Hypothalamic radial glia function as self-renewing neural progenitors in the absence of Wnt/β-catenin signaling

Robert N. Duncan, Yuanyuan Xie, Adam D. McPherson, Andrew V. Taibi, Joshua L. Bonkowsky, Adam D. Douglass, and Richard I. Dorsky

Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, Utah

Correspondence should be addressed to R.I.D. (richard.dorsky@neuro.utah.edu).

Phone: 801-581-6073

Keywords: Wnt signaling, Radial Glia, Neural Progenitors, Hypothalamus, Zebrafish
Abstract

The vertebrate hypothalamus contains persistent radial glia that have been proposed to function as neural progenitors. In zebrafish, a high level of postembryonic hypothalamic neurogenesis has been observed, but the role of radial glia in generating these new neurons is unclear. We have used inducible Cre-mediated lineage labeling to show that a population of hypothalamic radial glia undergoes self-renewal and generates multiple neuronal subtypes at larval stages. While Wnt/β-catenin signaling has been demonstrated to promote the expansion of other stem and progenitor cell populations, we find that pathway activity inhibits this process in hypothalamic radial glia, and is not required for their self-renewal. In contrast, Wnt/β-catenin signaling is required for the differentiation of a specific subset of radial glial neuronal progeny residing along the ventricular surface. We also show that partial genetic ablation of hypothalamic radial glia or their progeny causes a net increase in their proliferation, which is also independent of Wnt/β-catenin signaling. Hypothalamic radial glia in the zebrafish larva thus exhibit several key characteristics of a neural stem cell population, and our data support the idea that Wnt pathway function may not be homogeneous in all stem or progenitor cells.
Introduction

The postembryonic zebrafish brain is highly proliferative and regenerative, characteristics that have been attributed to the presence of radial glia that persist throughout the central nervous system (CNS) and generate neurons (Kizil et al., 2012; Than-Trong and Bally-Cuif, 2015). We previously characterized a population of neural progenitors in the postembryonic zebrafish hypothalamus, which produces multiple neuronal subtypes through adulthood (Wang et al., 2012). A similar process also occurs in the mammalian hypothalamus, where adult neurogenesis contributes to reproductive and feeding behaviors (Kokoeva et al., 2005; Lee et al., 2012; Cheng, 2013). However the underlying progenitor cell populations supporting hypothalamic neurogenesis remain poorly characterized. While radial glia have been proposed to fulfill this role in both zebrafish and mouse (Lee et al., 2012; Wang et al., 2012; Haan et al., 2013; Robins et al., 2013), their capacity for self-renewal and differentiation have not been comprehensively tested.

In addition, the molecular pathways regulating radial glial self-renewal, expansion, and neurogenesis in the hypothalamus are poorly understood. Previous work from our laboratory showed that Wnt/β-catenin signaling is required for postembryonic hypothalamic neurogenesis (Wang et al., 2012), and other studies have also led to the hypothesis that pathway activity promotes radial glial differentiation (Lee et al., 2006; Wang et al., 2011; Choe and Pleasure, 2012; Wang et al., 2012; Varela-Nallar and Inestrosa, 2013). In contrast, Wnt/β-catenin signaling has also been shown to promote the self-renewal and expansion of neural stem cells in the mammalian telencephalic subventricular zone and dentate gyrus (Qu et al., 2010). The specific function of Wnt/β-catenin activity in hypothalamic radial glia is therefore unclear, leaving an
open question as to whether a general role for the pathway exists for all neural stem and progenitor cell populations.

Here we take a genetic approach to identify the neural progenitor cell population in the larval zebrafish hypothalamus, and to characterize the response and regulation of hypothalamic radial glia during tissue growth and regeneration. Our data show that the radial glial population is both self-renewing and multipotent, and exhibits a proliferative response to partial ablation of themselves or their neuronal progeny. In addition, we use multiple perturbations of Wnt/β-catenin signaling to test the necessity and sufficiency of pathway activity for radial glial self-renewal, expansion, and neuronal differentiation. Consistent with studies of non-neural stem cells (Lowry et al., 2005; Blanpain and Fuchs, 2009; Farin et al., 2012), our data show that Wnt/β-catenin signaling is only necessary for the terminal differentiation of specific neuronal progeny. Furthermore, and as shown for radial glia in other brain regions (Wang et al., 2011), we find that ectopic Wnt/β-catenin activity inhibits the expansion of neurogenic radial glia in the hypothalamus. Together, these data suggest that the most generally conserved role for Wnt pathway activity in neural progenitors is in promoting neurogenesis, and that other functions may differ between diverse stem and progenitor cell populations.
Results

Radial glia are multipotent neural progenitors in the postembryonic hypothalamus

In an effort to identify molecular markers of radial glia in the zebrafish hypothalamic posterior recess (Fig. 1A), we found that at 5 days post-fertilization (dpf), Glutamine Synthetase (GS) and a her4.3:EGFP transgene (Fig. 1B, Yeo, et al., 2007) both label radially oriented cells contacting the ventricle along with a Et(Gal4-VP16;myl7:gfp)zc1066a enhancer trap line that we previously showed to be expressed in radial glia of the posterior recess (Figure 1C, Wang et al., 2012). While there was not complete co-expression likely due to transgene mosaicism, the majority of cells expressing GS also expressed her4.3:EGFP (Figure 1D). A consistent minority of cells labeled by her4.3:EGFP were also GS-, potentially representing non-glial progeny due to GFP perdurance (Briona and Dorsky, 2014). In contrast we did not observe expression of either GFAP (Wang et al., 2012), or S100ß (data not shown) in this region. Combined with previous evidence supporting the specificity of GS and her4.3:EGFP in labeling radial glia throughout the zebrafish brain (Ganz et al., 2010; Grupp et al., 2010; Kroehne et al., 2011), we concluded that both markers also effectively label this population in the posterior recess.

