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Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors

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SUMMARY

Severe traumatic injury to the adult mammalian CNS leads to life-long loss of function. By contrast, several non-mammalian vertebrate species, including adult zebrafish, have a remarkable ability to regenerate injured organs, including the CNS. However, the cellular and molecular mechanisms that enable or prevent CNS regeneration are largely unknown. To study brain regeneration mechanisms in adult zebrafish, we developed a traumatic lesion assay, analyzed cellular reactions to injury and show that adult zebrafish can efficiently regenerate brain lesions and lack permanent glial scarring. Using Cre-loxP-based genetic lineage-tracing, we demonstrate that *her4.1*-positive ventricular radial glia progenitor cells react to injury, proliferate and generate neuroblasts that migrate to the lesion site. The newly generated neurons survive for more than 3 months, are decorated with synaptic contacts and express mature neuronal markers. Thus, regeneration after traumatic lesion of the adult zebrafish brain occurs efficiently from radial glia-type stem/progenitor cells.

Key words: CNS, Adult neural stem cells, Brain injury, Genetic lineage-tracing, Neurogenesis, Teleost

INTRODUCTION

In mammals, severe injury to the adult brain has catastrophic effects and significant regeneration does not occur. By contrast, adult non-mammalian vertebrates can regenerate considerable regions of their central nervous system (CNS) (Endo et al., 2007; Font et al., 2001; Kaslin et al., 2008; Kizil et al., 2011; Tanaka and Ferretti, 2009). Although different means of cellular regeneration, such as transdifferentiation, dedifferentiation and activation of endogenous progenitors have been proposed, the mechanisms are currently not known (Echeverri and Tanaka, 2002; Kikuchi et al., 2010; Kirsche, 1965; Kragl et al., 2009; Yoshii et al., 2007). Work in frogs, lizards, salamanders and fish suggests that neural stem/progenitor cells at the ventricle react to CNS injury by increasing proliferation and neurogenesis, and might be the origin of regenerated neurons (Endo et al., 2007; Font et al., 2001; Kaslin et al., 2008; Kirsche and Kirsche, 1961; Parish et al., 2007; Tanaka and Ferretti, 2009; Zupanc and Clint, 2003). However, the experimental evidence for this notion is indirect: it relies on the observation of upregulated proliferation and neurogenesis after lesion in the ventricular zone and enhanced migration of newborn neurons to the lesion site (Endo et al., 2007; Font et al., 2001; Kaslin et al., 2008; Kirsche, 1965; Romero-Aleman et al., 2004; Zupanc and Ott, 1999). In addition, removal of the constitutive proliferation zones in the carp optic tectum prevents restoration of the tissue architecture (Kirsche and Kirsche, 1961). Short-term lineage-tracing analyses of ventricular radial glia (RG) in the brain and spinal cord suggested that RG could give rise to new neurons after injury. Because these studies relied on transient protein persistence, and did not permit long-term cell fate analysis (Berg et al., 2010; Echeverri and Tanaka, 2002; Reimer et al., 2008), the cellular basis of neuronal regeneration in the CNS remained unresolved.

To study the origin of newly generated neurons in regenerating brains, we developed a novel traumatic stab lesion model. We find that the adult zebrafish brain efficiently regenerates and restores the tissue architecture completely. Using conditional CreER^{T2}-loxP lineage-tracing (Hans et al., 2009), we show that ventricular RG expressing *her4.1* (an orthologue of mammalian *hes5*) serve as neuronal progenitors that respond to the lesion. Upon injury, these cells increase proliferation, upregulate neuronal-fate determining gene transcription, and give rise to neuroblasts that migrate into the periventricular zone and deeper into the parenchyma to the site of injury, where they differentiate into mature neurons.

MATERIALS AND METHODS

All animal experiments were conducted according to the guidelines and under supervision of the Regierungspräsidium Dresden (permit AZ 24D-9168.11-1/2008-1 and AZ 24D-9168.11-1/2008-4). All efforts were made to minimize animal suffering and the number of animals used.

Fish maintenance

Fish were kept under standard conditions as described previously (Brand et al., 2002). Wild-type fish were from the *gol-b1* line in the AB genetic background.

Stab lesion assay

Adult fish were 6-10 months old and had a body length of 25-32 mm. Fish were anaesthetized using Tricaine (Sigma). A canula (30 gauge, outer diameter 300 µm) was pushed through a nostril ~6-8 mm deep along the rostrocaudal body axis, through the olfactory bulb until reaching the caudal part of the telencephalon. The nostrils helped to guide the canula into the dorsal telencephalon (Fig. 1A). Stab-lesioned fish survived well (>90%) with only minor bleeding, and recovered in fresh fishwater. Sham-operated animals were treated equally, except that the canula was inserted into the nostril and not further into the brain.

Plasmid construction and germline transformation

To create the pTol *her4.1*:mCherryT2ACreER^{T2} plasmid, the *her4.1* promoter (Yeo et al., 2007) was PCR amplified (*her4*-for, 5'-agtaGGG-CCCctgtgtgagcagctcatgtt; *her4*-rev, 5'-cattGGCCGGCCgtcagatcagatctg-act) flanked by *Apal* and *FseI* restriction sites that allowed substitution of the *hsp70l* promoter of the pTol *her4.1*:mCherryT2ACreER^{T2} plasmid (Hans et al., 2011). To generate pTol *hsp70l*:*DsRed2(floxed)EGFP*

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plasmid, the *hsp70l* promoter (Halloran et al., 2000) was PCR amplified with primers *hsp70l*-for (5'-tataGGGCCCTtcagggtgctgcttggatttc-3') and *hsp70l*-rev (5'-tataGGCCGCGCCtttttagtcaacaagtgaattc-3') flanked by the unique restriction sites *ApaI* and *FseI*, respectively. After digestion, the PCR product was cloned into the vector pTol *EF1 α* loxP-DsRed2-loxP EGFP substituting the *EF1 α* promoter (Hans et al., 2009). For germ line transformation, plasmid DNA and transposase mRNA were injected into fertilized eggs (F0), raised to adulthood and crossed to AB wild-type fish as previously described (Kawakami et al., 2004). To identify the Tg(*her4.1*:mCherryT2ACreER^{T2}) driver line, F1 embryos were examined under a fluorescent microscope and positive embryos were raised. Five independent F0 fish were tested for recombination by crossing to Tg(*EF1 α* : loxP-DsRed2-loxP EGFP) and application of TAM (Hans et al., 2009). In all lines, recombination was induced after application of TAM. To identify transgenic Tg(*hsp70l*:DsRed2(*floxed*)EGFP), F1 embryos at 24 hpf were heat-shocked, examined after 24 hours and DsRed-positive embryos were raised. Thus, seven independent F0 lines were identified that all showed efficient recombination in the presence of Cre.

Tamoxifen and heat-shock treatments

Tamoxifen (TAM, T5648, Sigma) and heat-shock treatments during embryonic stages were performed as previously described (Hans et al., 2011; Hans et al., 2009). CreER^{T2}-mediated recombination in adult zebrafish was induced either by intra-peritoneal injection of 10 μ l of 10 mM TAM or by four or five soakings (10 hours each) in 5 μ M TAM in a DMSO/fishwater (1:1000) solution. For intra-peritoneal injections of TAM with a Hamilton syringe, the TAM stock solution was diluted 1:5 in sunflower oil and sonicated. For analysis, no sections were taken into account where unconditional recombination could have occurred (rare cases in the dorsal medial part of the caudal end of the telencephalon). Recombination was induced by a single TAM intra-peritoneal injection 1 day before lesion, or by soaking from 5 days to 1 day before lesion. Adult Tg(*hsp70l*:DsRed2(*floxed*)EGFP) fish were heat-shocked once daily on three consecutive days to reveal DsRed and GFP expression. For daily heat-shock treatments, water was heated to 37°C over 30 minutes and kept at this temperature for 30 minutes. Heating was then switched off. About 7 hours later, water temperature was back to 28°C. Six hours after the last heat-shock, fish were sacrificed for analysis.

