the cell closer to the ventricle whereas notch1a and notch1b show strong expression levels until 2 PCNA+/+ nuclei away from the ventricle. These measurements were done in single stacks corresponding to the images shown in Fig. 5A-C.

**(TIF)**

**Figure S4** notch3 expression in glia and proliferating cells of the adult zebrafish hypothalamus. Confocal images showing localization of notch3 mRNA by FISH (white), radial glia labelled with S100β (red), and PCNA+/+ proliferating cells (green). Cross-sections at the indicated levels through the diencephalon; hypothalamic area shown in the micrographs is indicated in the cross-section schematics. A, B, notch3 is expressed in most PCNA+/+/100β− cells and in PCNA−/S100β− cells (unfilled white arrowheads) of the Hv; unfilled yellow arrowheads indicate notch3−/PCNA+/S100β− cells. A–F, notch3 localizes with most S100β+/+ cells of Hd and Hc, PCNA+/+ (filled white arrowheads) or PCNA− (filled white arrows); filled yellow arrowhead indicates a notch3−/PCNA+/+S100β− cell. Notice the notch3 expression in the S100β+ cellular processes in Hc (in E). Asterisk indicates a S100β+ group of cells in Hd that is negative for notch3. Abbreviations: Hc, caudal zone of the periventricular hypothalamus; Hd, dorsal zone of the periventricular hypothalamus; Hv, ventral zone of the periventricular hypothalamus. Scale bar = 100 μm in A (applies to B). Scale bars = 50 μm.

**(TIF)**

**Figure S5** Overlapping and complementary notch1a/3 expression in the adult zebrafish hypothalamus. Confocal images of double FISH showing the localization of notch1a (white), notch3 (red), and PCNA (green). Cross-sections at the indicated level through the diencephalon; hypothalamic area shown in the micrographs is indicated in the cross-section schematics. A–C, notch1a is expressed in a subpopulation of notch3+/PCNA− cells in Hv, Hd and Hc (filled white arrowheads); yellow arrows indicate Notch receptor−/PCNA+/+ cells; unfilled yellow arrowheads indicate cells expressing notch3+ alone. notch1a expression partially overlaps with the notch3+/PCNA− population (unfilled white arrowheads); there are a few notch1a+/+/notch3−/PCNA− cells in Hv and Hc (filled white arrows). Abbreviations: Hv, posterior recess of the diencephalic ventricle. Scale bar = 100 μm in A (applies to B).

**(TIF)**

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