Consistent with our previous work (Wang et al., 2012), we found that only 5.5% ± 2.5% (S.E.M., n=11 optical sections from 2 brains) of GS+ cells expressed the Wnt reporter transgene 7xTCF-Xla.Siam:mCherry (Moro et al., 2012, Figure 1E). Furthermore, analysis of embryos homozygous for a null mutation in the Wnt effector lefl (Wang et al., 2012) at 5 dpf (Figure 1F), showed that the number of GS+ cells was in fact increased in the posterior recess relative to tissue size (29.5±1.4 in wild-
type vs. 32.0±2.7 in lefl mutants, S.E.M., n=3 equatorial optical sections each from 4 brains). These data, along with observations that her4.3:EGFP+ cells are also not decreased in lefl mutants (data not shown), support our prior conclusion that hypothalamic radial glia do not require Wnt/β-catenin activity for their formation or maintenance.

To determine the lineage of the radial glial population, we took advantage of an existing transgenic line that uses the her4.3 promoter/enhancer to drive the expression of tamoxifen-inducible Cre recombinase (Boniface et al., 2009). By crossing this line to the Cre-inducible ubi:switch reporter (Mosimann et al., 2011), which expresses mCherry following recombination, we were thus able to permanently label all progeny (Figure 2A). Addition of 5µM 4-hydroxytamoxifen (4-OHT) from 5-6 dpf resulted in conversion of 1-5 cells per posterior recess, and we did not observe any mCherry expression in untreated larvae. Analysis at 6 dpf showed that 97% ± 3% of mCherry+ cells (S.E.M., n=30 optical sections from 3 brains) were co-labeled by GS (Figure 2B, Supplementary Figure 1A). Six days after recombination, radial chains of labeled progeny were visible extending from the ventricle, including both GS+ and GS- cells (Figure 2C). By 6 weeks post-fertilization (wpf), labeled progeny had expanded into large radial clones (Figure 2D), which contained only 8.7% ± 1.5% GS+ cells (S.E.M, n=30 optical sections from 3 brains, Figure 2E, Supplementary Figure 1B). By using markers for differentiated neuronal cell types, we found that at 6 wpf the lineage included neurons labeled by HuC/D (Figure 2F, Supplementary Figure 1C), serotonin (Figure 2G, Perez et al., 2013), and a transgenic marker of dopaminergic fate [Tg(th2:Gal-VP16)zd202, McPherson et al., submitted, Figure 2H].

These data indicate that postembryonic neurons in the zebrafish hypothalamus arise
from a radial glial population that can self-renew, expand, and generate multiple types of progeny.

**Wnt/β-catenin signaling is only required for differentiation of a specific subset of neuronal progeny**

We next tested whether Wnt/β-catenin signaling is necessary for the self-renewal, expansion, or neuronal differentiation of radial glia. Using heat shock-mediated expression of the secreted Wnt signaling inhibitor, Dkk1 (Stoick-Cooper et al., 2007), following conversion of the Cre-labeled population with 4-OHT, we examined the effects on the lineage size and composition. After conversion from 5-6 dpf, Tg(hsp701:dkk1-GFP)y32 embryos were heat shocked once daily and fixed at 9 dpf. While Dkk1 expression effectively inhibited Wnt signaling as determined by in situ hybridization of the Wnt/β-catenin target sp5l (Weidinger et al., 2005 and Supplementary Figure 2), it did not result in a significant difference in the total number of labeled cells (Figure 3A,D), or in the percent of GS+ radial glia (Figure 3B,D) in the lineage. Dkk1 expression also did not inhibit the differentiation of HuC/D+ neurons from labeled radial glia (Figure 3C), and in fact caused a small but statistically insignificant increase in neurogenesis. These results suggest that radial glia can divide and produce neuronal progeny in the absence of Wnt pathway activity.

As an alternative method to inhibit Wnt signaling we examined the radial glial lineage in lef1 mutants. After 4-OHT-mediated conversion from 5-6 dpf and lineage analysis at 9 dpf, we found that loss of lef1 also did not significantly change the percentage of GS+ radial glia (Figure 3E) within mCherry-labeled progeny. As we reported previously, lef1 is critically required to generate a subset of HuC/D+ neurons in the posterior recess (Wang et al., 2012). Our lineage analysis confirmed that these lef1-dependent neurons arise from radial glia and showed that they specifically reside
within two cell diameters of the ventricle (Figure 3F,G). However they comprise only a small portion of radial glial progeny, and the number of non-ventricular neurons was not decreased (Figure 3F). Combined with the results of Dkk1 overexpression, these data lead us to conclude that Wnt/β-catenin signaling is not necessary for radial glial self-renewal or expansion. In addition, while Lef1-mediated Wnt activity is required for the differentiation of ventricular neurons, it is not required for the majority of neurogenesis in the hypothalamic posterior recess.