BrdU labeling

To label cells in S-phase of the cell cycle, zebrafish were immersed in 7.5 mM (incubation time >48 hours) or 10 mM (incubation time \leq 48 hours) BrdU solution [Sigma (Grandel et al., 2006)]. Short-pulses (2-48 hours) were carried out by immersing fish continuously, whereas long-pulses (12 days) were carried out immersing fish for 14-16 hours per day in a freshly prepared BrdU solution.

Histology

Cryosections (14 μ m) were prepared as described previously (Ganz et al., 2010; Grandel et al., 2006). For paraffin sections, brains were fixed within the skull for 24 hours at 4°C with 4% PFA and embedded using Paraffin Embedding Station STP 420 (Microm) and Embedding Center EG1160 (Leica). Semi-thin sections (1 μ m) were cut on an Ultracut microtome (Mikrom) and counterstained using Hematoxylin/Eosin (HE, Sigma).

For histological analysis a total of 45 zebrafish were stab lesioned, and sacrificed in groups of five at different time-points after lesion: 4 hpl; 1, 3, 7, 14, 30, 60, 92 and 360 dpl; plus five sham-injured animals. Bodian Silver/Cresyl Violet staining was as described previously (Nüsslein-Volhard and Dahm, 2002). For Acid Fuchsin/Orange G double staining (AFOG), paraffin sections were incubated in Bouin's solution (HT 10132, Sigma) at 60°C for 2 hours, plus 30 minutes at room temperature, washed for 30 minutes in distilled water, followed by 4.5 minutes in 1% phosphomolybdic acid (Sigma), 5 minutes in running tap water and 4.5 minutes in AFOG staining solution (pH=1.09) containing 1 g of Aniline Blue (Waldeck-Chroma), 2 g of Orange G (Fluka) and 3 g of Acid Fuchsin (Sigma) in 200 ml distilled water. After final washing in distilled water, sections were dehydrated and mounted with Entellan (Merck).

Immunohistochemistry

Immunohistochemistry was performed as described previously (Ganz et al., 2010; Grandel et al., 2006). Secondary antibodies (dilution 1:750) were conjugated to AlexaFluor 488, 555 and 633 (Invitrogen). All immunostainings were carried out on at least five individuals.

HuC/D, parvalbumin, MAP2a+b, Prox1 and NeuroD staining of sections

To retrieve the antigens of HuC/D, parvalbumin, MAP2a+b, Prox1 and NeuroD1, sections were pre-incubated in 50 mM Tris-buffer (pH 8.0) at 99°C for 5 minutes and cooled down to room temperature over 15 minutes and washed for 5 minutes in PBS and twice for 10 minutes in PBSTx.

Primary antibodies

Primary antibodies were mouse monoclonal antibodies against Vimentin (40-EC, 1:750, DSHB), GFAP (ZRF1, 1:500, zfin), PCNA (PC 10, 1:500, Dako), HuC/D (A-21271, 1:300, Invitrogen), parvalbumin (Mab1572, 1:2500, Chemicon), MAP2a+b (AP-20, 1:300, Sigma), NeuroD1 (ab60704, 1:1000, Abcam), synaptic vesicles (SV2, 1:750, DSHB), mGluR2 (MAB397, 1:100, Millipore) and glutamine synthetase (1:5000, MAB302, Millipore); polyclonal rabbit antibodies against L-Plastin (Lcp1, 1:7500, a kind gift from Michael Redd, University of Utah, Salt Lake City, UT, USA), S100 (Z0311, 1:500, Dako), Prox1 (AB5475, 1:2000, Millipore) and DsRed2/mCherry (632496, 1:500, Clontech); monoclonal rat antibody against BrdU (MCA2060, 1:500, Serotec); and polyclonal chicken antibody against GFP (ab13970, 1:4000, Abcam).

TUNEL assay

TUNEL assays were carried out using the ApopTag Red *In Situ* Apoptosis Detection Kit (Chemicon), according to the manufacturer's instructions. In every experimental series, positive [digestion with DNase I (3000-3 U/ml) in 50 mM Tris-HCl (pH 7.5), 1 mg/ml BSA] and negative (omitting the TdT enzyme) control slides were included.

In situ hybridization

Probe generation and in situ hybridization, on at least three individuals, were as described previously (Ganz et al., 2010; Kaslin et al., 2009). *ascl1*, *deltaD* and *her4.1* probes have been described previously (Cau and Wilson, 2003; Hans and Campos-Ortega, 2002; Takke et al., 1999). The *cre* in situ probe was generated from the *pCS2+* Cre vector (Langenau et al., 2005).

Image acquisition and processing

Confocal images were acquired with a Leica TCS-SP5 confocal microscope using HC PL APO CS 20 \times /0.7 NA, HCX PL APO 40 \times /1.25 NA and HCX PL APO 63 \times /1.2 NA objectives. To minimize channel crosstalk, images were acquired sequentially. Bright-field images were acquired with a Zeiss Axio Imager Z1. Images were processed and assembled using Fiji, Adobe Photoshop CS4 and Illustrator CS4.

Data quantification and statistical analysis

Areas in histological paraffin sections were quantified using approximately every seventh serial cross-section between the caudal end of the olfactory bulb and the anterior commissure using AxioVisionLE software (V4.6.3.0, Carl Zeiss). For cell counts, approximately every second serial cryosections of the same region was analyzed. For co-expression studies, co-localization was verified by analyzing high-resolution confocal stacks. The optical sections were taken with 0.5-0.7 μ m intervals using 40 \times or 63 \times objectives. Microsoft Excel was used to process the measured data. To analyze significance, *P* values were determined with GraphPad Prism using the paired Student's *t*-test (comparisons within single animals) and by one-way ANOVA, followed by Tukey post-hoc test (comparisons between different animals). Error bars represent s.e.m. ****P* \leq 0.001; ***P* \leq 0.01; **P* \leq 0.05. *P*>0.05 was not considered significant.

RESULTS

The stab lesion assay and histological regeneration of the telencephalon

To study adult brain regeneration in zebrafish, we developed a novel stab lesion assay that reproducibly injures the central dorsal telencephalon (DT), i.e. the inner parenchyma, by nasal insertion

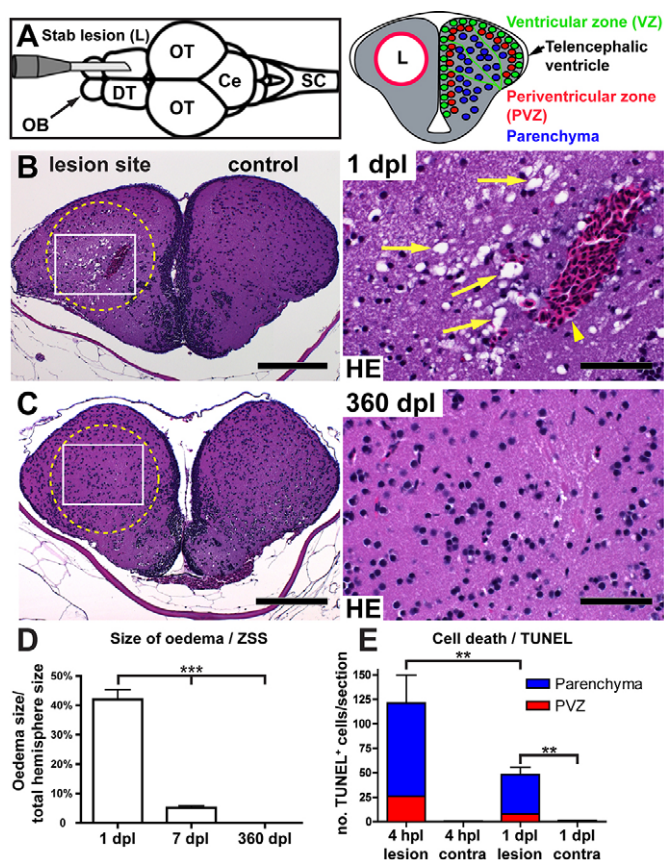


Fig. 1. The stab lesion assay and restoration of tissue integrity of the adult telencephalon. (A) Insertion of a canula into the telencephalon of an adult zebrafish causes a lesion canal (L) in parallel to the rostrocaudal body axis along one hemisphere (dorsal view; OB, olfactory bulb; DT, dorsal telencephalon; OT, optic tectum; Ce, cerebellum; SC, spinal cord). In cross-sections (right), three layers can be functionally distinguished in DT: contacting the ventricle is the neural progenitor containing ventricular zone (VZ, green). During constitutive neurogenesis, all newborn neurons integrate into the periventricular zone (PVZ, red, one or two cell diameters adjacent to the VZ). The uninjured central parenchyma (blue) does not receive new neurons. (B) Tissue within the stab canal is strongly vacuolated (arrows), and often contains a prominent blood clot (arrowhead) as seen in Haematoxylin/Eosin (HE) stained sections 1 day post lesion (dpl). (C) Complete morphological restoration of the tissue architecture is seen by 360 dpl. Scale bars: 200 μ m in B,C overviews; 50 μ m in B,C insets. Circles indicate the lesion canal. (D) Quantification of the size of the vacuolated zona status spongiosus (ZSS) at 1, 7 and 360 dpl ($n=3$, $n=4$ and $n=3$, respectively). (E) Quantification of TUNEL⁺ cells at 4 hours post lesion (hpl) ($n=3$) and at 1 dpl ($n=4$). Data are mean \pm s.e.m. *** $P<0.001$; ** $P<0.01$.