**Partial genetic ablation of radial glia leads to increased proliferation of GS+ cells**

To test whether hypothalamic radial glia show a regenerative response similar to other neural stem cell populations, we used the Et(Gal4-VP16,myl7: GFP)\(^{c1066a}\) enhancer trap line in combination with the Tg(UAS-E1b:NTR-mCherry)\(^{h17}\) effector line to express Nitroreductase (NTR) specifically in radial glia of the posterior recess (Otsuna et al., 2015), and thus ablate cells using metronidazole (MTZ, Davison et al., 2007; Pisharath et al., 2007). Consistent with previous studies, incubation of non-transgenic larvae in 1mM MTZ did not significantly affect cell proliferation or cell death (data not shown). In contrast, after incubation of NTR-expressing larvae in 1mM MTZ from 5-6 dpf we observed partial ablation of radial glia (Figure 4A,B, Supplementary Figure 3), and the remaining radial glia, labeled either by GS (Figure 4C,D) or her4.3:EGFP, showed variable but significantly increased BrdU labeling from 7-8 dpf (Figure 4C-F). This result suggested that radial glia can react to a decrease in their own population with a corresponding increase in self-renewal.

To determine if the proliferative response of radial glia to partial ablation requires Wnt/β-catenin signaling, we repeated our experiments in the presence of Dkk1 overexpression and in lefl mutants. In both cases we observed a similar increase in BrdU incorporation within GS+ cells as in control animals (Figure 4G,H). These data
indicate that just as in during normal growth, the regenerative expansion of hypothalamic radial glia is also Wnt/β-catenin independent.

**Radial glia proliferate in response to genetic ablation of progeny**

We next wanted to determine whether hypothalamic radial glia exhibit a proliferative response to the loss of a progeny cell type. Since we had observed that the lineage included dopaminergic neurons labeled by the *th2* enhancer/promoter (Figure 2F), and we found that the enhancer was not expressed in GS+ cells (Figure 5A), we used a transgenic line [Tg(*th2*:Gal-VP16)]z20, McPherson et al., submitted] to drive UAS:NTR-mCherry expression (Figure 5B). Following incubation in a high dose (2.5mM) of MTZ from 5-6 dpf to maximize the level of ablation, we observed a significant increase in BrdU labeling within the GS+ population at 8-9 dpf (Figure 5C-E) but not one day earlier or later (Fig. 5C). The proliferative response coincided with a decrease in the overall number of GS+ cells at 9 dpf (Figure 5F). Along with an increase in BrdU labeled GS- cells (Figure 5D), which are likely non-glial Sox3+ neural progenitors (Wang et al., 2012), these data are consistent with the depletion of radial glia observed during regeneration in the adult zebrafish telencephalon (Barbosa et al., 2015).

**Wnt activation blocks expansion of the radial glial population**

Based on evidence that ectopic Wnt/β-catenin signaling leads to a decrease in the number of radial glia (Wang et al., 2011; Wang et al., 2012) we wanted to determine whether pathway activity specifically inhibits the normal expansion of their progeny. Following induction of *wnt8a* at 5 dpf using the heat shock-inducible transgenic line Tg(hsp70l:*wnt8a*-GFP)w34 (Weidinger et al., 2005), we observed a significant decrease in the number of GS+ cells at 6 dpf compared to controls (Figure 6A).
Continuous *wnt8a* expression over multiple days resulted in lethality, so to test the longer-term consequences of pathway activation we incubated animals in 4µM BIO, a pharmacological activator of Wnt/β-catenin signaling (Sato et al., 2004; Shimizu et al., 2012; Lush and Piotrowski, 2014) from 6-9 dpf. This experiment also produced a small but significant decrease in the number of GS+ cells compared to controls (Figure 6B,C), suggesting that Wnt signaling either inhibits radial glial expansion or causes the loss of GS expression. To specifically test these possibilities, we next performed lineage analysis in the presence of BIO from 6-9 dpf after recombination from 5-6 dpf. We found that the total number of mCherry+ cells in animals treated with BIO was significantly decreased compared to controls (Figure 6D), coupled with a relative increase in the proportion of GS+ cells within the labeled population (Figure 6E). The smaller number of progeny was not due to cell death, as neither *wnt8a* induction (Wang et al., 2012) nor BIO treatment (data not shown) caused a significant increase in apoptosis. Our results could therefore by explained by a decrease in the number of radial glia undergoing amplifying divisions, combined with the decrease in neurogenesis that we previously demonstrated to result from constitutive Wnt activation (Wang et al., 2012).

**Discussion**

**Hypothalamic radial glia exhibit multiple features of neural stem cells**

Our results demonstrate that hypothalamic radial glia in zebrafish are self-renewing neural progenitors that can undergo a regenerative response, characteristics that are hallmarks of a stem cell population. Because we were not able to follow the lineage of single cells, we cannot determine whether individual radial glia are multipotent with
respect to neuronal fate. However the expansion that we observe in the lineage over a 5 week labeling period indicates that radial glia contribute significantly to the growth in size of the posterior recess, and our marker analysis shows that the population as a whole generates several neuronal subtypes.

While previous studies from our laboratory and others have observed the presence of proliferating neural progenitors in the adult zebrafish hypothalamus (Wang et al., 2012; Perez et al., 2013), the work described here focused on an earlier period of larval development. The behavior of radial glia during this period is therefore not strictly equivalent to other adult stem cell populations, which are typically quiescent or support tissue homeostasis rather than growth. Future studies testing the lineage and injury response of radial glia in the adult zebrafish posterior recess will provide more insight into whether they function as true neural stem cells.

**Wnt/β-catenin signaling is not necessary for hypothalamic radial glial self-renewal or expansion**

Studies in the CNS (Piccin and Morshead, 2011) and other tissues (Nusse, 2008; Holland et al., 2013) have resulted in the hypothesis that Wnt/β-catenin signaling may function generally to promote stem and progenitor cell proliferation. In order to achieve the increase in population size that we observe in our lineage analysis, radial glia must undergo amplifying self-renewing divisions, and as has been shown in the telencephalon (Barbosa et al., 2015), regeneration may require these divisions at an even higher frequency. However our data indicate that ectopic Wnt activity in fact inhibits the expansion of hypothalamic radial glia population size, while other studies suggest that signals such as FGF (Kaslin et al., 2009; Robins et al., 2013), and Sonic Hedgehog (Dave et al., 2011; Shikata et al., 2011; Komada, 2012) likely promote this process.
We found that a specific subset of lef1-dependent neurons located near the hypothalamic ventricle arise from the radial glial lineage (Wang et al., 2012). Combined with other studies in the retina (Agathocleous et al., 2009), cerebral cortex (Munji et al., 2011; Zhang et al., 2014), hippocampus (Seib et al., 2013), and midbrain (Castelo-Branco et al., 2003), our data suggest that the most widely conserved role for Wnt/β-catenin signaling in the CNS may be to regulate the differentiation of specific subsets of committed neural progenitors.

**Wnt/β-catenin activity does not act identically in all neural stem and progenitor cells**

Our experiments support the idea that diverse neural stem and progenitor cell populations likely exhibit different responses to Wnt/β-catenin signaling. While Wnt ligands and reporters are expressed at high levels in the hypothalamic ventricular zone (Wang et al., 2012), radial glia largely fail to respond to these signals. This low activity state could be regulated by extracellular or intracellular pathway antagonists, or radial glia may simply fail to express the appropriate receptors to transduce Wnt signals. Regardless of the mechanism, it appears that this characteristic of hypothalamic radial glia is similar to other radial glial populations in the zebrafish retina and spinal cord (Goldman, 2014; Briona et al., 2015), but different from radial glia in the mammalian dentate gyrus (Qu et al., 2010). Other studies have similarly shown that neural progenitor populations vary dramatically in their interpretation of pathway activity (Poschl et al., 2013). Understanding these differences may help provide insight into the basis of radial glial, and neural stem/progenitor cell, heterogeneity.
Materials and Methods

Use of zebrafish

Embryos were obtained from the following zebrafish lines: Tg(her4.3:EGFP)y83 (Yeo et al., 2007), Tg(ubi:loxP-eGFP-loxP-mCherry)cz1701 (Mosimann et al., 2011), Tg(-3her4.1:ERT2-Cre-ERT2)u298 (Boniface et al., 2009; Mosimann et al., 2011), Et(Gal4-VP16,myl7:gfp)zc1066a (Wang et al., 2012), Tg(UAS-E1b:NTR-mCherry)h17 (Davison et al., 2007; Pisharath et al., 2007), Tg(7xTCF-Xla.Siam:GFP)j44 (Moro et al., 2012), Tg(hsp701:dkk1-GFP)w32 (Stoick-Cooper et al., 2007), Tg(hsp70l:wnt8a-GFP)w34 (Weidinger et al., 2005), lef1zd11 (Wang et al., 2012), Tg(th2:GFP-aequorin)zd201 (McPherson et al., submitted), and Tg(th2:Gal-VP16)zd202 (McPherson et al., submitted). Embryos were staged according to (Kimmel et al., 1995). All experiments were approved by the University of Utah Institutional Animal Care and Use Committee.

Transgenic embryos were identified by GFP tag expression following heat shock induction of wnt8 or dkk1, or by PCR amplification of trunk tissue for dkk1 induction in the presence of the –3.5ubi:loxP-EGFP-loxP-mCherry reporter using the following primers: (dkk1 forward: tcgactcaaggatcaccaca, mgfp5 reverse: tccctcaaacttgacttcagc). lef1 mutant animals were identified by the absence of posterior neuromasts as labeled with DASPEI (McGraw et al., 2011; Wang et al., 2012).

Treatment of embryos and larvae

Cre-mediated recombination was performed by incubation in 5µM 4-Hydroxytamoxifen (4-OHT) (Sigma, CAS RN 68047-06-3) in 1% DMSO from 5-6 dpf. Ablations were performed by incubation in 1mM Metronidazole (MTZ) (Fluka 46461) from 5-6 dpf. To activate Wnt/β-catenin signaling, larvae were incubated in
4µM BIO (Sigma B1686) from 6-9 dpf, with fresh solution added each day. For BrdU labeling, larvae were incubated in 10mM BrdU for one day prior to fixation for all experiments. Heat shock experiments were performed by incubating larvae in 50mL conical tubes in a 39.5C water bath for 20 minutes. For experiments from 5-9 dpf, larvae were not fed.