of a canula along the rostrocaudal body axis (Fig. 1A). A characteristic of the zebrafish brain is its widespread constitutive life-long neurogenesis. Nevertheless, non-neurogenic regions do also exist in the adult zebrafish brain. In DT, it has been proposed that neural stem/progenitor cells line the ventricle (ventricular zone, VZ) and give rise to neuroblasts that migrate only one to two cell diameters into the periventricular zone (PVZ) (Adolf et al., 2006; Ganz et al., 2010; Grandel et al., 2006). Hence, the inner telencephalic parenchyma does not receive new neurons from constitutive neurogenesis and is therefore ideally suited to study regenerative neurogenesis (Fig. 1A).

The stab lesion assay has several advantages: (1) it spares the VZ where the presumptive neural progenitor cells, radial glia (RG) reside; (2) the contralateral hemisphere serves as an internal control; and (3) treated fish show a high survival rate (>90%). Initially, 4 hours post lesion (4 hpl) to 1 day post lesion (1 dpl), the lesion is demarcated by a blood clot, cerebral oedema and bulk loss of neuronal processes (Fig. 1B; supplementary material Fig. S1A, Fig. S2A). The typical spongy appearance of injured nervous tissue is caused by intra- and extracellular oedema (Adornato and Lampert, 1971; Noack et al., 1971). The initial (1 dpl) size of the vacuolated zona status spongiosus (ZSS), i.e. the lesion perimeter, is slightly larger (10%) than the perimeter of the canula and makes up 42.0% \pm 3.3% (mean \pm s.e.m., $n=3$) of the total volume of the lesioned hemisphere (Fig. 1B,D). However, the extent of the ZSS declines quickly within the first 7 dpl to 5.2% \pm 0.7% ($n=4$, Fig. 1D; supplementary material Fig. S1B,C). Histologically, the architecture of the lesioned telencephalon is largely restored by 30 dpl and completely reconstituted 360 dpl ($n=3$, supplementary material Fig. S1D; Fig. 1C,D). Furthermore, neuronal processes are partially re-established 14 dpl within the lesion site and are indistinguishable on a histological level from an unlesioned hemisphere at 360 dpl (supplementary material Fig. S2B,C).

Using the TUNEL assay, we find that cell death is an early response after injury (Fig. 1E; supplementary material Fig. S3). At 4 hpl and 1 dpl, many TUNEL⁺ cells are detected within the ZSS that show characteristics of both necrotic (i.e. cytoplasmic TUNEL signal) and apoptotic (i.e. cytoplasmic and nuclear condensation) cell death (Fig. 1E; supplementary material Fig. S3). However, the distinction between necrosis and apoptosis after CNS injuries is difficult, and also hybrid forms of cell death have been reported (Martin et al., 1998). Neuronal cell death is not confined to the ZSS because we detect TUNEL⁺/HuC/D⁺ cells within the whole hemisphere, and also close to the ventricle in the PVZ (Fig. 1E). Cell death recedes quickly and reaches the low levels of sham-lesioned and contralateral hemispheres (i.e. approximately one cell or less per section on average) by 3 dpl (Fig. 1E).

Acute inflammatory response and gliosis but no chronic inflammation or glial scarring

In mammals, severe injuries to the CNS cause reactive gliosis and intense inflammation, leading to tissue loss and formation of a compact glial scar (Fitch and Silver, 2008; Silver and Miller, 2004; Sofroniew, 2009). Therefore, we analyzed glia- and leukocyte-specific markers in the zebrafish brain after stab lesion. Early after injury, we find characteristics of reactive gliosis, such as upregulated expression of the intermediate filamentous proteins GFAP and vimentin, swelling of glial processes (hypertrophy) and glial proliferation, in the lesioned hemisphere (Fig. 2A, Fig. 3D). The number of L-plastin-positive leukocytes (Redd et al., 2006) is strongly increased at 4 dpl, indicating an acute inflammatory response in the lesioned hemisphere (Fig. 2B). Upregulation of glial markers and hypertrophy of glial processes are still detected at 30 dpl (supplementary material Fig. S4). However, at later time-points neither characteristics of persistent gliosis and fibrotic scar formation, such as accumulation of reactive glial cells and processes (Fig. 2C) and ectopic extracellular matrix (ECM) deposition (supplementary material Fig. S5), nor characteristics of chronic inflammation, such as persistence of ectopic leukocytes at the lesion site, are evident (Fig. 2C). Occasionally, we observe a tiny residual cyst (0.09% \pm 0.06% of the lesioned telencephalic

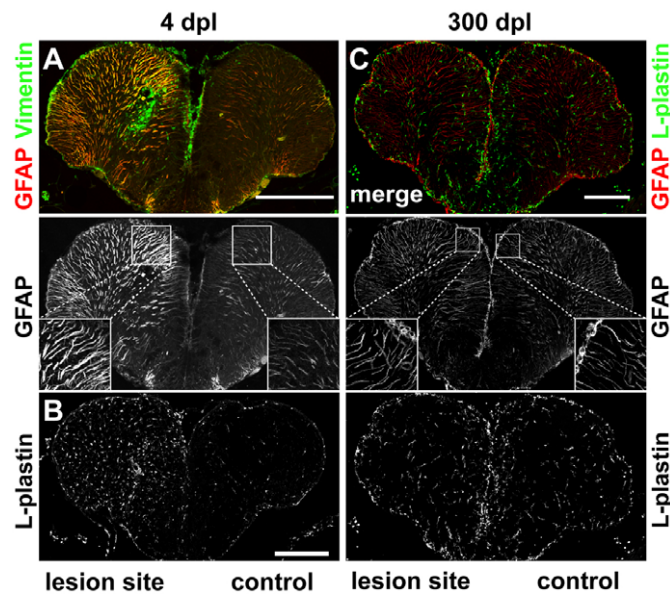


Fig. 2. Reactive gliosis, inflammatory response and scarring. (A) The glial markers GFAP (red) and vimentin (green) are strongly upregulated in the lesioned hemisphere early after injury (4 dpl). GFAP⁺ radial fibres in the lesioned hemisphere are thicker compared with the control hemisphere. (B) The pan-leukocyte marker L-plastin is strongly upregulated in the lesioned hemisphere at 4 dpl. (C) No difference in number and appearance of GFAP⁺ (red) radial processes is detected comparing lesioned and control hemispheres 300 dpl. Staining for L-plastin (green) shows no difference in the number and distribution of leukocytes between the lesioned and the control hemisphere. Scale bars: 200 μ m.

hemisphere volume, $n=3$) that contains collagen, glial cells and neuronal processes in the caudal part of the telencephalon (supplementary material Fig. S6).

Radial glia and leukocytes react to stab lesion by upregulating proliferation

As we detected an almost complete re-establishment of the brain architecture after stab lesion, we asked whether progenitor cell proliferation is stimulated after injury. In the unlesioned adult zebrafish telencephalon, mitotic activity is confined to the VZ, where presumptive neural progenitor cells are located (Adolf et al., 2006; Ganz et al., 2010; Grandel et al., 2006), but typically not in the parenchyma. Importantly, after injury, strongly increased proliferation is found in the VZ and in the parenchyma of the lesioned hemisphere (Fig. 3A). Reactive proliferation peaks at 3 dpl, remains elevated until 14 dpl (Fig. 3E), but has returned to control levels by 30 dpl (Fig. 3E). In the parenchyma, proliferating cells express either the pan-leukocyte marker L-plastin, identifying them as blood-derived leukocytes and resident microglia (Fig. 3B; supplementary material Fig. S7A), or are *fli1*:GFP-expressing endothelial cells (Lawson and Weinstein, 2002) or oligodendrocyte-precursor cells (OPCs, Fig. 3B,C; supplementary material Fig. S7) expressing *olig2*:GFP (Shin et al., 2003). In the uninjured DT of zebrafish, approximately two-thirds of all proliferating VZ progenitors are radial glia (Ganz et al., 2010). This population specifically reacts to stab lesion by upregulating proliferation; 86.5% \pm 3.1% ($n=4$) of all actively cycling cells in the dorsal VZ express the glial marker S100B at the peak of proliferation at 3 dpl (Fig. 3D; supplementary material Fig. S8).

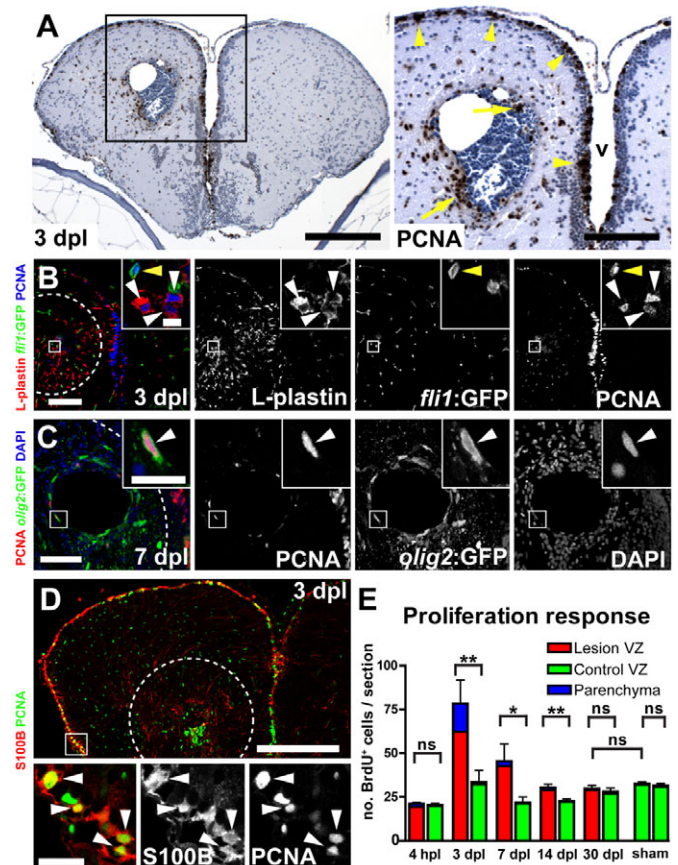


Fig. 3. Reactive proliferation. (A) Many mitotic, PCNA⁺ (brown) cells are detected close to the lesion (3 dpl) in the parenchyma (arrows), as well as in the VZ (arrowheads). In the contralateral hemisphere, fewer PCNA⁺ cells are detected in the VZ and only very few in the parenchyma (v, ventricle). Nuclei are counterstained with Nissl (blue). (B) Using a pan-leukocyte marker (L-plastin, red) combined with PCNA (blue) identifies leukocytes as the major proliferating cell population in the parenchyma (white arrowheads). PCNA⁺ endothelial cells are also found in the parenchyma (yellow arrowhead), as shown by analysis for *fli1*:GFP expression (green). (C) *Olig2*:GFP⁺ (green) oligodendrocyte-precursor cells are ectopically proliferating (PCNA, red, arrowhead) in the parenchyma. (D) S100B⁺ (red) radial glia increase proliferation (PCNA, green, arrowheads) within the VZ in lesioned hemispheres. Broken outlines represent the lesion canal. Scale bars: 200 μ m in A, inset 100 μ m; 100 μ m in B, inset 10 μ m; 50 μ m in C, inset 10 μ m; 200 μ m in D, inset 20 μ m. (E) Quantification of the number of BrdU⁺ cells (2 hours BrdU pulse) shows a significant upregulation of the total number of proliferating cells in the lesioned (red bars) versus the control hemisphere (green bars) at 3, 7 and 14 dpl ($n=5$, $n=4$ and $n=5$, respectively). At 4 hpl and at 30 dpl, no significant difference is detected between lesioned, contralateral and sham-operated hemispheres ($n=3$, $n=4$ and $n=3$, respectively). At 3 dpl, reactive proliferation reaches its peak, and the majority of mitotic cells are located within the VZ (79.5% \pm 14.8%, red). Data are mean \pm s.e.m. ** $P\leq 0.01$; * $P\leq 0.05$.

Reactive proliferation results in increased neurogenesis and long-lived mature neurons

To test whether the lesion-induced increase in proliferative activity also causes increased neurogenesis, we performed BrdU pulse-chase experiments. Shortly after lesion (4 dpl), newborn BrdU⁺, migrating cells express the neuronal marker HuC/D close to the

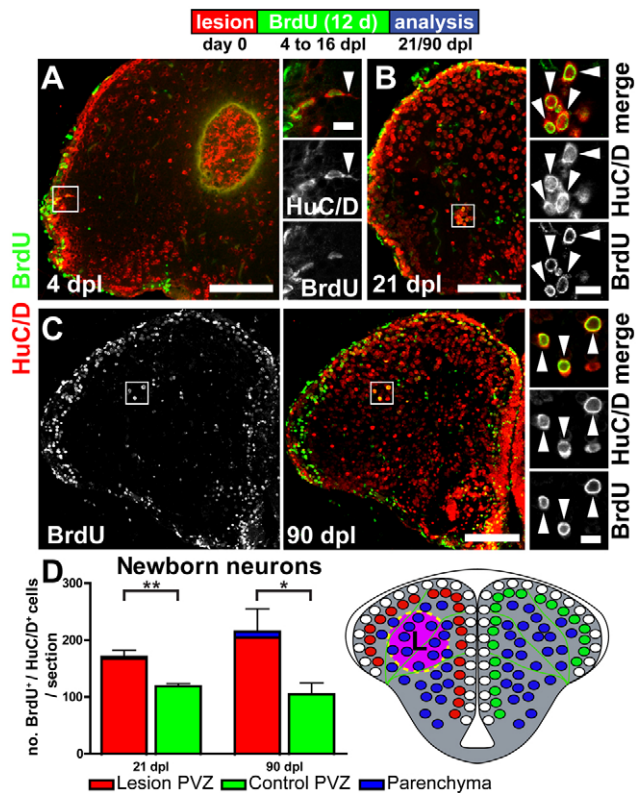


Fig. 4. Reactive neurogenesis leads to many long-lived, newly generated neurons. (A) 4 dpl newborn BrdU⁺(green)/HuC/D⁺(red) neuroblasts, generated at the time of the radial glia proliferation peak (BrdU pulse 48–92 hpl), are leaving the VZ and migrate towards the lesion site. Note the prominent leading edge profile (arrowhead), a typical feature of migrating neuroblasts. (B) Newborn BrdU⁺/HuC/D⁺ neurons that were born between 4 and 16 dpl can be found close to the lesion site in the parenchyma 21 dpl (arrowheads). (C) Many of these injury-induced ectopic neurons survive for at least 90 dpl close to the lesion site deep in the parenchyma, as well as in the PVZ, as shown by BrdU/HuC/D double staining (arrowheads). Scale bars: 100 μ m in A–C; 10 μ m in insets. (D) Quantification of BrdU⁺/HuC/D⁺ cells shows a significant net gain of newly generated neurons in the lesioned hemisphere at 21 ($n=5$) and 90 dpl ($n=3$), compared with the control hemisphere (Fig. 4D). HuC/D is expressed from early stages of neuronal differentiation onwards and therefore does not distinguish between mature and immature neurons/neuroblasts. We, thus, performed analysis for the co-localization of BrdU⁺ cells also with markers for more mature neurons, using the Ca²⁺-binding protein parvalbumin, and high-molecular isoforms of MAP2 (MAP2a+b) as a dendritic marker. We find that periventricular and parenchymal BrdU⁺ neurons, when located within parvalbumin⁺ brain nuclei, also express parvalbumin, indicating that they acquire a mature neuronal state and a correct neuronal identity (Fig. 5A;