**Immunohistochemistry and in situ hybridization**

Embryos were fixed in 4% paraformaldehyde with 5% sucrose overnight at 4C. Brains were then dissected for immunohistochemistry and trunks were placed in PCR tubes for genotyping. Whole brains were washed in water, incubated in 2N HCl for 20 minutes at room temperature (for BrdU detection), washed, and permeabilized with one unit of Dispase (Gibco 17105-041) for 90 minutes at room temperature. Primary antibodies were all used at 1:500 dilutions and incubated overnight at 4C: mouse anti Glutamine Synthetase (Millipore MAB302), rabbit anti DsRed (Clontech 632496), chicken anti GFP (Aves Labs GFP-1020), chicken anti BrdU (Immunology Consultants Laboratory CBDU-65A-Z), rabbit anti 5-HT (ImmunoStar 541016), mouse anti HuC/D (Molecular Probes A21271), goat anti L-Plastin (Santa Cruz Biotechnology sc-16657). Following washes, fluorescent secondary antibodies (diluted 1:500 in solution containing Hoechst 33342) were incubated overnight at 4C. Brains were imaged on a Nikon A1 confocal microscope with a 60X oil objective. The entire posterior recess was imaged using 3µm steps encompassing roughly 40µm total, cell counting was performed, and images were exported to Adobe Photoshop, Adobe Illustrator and ImageJ (NIH) for figure generation.

*In situ* hybridization was performed as described previously (Wang et al., 2012) using an antisense probe for *sp5l* generated from a PCR-amplified cDNA template. A T7 RNA polymerase initiation sequence was added to the 5’ end of the reverse primer.
statistical analyses

Microsoft Excel was used to perform two-tailed equal variance T-tests, and p-values < 0.05 were interpreted as statistically significant.
Acknowledgements

R.N.D., A.D.M., and A.V.T. were supported by NIH T32 NS07067, and A.D.M. was supported by NIH T32 HD07491. R.I.D. was supported by NIH R01 NS082645. Imaging was performed in the Fluorescence Microscopy Core Facility at the University of Utah, which is funded by NIH 1S10RR024761. We thank the University of Utah Centralized Zebrafish Animal Resource (CZAR) for assistance with animal care, and Wenbiao Chen (Vanderbilt U.), Christian Mosimann (U. Zürich), and Ajay Chitnis (NICHD) for sharing zebrafish lines.

Author Contributions

Experiments were performed by R.N.D., Y.X., A.D.M., and A.V.T. Transgenic lines were made by A.D.D. \[Tg(th2:GFP-Aequorin)^zd201]\], and by J.L.B. \[Tg(th2:Gal-VP16)^zd202]\]. R.N.D. wrote the manuscript, and R.I.D. supervised all the experiments and edited the manuscript.
References

Agathocleous, M., Iordanova, I., Willardsen, M. I., Xue, X. Y., Vetter, M. L., Harris, W. A. and Moore, K. B. (2009). A directional Wnt/beta-catenin-Sox2-proneural pathway regulates the transition from proliferation to differentiation in the Xenopus retina. Development 136, 3289-3299.

Barbosa, J. S., Sanchez-Gonzalez, R., Di Giaimo, R., Baumgart, E. V., Theis, F. J., Gotz, M. and Ninkovic, J. (2015). Neurodevelopment. Live imaging of adult neural stem cell behavior in the intact and injured zebrafish brain. Science 348, 789-793.

Blanpain, C. and Fuchs, E. (2009). Epidermal homeostasis: a balancing act of stem cells in the skin. Nature reviews. Molecular cell biology 10, 207-217.

Boniface, E. J., Lu, J., Victoroff, T., Zhu, M. and Chen, W. (2009). FlEx-based transgenic reporter lines for visualization of Cre and Flp activity in live zebrafish. Genesis 47, 484-491.

Briona, L. K. and Dorsky, R. I. (2014). Radial glial progenitors repair the zebrafish spinal cord following transection. Exp Neurol 256, 81-92.

Briona, L. K., Poulain, F. E., Mosimann, C. and Dorsky, R. I. (2015). Wnt/β-catenin signaling is required for radial glial neurogenesis following spinal cord injury. Dev Biol.

Castelo-Branco, G., Wagner, J., Rodriguez, F. J., Kele, J., Sousa, K., Rawal, N., Pasolli, H. A., Fuchs, E., Kitajewski, J. and Arenas, E. (2003). Differential regulation of midbrain dopaminergic neuron development by Wnt-1, Wnt-3a, and Wnt-5a. Proc. Natl. Acad. Sci. U. S. A. 100, 12747-12752.

Cheng, M. F. (2013). Hypothalamic neurogenesis in the adult brain. Frontiers in neuroendocrinology 34, 167-178.

Choe, Y. and Pleasure, S. J. (2012). Wnt signaling regulates intermediate precursor production in the postnatal dentate gyrus by regulating CXCR4 expression. Developmental neuroscience 34, 502-514.

Dave, R. K., Ellis, T., Toumpas, M. C., Robson, J. P., Julian, E., Adolphe, C., Bartlett, P. F., Cooper, H. M., Reynolds, B. A. and Wainwright, B. J. (2011). Sonic hedgehog and notch signaling can cooperate to regulate neurogenic divisions of neocortical progenitors. PloS one 6, e14680.