VZ, suggesting that these are neuroblasts leaving the VZ in direction of the lesion (Fig. 4A). After long-term BrdU pulse-chase experiments, a significant increase in BrdU⁺/HuC/D⁺ neurons is observed at the lesion site in the parenchyma, as well as in the PVZ at 21 and 90 dpl (Fig. 4B,C). The number of newly generated neurons is increased in the lesioned hemisphere 1.4-fold at 21 dpl ($n=5$) and twofold at 90 dpl ($n=3$), compared with the control hemisphere (Fig. 4D). HuC/D is expressed from early stages of neuronal differentiation onwards and therefore does not distinguish between mature and immature neurons/neuroblasts. We, thus, performed analysis for the co-localization of BrdU⁺ cells also with markers for more mature neurons, using the Ca²⁺-binding protein parvalbumin, and high-molecular isoforms of MAP2 (MAP2a+b) as a dendritic marker. We find that periventricular and parenchymal BrdU⁺ neurons, when located within parvalbumin⁺ brain nuclei, also express parvalbumin, indicating that they acquire a mature neuronal state and a correct neuronal identity (Fig. 5A;

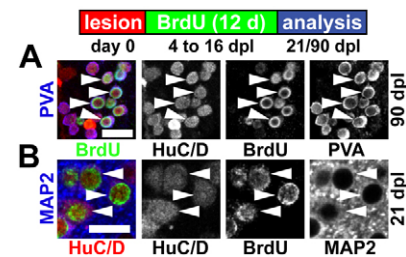


Fig. 5. Newly generated neurons attain a mature neuronal state. Newly generated, BrdU⁺(green)/HuC/D⁺(red), periventricular and parenchymal neurons show mature neuronal marker expression. (A) Parvalbumin (PVA, blue, a mature marker of an interneuron subtype). (B) High-molecular weight microtubule-associated protein-2 isoforms (MAP2, blue, pan-neuronal marker). Scale bars: 50 μ m in A; 10 μ m in B. Arrowheads indicate triple-positive cells.

supplementary material Fig. S9). Furthermore, many newly generated neurons express MAP2a+b (Fig. 5B), suggesting neuronal maturation (see also below).

Newly generated neurons derive from radial glial

In the developing mammalian CNS, RG function as neuronal progenitor cells (Anthony et al., 2004; Malatesta et al., 2003; Malatesta et al., 2000; Noctor et al., 2001). In many non-mammalian species, RG persist into adulthood and therefore could be a source of adult-born neurons (Kaslin et al., 2008). In salamanders, lizards and bony fishes, it has been suggested, but not shown, that ventricular RG might be the source of regenerated neurons in the lesioned brain and spinal cord (Berg et al., 2010; Echeverri and Tanaka, 2002; Parish et al., 2007; Reimer et al., 2008; Romero-Aleman et al., 2004). Very recently it has been shown by viral lineage-tracing in the zebrafish telencephalon that RG can indeed act as constitutive neural stem cells in the uninjured brain (Rothenaigner et al., 2011). However, the adult zebrafish telencephalon contains heterogeneous populations of presumptive ventricular neural progenitor cells both with and without radial glial marker expression (Ganz et al., 2010). Hence, the regenerated neurons that we found might derive from several neural and non-neuronal progenitor populations. To test the role of RG progenitors directly, we marked them genetically, by applying conditional Cre-LoxP-technology to trace their progeny (Hans et al., 2009). Using this lineage-tracing method, we asked whether RG serve as neuronal progenitors in the adult zebrafish telencephalon, during both constitutive and lesion-stimulated neurogenesis.

In the uninjured adult zebrafish telencephalon, RG express S100B/GFAP/vimentin/glutamine synthetase and also *her4.1*, a notch target gene (Fig. 2, Fig. 3D, Fig. 6C; supplementary material Figs S4, S10) (Ganz et al., 2010). After injury, a proliferation response is detected in RG expressing *her4.1:GFP* (supplementary material Fig. S10). To mark these cells genetically, we generated a transgenic line expressing a bicistronic mRNA coding for mCherry and CreER^{T2} recombinase under the control of the zebrafish *her4.1* promoter that closely recapitulates the endogenous *her4.1* expression pattern (supplementary material Fig. S10) (Yeo et al., 2007). In these fish, mCherry expression in the adult telencephalon is restricted to cells that co-express glutamine synthetase (Fig. 6C; supplementary material Fig. S11A). Furthermore, *cre* mRNA expression is restricted to the VZ and resembles the endogenous *her4.1* expression (Fig. 6C; supplementary material Fig. S10A).