Davison, J. M., Akitake, C. M., Goll, M. G., Rhee, J. M., Gosse, N., Baier, H., Halpern, M. E., Leach, S. D. and Parsons, M. J. (2007). Transactivation from Gal4-VP16 transgenic insertions for tissue-specific cell labeling and ablation in zebrafish. Developmental biology 304, 811-824.

Farin, H. F., Van Es, J. H. and Clevers, H. (2012). Redundant sources of Wnt regulate intestinal stem cells and promote formation of Paneth cells. Gastroenterology 143, 1518-1529 e1517.

Ganz, J., Kaslin, J., Hochmann, S., Freudenreich, D. and Brand, M. (2010). Heterogeneity and FGF dependence of adult neural progenitors in the zebrafish telencephalon. Glia 58, 1345-1363.
Goldman, D. (2014). Muller glial cell reprogramming and retina regeneration. *Nat Rev Neurosci* 15, 431-442.

Grupp, L., Wolburg, H. and Mack, A. F. (2010). Astroglial structures in the zebrafish brain. *The Journal of comparative neurology* 518, 4277-4287.

Haan, N., Goodman, T., Najdi-Samiei, A., Stratford, C. M., Rice, R., El Agha, E., Belluscio, S. and Hajihosseini, M. K. (2013). Fgf10-expressing tanyctyes add new neurons to the appetite/energy-balance regulating centers of the postnatal and adult hypothalamus. *J Neurosci* 33, 6170-6180.

Holland, J. D., Klaus, A., Garratt, A. N. and Birchmeier, W. (2013). Wnt signaling in stem and cancer stem cells. *Current opinion in cell biology* 25, 254-264.

Kaslin, J., Ganz, J., Geffarth, M., Grandel, H., Hans, S. and Brand, M. (2009). Stem cells in the adult zebrafish cerebellum: initiation and maintenance of a novel stem cell niche. *J Neurosci* 29, 6142-6153.

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. and Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Dev Dyn* 203, 253-310.

Kizil, C., Kaslin, J., Kroehne, V. and Brand, M. (2012). Adult neurogenesis and brain regeneration in zebrafish. *Dev Neurobiol* 72, 429-461.

Kokoeva, M. V., Yin, H. and Flier, J. S. (2005). Neurogenesis in the hypothalamus of adult mice: potential role in energy balance. *Science* 310, 679-683.

Komada, M. (2012). Sonic hedgehog signaling coordinates the proliferation and differentiation of neural stem/progenitor cells by regulating cell cycle kinetics during development of the neocortex. *Congenital anomalies* 52, 72-77.

Kroehne, V., Freudenreich, D., Hans, S., Kaslin, J. and Brand, M. (2011). Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. *Development* 138, 4831-4841.

Lee, D. A., Bedont, J. L., Pak, T., Wang, H., Song, J., Miranda-Angulo, A., Takiar, V., Charubhumi, V., Balordi, F., Takebayashi, H. et al. (2012). Tanyctyes of the hypothalamic median eminence form a diet-responsive neurogenic niche. *Nature neuroscience* 15, 700-702.

Lee, J. E., Wu, S. F., Goering, L. M. and Dorsky, R. I. (2006). Canonical Wnt signaling through Lef1 is required for hypothalamic neurogenesis. *Development* 133, 4451-4461.

Lowry, W. E., Blanpain, C., Nowak, J. A., Guasch, G., Lewis, L. and Fuchs, E. (2005). Defining the impact of beta-catenin/Tcf transactivation on epithelial stem cells. *Genes Dev.* 19, 1596-1611.

Lush, M. E. and Piotrowski, T. (2014). ErbB expressing Schwann cells control lateral line progenitor cells via non-cell-autonomous regulation of Wnt/beta-catenin. *eLife* 3, e01832.

McGraw, H. F., Drerup, C. M., Culbertson, M. D., Linbo, T., Raible, D. W. and Nechiporuk, A. V. (2011). Lef1 is required for progenitor cell identity in the zebrafish lateral line primordium. *Development* 138, 3921-3930.

Moro, E., Ozhani-Kizil, G., Mongera, A., Beis, D., Wierzbicki, C., Young, R. M., Bournele, D., Domenichini, A., Valdivia, L. E., Lum, L. et al. (2012). In vivo Wnt
signaling tracing through a transgenic biosensor fish reveals novel activity domains. *Developmental biology.*

**Mosimann, C., Kaufman, C. K., Li, P., Pugach, E. K., Tamplin, O. J. and Zon, L. I.** (2011). Ubiquitous transgene expression and Cre-based recombination driven by the ubiquitin promoter in zebrafish. *Development* **138**, 169-177.

**Munji, R. N., Choe, Y., Li, G., Siegenthaler, J. A. and Pleasure, S. J.** (2011). Wnt signaling regulates neuronal differentiation of cortical intermediate progenitors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **31**, 1676-1687.

**Nusse, R.** (2008). Wnt signaling and stem cell control. *Cell research* **18**, 523-527.

**Otsuna, H., Hutcheson, D., Duncan, R., McPherson, A., Scoresby, A., Gaynes, B., Tong, Z., Fujimoto, E., Kwan, K., Chien, C. et al.** (2015). High-resolution analysis of CNS expression patterns in zebrafish Gal4 enhancer-trap lines. *Dev Dyn.*

**Piccin, D. and Morshead, C. M.** (2011). Wnt signaling regulates symmetry of division of neural stem cells in the adult brain and in response to injury. *Stem cells* **29**, 528-538.