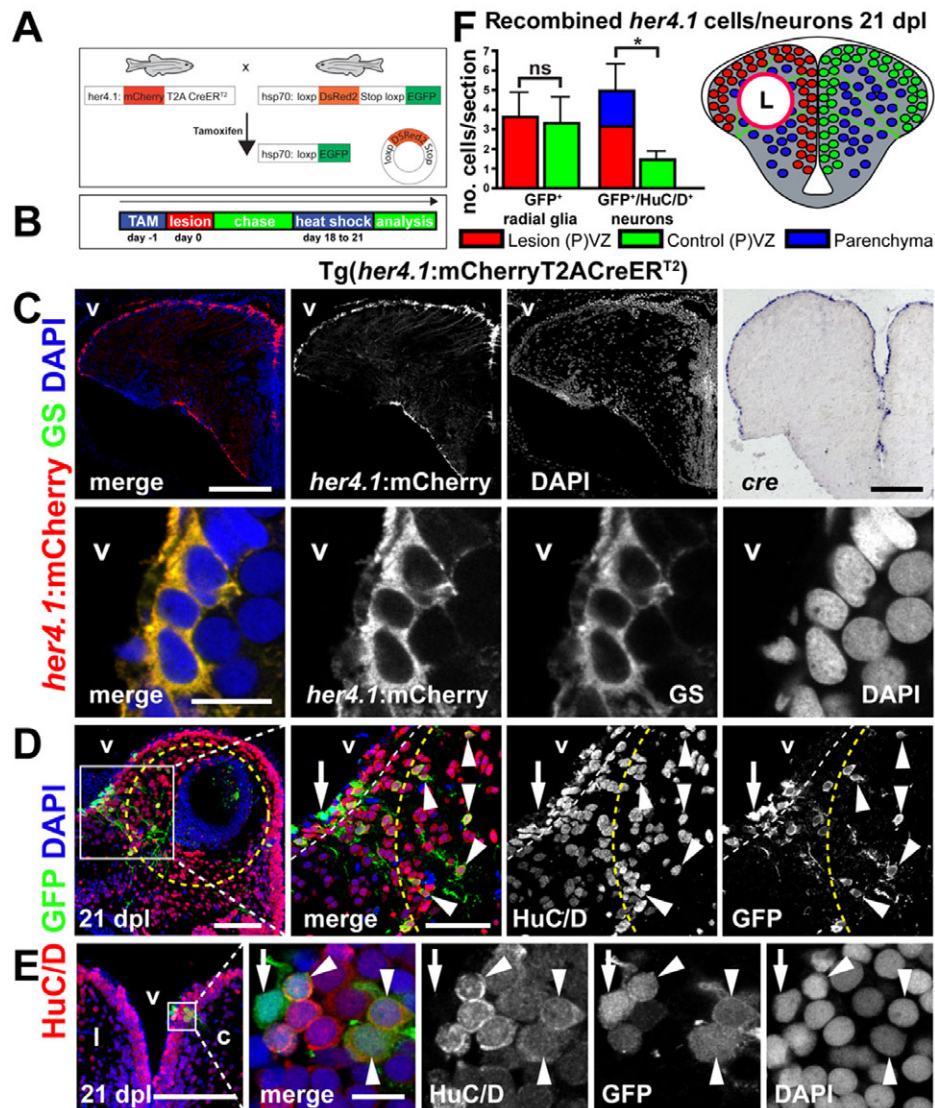


Fig. 6. Newly generated neurons derive from a *her4.1*-expressing radial glia-type progenitor. (A) Generation of double-transgenic fish used for cell fate analysis: fish expressing CreER^{T2} controlled by the *her4.1*-promoter were crossed to red-to-green reporter fish. In double-transgenics, GFP expression can be specifically induced in *her4.1*-expressing radial glia and their progeny by tamoxifen application and heat shock. (B) Recombination was induced specifically in *her4.1*-expressing radial glia by tamoxifen injection 1 day before lesion. Fish (18 to 21 dpl) were heat-shocked to induce GFP expression in recombined cells only. (C) Cre-recombinase expression is restricted to radial glia in the adult telencephalon of Tg(*her4.1*:mCherryT2ACreER^{T2}) fish: the expression of mCherry (red) is restricted to ventricular cells that resemble radial glia by morphology. *Cre* mRNA expression (blue) is restricted to the VZ, as shown by in situ hybridization. *her4.1*:mCherry is expressed in ventricular cells that co-express glutamine synthetase (GS, green), identifying them as radial glia. (D) By 21 dpl, many recombined GFP⁺(green)/HuC/D⁺(red) neurons/neuroblasts (arrowheads) are found within the lesion-canal (yellow outline) and in the PVZ (white outline). In the VZ recombined GFP⁺/HuC/D⁻ cells with radial processes, i.e. radial glia (arrow), are found. (E) Recombined neurons (arrowheads) derived from *her4.1*⁺ radial glia (arrow) are exclusively found in the PVZ in the dorsal part of unlesioned control hemispheres 21 dpl. Scale bars: 200 μ m in C overviews, inset 10 μ m; 100 μ m in D, inset 50 μ m; 100 μ m in E, inset 10 μ m. v, ventricle; l, lesioned hemisphere; c, control hemisphere. (F) Quantification of cell fate analysis 21 dpl ($n=7$). The number of recombined GFP⁺/HuC/D⁻ ventricular radial glia is not significantly different in lesioned and control hemispheres. However, the number of lineage-traced neurons is significantly increased in the lesioned hemisphere, showing that radial glia in the lesioned hemisphere produce more neurons and, in particular, give rise to neurons that migrated into the parenchyma. Data are mean \pm s.e.m. * $P\leq 0.01$.

Therefore, *cre* expression in the adult zebrafish telencephalon is restricted to RG in the transgenic *her4.1*:mCherryT2ACreER^{T2} line. For lineage-tracing experiments, these fish were crossed to a red-to-green reporter line to detect Cre-dependent recombination (Hans et al., 2009). In the absence of Cre, the reporter line expresses DsRed2 under the control of the ubiquitous, temperature inducible *hsp70* promoter, but switches permanently to EGFP after

a successful recombination event (Fig. 6A) (Hans et al., 2011). In double-transgenic fish, recombination can therefore be induced specifically in *her4.1*/S100B-expressing RG progenitors by the application of tamoxifen (supplementary material Fig. S11B).

Importantly, when we induced recombination in RG in double-transgenic fish 1 day before injury, we find that these cells gave rise to telencephalic neurons at 21 dpl. In lesioned hemispheres, the

newly generated GFP-marked neurons migrated into two locations: the PVZ and the inner parenchyma, i.e. the lesion site (Fig. 6D,F). By contrast, in unlesioned control hemispheres of the same animals, we detect GFP-marked neurons exclusively within the PVZ (Fig. 6E,F), showing that neuroblasts migrate only short distances in the unlesioned adult DT (Grandel et al., 2006). Thus, after injury, GFP-marked neurons derived from RG progenitors are found in two locations: (1) the constitutive target site in the PVZ at 21 dpl ($63.5 \pm 16.9\%$, $n=7$, Fig. 6F), but additionally (2) many recombined cells ($36.5 \pm 17.8\%$, $n=7$) migrated deep into the lesioned parenchyma, where newly generated neurons are not normally found. As expected, the recombination efficiency of GFP⁺ ventricular RG is not significantly different between lesioned and control hemispheres (3.6 ± 1.2 versus 3.3 ± 1.3 cells/section, $n=7$). Importantly, neurogenesis from genetically marked progenitors is significantly stimulated in the lesioned hemisphere ($342.4\% \pm 95.1\%$ of control hemispheres). The regeneration-stimulated neurogenesis was associated with strongly increased expression of the proneural gene *ascl1a* and the neurogenic gene *deltaD* specifically in the VZ of the lesioned hemisphere 3 dpl (supplementary material Fig. S12).

In the mammalian brain, it has been suggested that lesion-induced newly generated neurons fail to differentiate into correct neuronal subtypes and do not acquire appropriate regional identities after injury (Liu et al., 2009). We therefore sought to investigate whether newly generated, RG-derived neurons are also maintained long term after lesion and whether they differentiate appropriately. Indeed, we find that many GFP⁺/HuC/D⁺ neurons survive in the PVZ and the parenchyma until 100 dpl, suggesting that these cells survive long term and are integrated into the brain circuitry (Fig. 7A). To examine neuronal subtype and regional marker expression, we studied two neuronal subpopulations in our lineage-tracing experiments: (1) a neuronal subpopulation that expresses the transcription factor NeuroD1 and (2) a subpopulation of interneurons that co-expresses parvalbumin and Prox1 (supplementary material Figs S9, S13) (Ganz et al., 2011; Grandel et al., 2006). We find that recombined neurons express NeuroD1, parvalbumin and Prox1 correctly within the areas defined by these markers (Fig. 7B-D).

To address the maturation status of newly generated *her4.1*-derived neurons, we examined the expression of MAP2a+b and synaptic vesicle-associated protein 2 (SV2) in lesioned brains. We find that recombined cells in the parenchyma express MAP2a+b and are decorated with synaptic vesicular contacts (Fig. 7E,F). Furthermore, we find that a subpopulation of lineage-traced cells in the parenchyma expresses glutamate receptors as pre- and postsynaptic markers (Fig. 7G).

Taken together, our results show that a subpopulation of *her4.1*-expressing RG generates new neurons in the adult zebrafish brain, both during constitutive neurogenesis and after stimulation by a lesion. After injury, newborn neurons migrate into the lesion site and express mature regional and subtype-specific markers appropriately. Furthermore, many newly generated neurons are maintained for more than 100 dpl and are covered with synaptic contacts. Some lineage-traced neurons express metabotropic glutamate receptors, suggesting that RG-derived neurons are characterized by active signalling and a mature physiology.

DISCUSSION

After a stab lesion in the adult zebrafish telencephalon, the brain architecture is quickly restored at the histological level. Gliotic and acute inflammatory reactions occur early after the lesion; however,

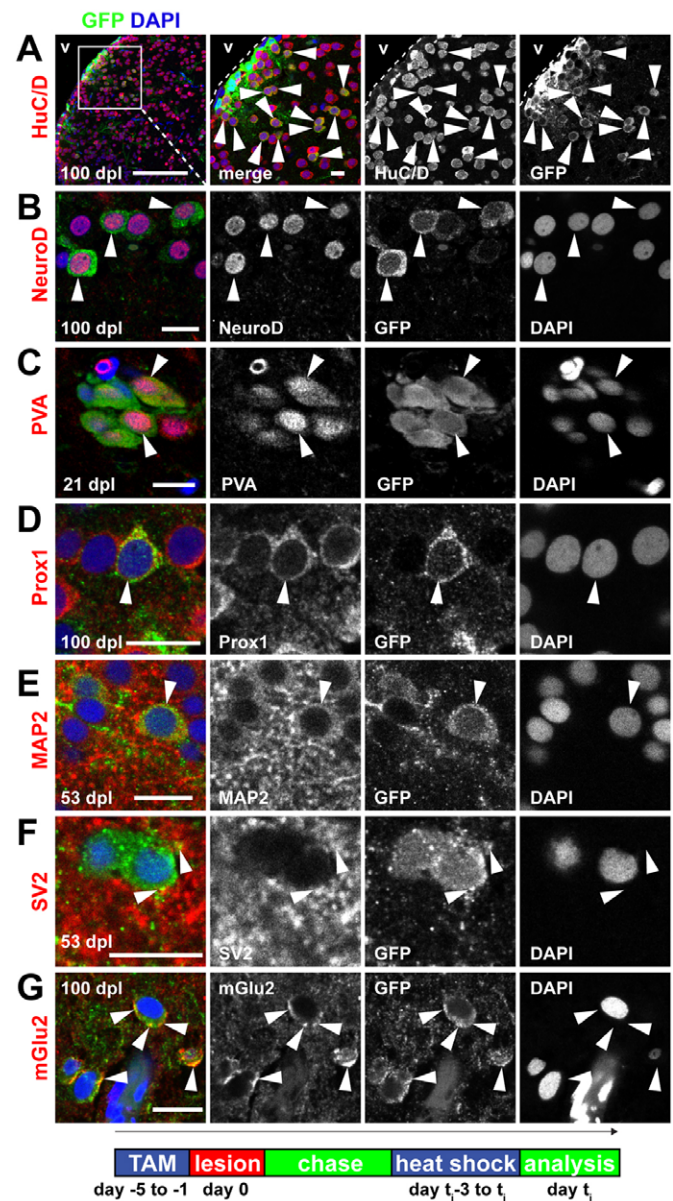


Fig. 7. Radial glia-derived neurons are maintained long term, show a correct regional identity and display features of mature functional neurons. (A) Many recombined GFP⁺(green)/HuC/D⁺(red) neurons (arrowheads) are maintained in the parenchyma and in the PVZ until 100 dpl. (B-G) Examples of radial glia-derived (GFP⁺, green) neurons in the dorsal parenchyma that express: (B) the dorsal marker NeuroD1 (arrowheads, red) at 100 dpl; (C) the mature interneuron and regional marker parvalbumin (arrowheads, PVA, red) at 21 dpl; (D) the regional marker Prox1 (arrowhead, red) at 100 dpl; (E) the mature neuronal marker MAP2a+b (arrowhead, MAP2, red) at 53 dpl; (F) the synaptic vesicle marker SV2 (arrowheads, SV2, red) at 53 dpl; and (G) the synaptic marker metabotropic glutamate receptor 2 (arrowheads, mGlu2, red) at 100 dpl. Scale bars: 100 μ m in A overview; 10 μ m in A inset; in 10 μ m in B-G. v, ventricle. Timeline shows experimental procedures.

both are resolved and do not lead to permanent glial scarring. This is in contrast to the situation in mammals (summarized in Table 1). Brain injury results in a marked increase in proliferation of ventricular zone progenitors, whereas in the parenchyma itself

Table 1. Comparison of reactive gliosis, inflammation and scarring in zebrafish and mammals

Cellular reaction	Acute phase (hours to days)					Sub-acute and chronic phase (weeks to months)					
	Reactive gliosis			Accumulation of glial cells	Inflammation		Glial and fibrotic scarring				
	Glial hypertrophy	GFAP/vimentin upregulation	Glial proliferation		Leukocyte proliferation	Infiltration/accumulation of leukocytes	Accumulation of glia	Accumulation of leukocytes	Ectopic ECM deposition	Inhibition of axonal/neuronal regeneration	
Zebrafish	+	+	+ (RG)	–	+	+	–	–	–	–	
Mammals	+	+	+ (AS)	+	+	+	+	+	+	+	

Certain characteristics of reactive gliosis and inflammation acutely after brain injury seem to be similar in zebrafish and mammals, such as hypertrophy of glial somata and processes, glial upregulation of GFAP and vimentin, infiltration and accumulation of leukocytes (mostly macrophages and resident microglia), and glial and leukocyte proliferation. However, the cellular nature of glial cells is different: typical star-shaped astrocytes represent the major glial cell population in mammals. By contrast, radial glia are the major glial cells detected in the dorsal telencephalon. This difference probably also accounts for the difference in migration behaviour of reactive glial cells after lesion. Although in mammals reactive astrocytes migrate massively into the border regions of an injury, no accumulation of glial cell bodies around the lesion in the parenchyma is seen in zebrafish.

In sub-acute and chronic stages after injury, migrated and newly generated reactive astrocytes, together with microglia/macrophages and ectopic ECM molecules, form a barrier to axonal and neuronal regeneration in mammals, the glial scar. By contrast, the nature of reactive gliosis and inflammation in the adult zebrafish telencephalon is transient, and no ectopic glial cells, leukocytes or ectopic ECM deposition is seen after 300 dpl. This shows that the adult zebrafish telencephalon provides a permissive environment and allows for axonal and neuronal regeneration.

+, present; –, not present; RG, radial glia; AS, astrocytes.

proliferation is limited to infiltrating leukocytes, microglia, endothelial cells and oligodendrocytes. Using conditional Cre-loxP lineage tracing, we find that ventricular radial glia function as a lesion-responsive neuronal progenitor population: after injury, these cells upregulate proliferation and neurogenic gene transcription, and give rise to neuroblasts that migrate into the PVZ and deeper into the injured parenchymal territory, where they appear to differentiate appropriately and survive for at least 100 dpl. Thus, the adult zebrafish brain has a remarkable potential for neuronal repair from endogenous adult stem/progenitor cells (summarized in Fig. 8).

Regeneration-permissive features of the adult zebrafish telencephalon

In mammals, reactive gliosis of astrocytes, injury-induced inflammation and the formation of a compact glial scar are considered to be major obstacles to successful brain regeneration (Fitch and Silver, 2008; Silver and Miller, 2004; Sofroniew, 2009). Although typical star-shaped parenchymal astrocytes are not present in the adult zebrafish telencephalon (Ganz et al., 2010; Kalman, 1998; März et al., 2010), radial glia (RG) show, similar to mammalian astroglia, hallmarks of a reactive gliosis early after injury (Figs 2, 3; supplementary material Figs S8, S10). Although reactive astrocytes remain strictly non-neurogenic in the adult mammalian brain, they can give rise to multipotent neurospheres and neurons *in vitro*, emphasizing the important role of the restrictive, non-permissive environment *in vivo* (Buffo et al., 2008; Costa et al., 2010; Heinrich et al., 2010; Robel et al., 2011; Seidenfaden et al., 2006). By contrast, zebrafish reactive radial glia do function as endogenous neurogenic progenitors after injury, producing neuroblasts that migrate both into the constitutive, periventricular target sites, as well as deep into in the parenchyma to the lesion site (which is normally not seen in unlesioned brains).

In mammals, activated microglia and macrophages cause a wave of secondary neuronal degeneration by interacting with reactive glial cells, leading to massive tissue loss and expansion of the lesion cavity (Fitch and Silver, 2008). Besides these deleterious interactions with reactive glia, inflammatory processes have also been implicated in negatively regulating neurogenesis directly (Ekdahl et al., 2003; Ekdahl et al., 2009; Monje et al., 2003). In the adult zebrafish telencephalon, we detect, as in mammals, a massive inflammatory response early after lesion (Figs 2, 3). However,

tissue integrity of the lesioned hemisphere is re-established quickly and a pronounced neurogenic response ensues in the ventricular RG population. In mammals, inflammatory cells, reactive astrocytes and ECM molecules all contribute to the formation of a glial scar, which persists until long after the initial injury (Rolls et al., 2009; Schwab and Bartholdi, 1996; Silver and Miller, 2004; Sofroniew, 2009). In zebrafish, we do not observe the formation of a noticeable glial scar, characterized by sustained accumulation of inflammatory cells, reactive glia or ectopic ECM deposition that might be an impediment to cell migration and/or axon regeneration. This suggests that the zebrafish brain environment may be more permissive to regeneration. The molecular mechanisms that underlie this permissiveness await further studies. In mammals, two major ECM components of the glial scar that exert growth-inhibiting cues to axons are chondroitin sulphate proteoglycans (CSPGs) and myelin-derived proteins, such as members of the Nogo family (Silver and Miller, 2004). Interestingly, in zebrafish, CSPGs appear not to inhibit, but rather to guide optic nerve regeneration in zebrafish (Becker and Becker, 2002). Furthermore, orthologues of mammalian growth-inhibitory molecules and their receptors, like Nogo-family members, have been identified in fish (Abdesselem et al., 2009; Diekmann et al., 2005; Klinger et al., 2004). However, zebrafish NogoA lacks a specific domain that inhibits neurite outgrowth in mammals (Diekmann et al., 2005). These examples from axonal regeneration illustrate how the greater permissiveness of fish CNS for regeneration might be encoded at a molecular level.