**Pisharath, H., Rhee, J. M., Swanson, M. A., Leach, S. D. and Parsons, M. J.** (2007). Targeted ablation of beta cells in the embryonic zebrafish pancreas using E. coli nitroreductase. *Mechanisms of development* **124**, 218-229.

**Poschl, J., Grammel, D., Dorostkar, M. M., Kretzschmar, H. A. and Schuller, U.** (2013). Constitutive activation of beta-catenin in neural progenitors results in disrupted proliferation and migration of neurons within the central nervous system. *Developmental biology* **374**, 319-332.

**Qu, Q., Sun, G., Li, W., Yang, S., Ye, P., Zhao, C., Yu, R. T., Gage, F. H., Evans, R. M. and Shi, Y.** (2010). Orphan nuclear receptor TLX activates Wnt/beta-catenin signalling to stimulate neural stem cell proliferation and self-renewal. *Nat Cell Biol* **12**, 31-40; sup pp 31-39.

**Robins, S. C., Stewart, I., McNay, D. E., Taylor, V., Giachino, C., Goetz, M., Ninkovic, J., Briancon, N., Maratos-Flier, E., Flier, J. S. et al.** (2013). alpha-Tanyctyes of the adult hypothalamic third ventricle include distinct populations of FGF-responsive neural progenitors. *Nature communications* **4**, 2049.

**Sato, N., Meijer, L., Skaltsounis, L., Greengard, P. and Brivanlou, A. H.** (2004). Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med* **10**, 55-63.

**Seib, D. R., Corsini, N. S., Ellwanger, K., Plaas, C., Mateos, A., Pitzer, C., Niehrs, C., Celikel, T. and Martin-Villalba, A.** (2013). Loss of Dickkopf-1 restores neurogenesis in old age and counteracts cognitive decline. *Cell stem cell* **12**, 204-214.
Shikata, Y., Okada, T., Hashimoto, M., Ellis, T., Matsumaru, D., Shiroishi, T., Ogawa, M., Wainwright, B. and Motoyama, J. (2011). Ptch1-mediated dosage-dependent action of Shh signaling regulates neural progenitor development at late gestational stages. Developmental biology 349, 147-159.

Shimizu, N., Kawakami, K. and Ishitani, T. (2012). Visualization and exploration of Tcf/Lef function using a highly responsive Wnt/beta-catenin signaling-reporter transgenic zebrafish. Dev Biol 370, 71-85.

Stoick-Cooper, C. L., Weidinger, G., Riehle, K. J., Hubbert, C., Major, M. B., Fausto, N. and Moon, R. T. (2007). Distinct Wnt signaling pathways have opposing roles in appendage regeneration. Development 134, 479-489.

Than-Trong, E. and Bally-Cuif, L. (2015). Radial glia and neural progenitors in the adult zebrafish central nervous system. Glia.

Varela-Nallar, L. and Inestrosa, N. C. (2013). Wnt signaling in the regulation of adult hippocampal neurogenesis. Frontiers in cellular neuroscience 7, 100.

Wang, X., Imura, T., Sofroniew, M. V. and Fushiki, S. (2011). Loss of adenomatous polyposis coli in Bergmann glia disrupts their unique architecture and leads to cell nonautonomous neurodegeneration of cerebellar Purkinje neurons. Glia 59, 857-868.

Wang, X., Kopinke, D., Lin, J., McPherson, A. D., Duncan, R. N., Otsuna, H., Moro, E., Hoshijima, K., Grunwald, D. J., Argenton, F. et al. (2012). Wnt signaling regulates postembryonic hypothalamic progenitor differentiation. Developmental cell 23, 624-636.

Weidinger, G., Thorpe, C. J., Wuennenberg-Stapleton, K., Ngai, J. and Moon, R. T. (2005). The Sp1-related transcription factors sp5 and sp5-like act downstream of Wnt/beta-catenin signaling in mesoderm and neuroectoderm patterning. Curr. Biol. 15, 489-500.

Yeo, S. Y., Kim, M., Kim, H. S., Huh, T. L. and Chitnis, A. B. (2007). Fluorescent protein expression driven by her4 regulatory elements reveals the spatiotemporal pattern of Notch signaling in the nervous system of zebrafish embryos. Developmental biology 301, 555-567.

Zhang, S., Li, J., Lea, R., Vleminkx, K. and Amaya, E. (2014). Fezf2 promotes neuronal differentiation through localised activation of Wnt/beta-catenin signalling during forebrain development. Development 141, 4794-4805.
Figure 1. Radial glia in the hypothalamic posterior recess are not Wnt-responsive.

circle. (A) Schematic diagram of the hypothalamic posterior recess from a ventral view of a 5 dpf brain. Radial glia are labeled in green, red box indicates the area depicted in panel (B). (B) High-magnification optical section of her4.3:EGFP expression, showing the position of cell soma and radial processes. (C) Radial glial cells in the 5 dpf posterior recess can be labeled with anti-Glutamine Synthetase (GS, blue), a her4.3:EGFP transgene (green), and a Gal4 enhancer trap line (red). Yellow box indicates region shown in lower panels. A triple labeled cell is indicated by red circles. (D) Most cells expressing GS or her4.3:EGFP also express the other marker. Error bars = S.E.M., n=22 optical sections from 2 brains. (E) Most GS+ and her4.3:EGFP+ radial glia do not express the Wnt reporter transgene 7xTCF-Xla.Siam:GFP. Yellow box indicates region shown in lower panels. A reporter-negative radial glial cell is indicated by green circles, and a reporter-expressing non-glial cell is indicated by red circles. (F) GS+ cells are not reduced in lef1 mutants at 5 dpf. Images are single optical sections from ventral views of whole-mount brains. Scale bars=10µM.
Figure 2. Hypothalamic radial glia are self-renewing neural progenitors. (A) Timeline and schematic cartoon of experiments. Cells expressing -3her4.1:ERT2-CreERT and ubi:loxP-eGFP-loxP-mCherry were genetically labeled by the addition of 5µM 4-OHT from 5-6dpf. Labeled progeny (red) comprise expanding radial units spanning hemispheres of the posterior recess. Processes of GS+ radial glia (green) span the tissue, and nuclei occupy variable positions from the ventricle to the outer
edge. Neurons (blue) are located either adjacent to the ventricle or at the outer edge. (B) Immediately following conversion, the few labeled mCherry+ cells are also GS+. Yellow boxes indicate regions shown in lower panels. (C) Six days after conversion, labeled cells extend radially and include GS+ (yellow box) and GS- (green box) cells. (D) Five weeks after 4-OHT addition, discrete groups of mCherry+ cells extend from the ventricle (dashed line). (E-H) mCherry+ progeny 5 weeks after recombination include GS+ radial glia (E), HuC/D+ neurons (F), 5HT+ neurons (G), and th2:gfp+ dopaminergic neurons (H). Yellow boxes indicate regions shown in lower panels, and double-labeled cells are indicated by red circles. Green signal in (E,F) is ubi:GFP from unconverted cells. Images are single optical sections from ventral views of whole-mount brains. Scale bars=10µM.
Figure 3. Wnt/β-catenin signaling is only required for differentiation of a specific subset of ventricular neurons. (A-D) Following recombination of -3her4.1:ERT2-Cre-ERT+ progeny from 5-6 dpf, expression of Dkk1 from 6-9 dpf does not affect the average number of labeled cells (A), or the percentage of GS+ cells (B) in the lineage. (C) The differentiation of HuC/D+ neurons from labeled radial glia is not inhibited by Dkk1 expression. (D) Representative images of labeling for lineage and GS in control and Dkk1-expressing larvae. (E) In lef1 mutants there is no change in the percentage of GS+ cells after conversion from 5-6 dpf and analysis at 9 dpf, (F,G) lef1-dependent ventricular neurons fail to arise from the radial glial lineage, while other non-ventricular neurons are not significantly affected. Yellow boxes indicate areas shown in side panels. Images are single optical sections from ventral views of whole-mount brains. Scale bars=10μM. Error bars=S.E.M., n=50 confocal slices from 5 brains for each experiment.
Figure 4. Hypothalamic radial glia respond to partial ablation by increasing proliferative activity. (A-B) Partial ablation of NTR-mCherry-expressing radial glia (red) by incubation in 1mM MTZ from 5-6 dpf. (C-F) After partial ablation from 5-6
dpf and BrdU labeling from 7-8 dpf (C-D, yellow box indicates region shown in lower panels), there is a significant increase in the number of BrdU+ radial glia labeled either by GS (C-E), or by her4.3:EGFP (F) expression. (G-H) Inhibition of Wnt signaling by Dkk1 expression (G), or lef1 mutation (H), does not block the increase in BrdU labeling following partial ablation. Images are maximum-intensity Z-projections (A,B) or single optical sections (C,D) from ventral views of whole-mount brains. Scale bars=10µM. Error bars=S.E.M., n=40 optical sections from 4 brains for each experiment.
Figure 5. Hypothalamic radial glia proliferate in response to ablation of dopaminergic progeny. (A-B) The *th2:GFP* (5 dpf, A) and *th2:Gal4* (9 dpf, B) transgenes do not label GS+ radial glia. Yellow boxes indicate regions shown in lower panels. (C) Ablation of *th2:Gal4+* cells from 5-6 dpf leads to increased BrdU
labeling only at 8-9 dpf. (D-E) Ablation of th2:Gal4+ cells increases the number and percentage of GS+ radial glia, as well as the number of GS- cells, labeled with BrdU from 8-9 dpf. (F) The overall number of GS+ radial glia is decreased at 9 dpf following ablation of th2:Gal4+ cells. Images are single optical sections from ventral views of whole-mount brains. Scale bars=10µM. Error bars = S.E.M., n=50 optical sections from 5 brains for each experiment.
Figure 6. Wnt/β-Catenin signaling activation blocks expansion of the radial glial population. (A) Induction of Wnt8a at 5 dpf leads to a decrease in the number of GS+ radial glia at 6 dpf. (B) Addition of 4μM BIO, a GSK3β inhibitor, daily from 6-9 dpf leads to a decrease in GS+ radial glia. (C) Representative images of labeling for GS in control and BIO-treated larvae. Images are single optical sections from ventral views of whole-mount brains. Scale bar=10μM. (D) Following recombination from 5-6 dpf, incubation in BIO causes a significant decrease in the number of labeled progeny at 9 dpf. (E) Incubation in BIO causes a significant increase in the percentage of GS+ cells within the labeled lineage. Error bars = S.E.M. N=40 optical sections from 4 brains for each experiment.