Contribution of radial glia-type progenitor cells to brain regeneration

This study identifies a subpopulation of *her4.1*-expressing RG progenitor cells as the main neurogenic population reacting to lesion, and the primary source of the newly generated parenchymal neurons. A common principle in the regeneration of tissues such as appendages, heart or barbel in adult axolotl and zebrafish is the generation of a population of progenitor cells within the wound blastema, which are generated at least partly through dedifferentiation of differentiated cells (Jopling et al., 2010; Kikuchi et al., 2010; Kragl et al., 2009; LeClair and Topczewski, 2010). By contrast, in our stab-lesion paradigm, most proliferating cells belong to either the RG- or the leukocyte lineage, and the formation of a typical regeneration blastema consisting of undifferentiated mesenchymal cells is not evident. As yet, we

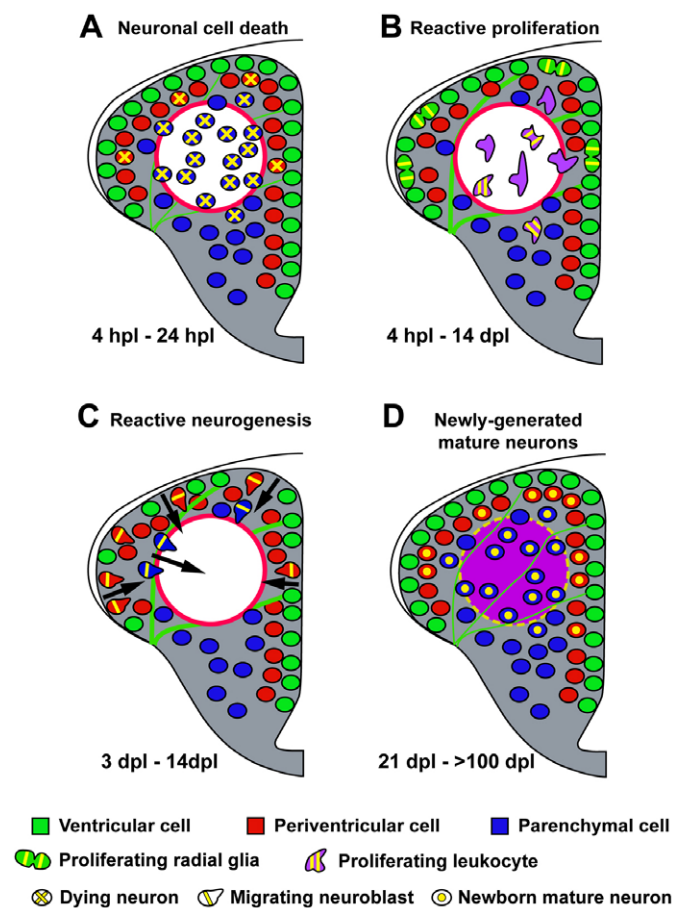


Fig. 8. Key events in regeneration of the adult zebrafish telencephalon. (A) Four to 24 hpl neurons in the parenchyma and the PVZ of the injured hemisphere enter cell death. (B) Resident microglia, invading leukocytes and ventricular radial glia enter a phase of reactive proliferation from 4 hpl to 14 dpl (peaking at 3 dpl). Radial glia show characteristics of reactive gliosis. (C) From 3 to 14 dpl, radial glia upregulate proliferation and subsequently undergo neurogenesis (reactive neurogenesis). The newborn neuroblasts leave the VZ and migrate towards the lesion. (D) From 21 to 100 dpl, many newly generated mature and active neurons are detected within the lesion site in the parenchyma and in the PVZ.

cannot rule out that cells other than the *her4.1*-expressing RG population also contribute to neuronal regeneration. However, our results suggest that this class of progenitors accounts for most regenerated neurons (see below), and that de-differentiation of otherwise non-neurogenic cells plays only a minor role, if any. We estimate the contribution of *her4.1*-positive progenitors to regeneration as follows. In the dorsal telencephalon, we previously found two populations of long-term label-retaining ventricular progenitors: RG and glia marker-negative cells (Ganz et al., 2010). In our present BrdU tracing experiments, we detected overall a 1.4-fold increase of neurogenesis within the lesioned hemisphere at 21 dpl (Fig. 4D). Specifically within the *her4.1*-expressing RG population, neurogenesis is increased 3.4-fold in the lesioned hemisphere at 21 dpl (Fig. 6F). Furthermore, roughly one-third (36.5%) of the newborn neurons deriving from the *her4.1*-lineage are located at the lesion site in the parenchyma. This suggests that the *her4.1*-expressing RG may be the major source of the newly generated parenchymal neurons.

Dedifferentiation of radial glia?

Do telencephalic RG cells dedifferentiate when they act as progenitors in regeneration? Dedifferentiation is thought to occur, for example, during regeneration of the teleost retina by Müller glia cells that share several morphological and molecular properties with RG (Becker and Becker, 2008; Bernardos et al., 2007; Ganz et al., 2010; Hitchcock and Raymond, 2004; Qin et al., 2009; Stenkamp, 2007; Thummel et al., 2008), but also in axolotl limb regeneration (Kragl et al., 2009) and during zebrafish bone regeneration (Knopf et al., 2011). For adult RG, the answer probably requires a more rigorous understanding of dedifferentiation. In telencephalic RG progenitors, the expression of all examined radial glial markers is retained or increased during the first stages of regeneration, up to 30 dpl, in spite of their proliferative activity (Figs 2, 3; supplementary material Fig. S4). This would suggest that there is little or no dedifferentiation. On the other hand, if dedifferentiation is taken to mean general re-acquisition of a progenitor potential, it is conceivable that a subset of adult RG cells also undergoes a partial dedifferentiation.

Efficiency of regeneration

In adult mammals, the overall contribution of any injury-induced neurogenesis to CNS reconstitution is very low. Non-traumatic brain injury, caused by, for example, stroke or neurodegeneration, first induces a proliferative response of neural progenitors that is followed by reactive neurogenesis. However, the great majority of newborn neurons is not maintained (>42 dpl), most probably owing to the non-permissive environment and lack of functional integration into the circuitry (Arvidsson et al., 2002; Magavi et al., 2000; Nakatomi et al., 2002; Yamashita et al., 2006). The regenerative outcome after traumatic injuries, such as stab lesions, appears even more restricted. Although a marked glial proliferation response is observed, only new astroglia are produced and no newborn neurons are found in vivo (Buffo et al., 2008; Buffo et al., 2005). By contrast, we find that regeneration in the adult zebrafish telencephalon occurs efficiently, yielding an essentially normal morphology again. At the cellular level, the total number of newborn neurons that survive until 90 dpl is more than doubled in the lesioned versus the control hemisphere (Fig. 4D), suggesting that a significant proportion of additional, newly generated neurons are maintained for extended time-periods in the regenerating zebrafish brain.

To study the status of newly generated neurons after lesion in detail, we examined the expression of established mature neuronal markers, such as MAP2a+b, parvalbumin, SV2 and metabotropic glutamate receptor 2 (mGlu2) (Buckley and Kelly, 1985; Kamiya et al., 1996; Kawaguchi et al., 1987; Pernet and Di Polo, 2006; Przyborski and Cambray-Deakin, 1995; Puyal et al., 2002; Reimer et al., 2008; Romero-Aleman et al., 2010; Scanziani et al., 1997; Yang et al., 2002). In the stab-lesioned zebrafish telencephalon, periventricular and parenchymal neurons derived from RG express MAP2a+b and parvalbumin (Fig. 7C,E). This suggests that newly generated neurons in and around the lesion site differentiate into mature neurons. Furthermore, we observed parenchymal neurons derived from RG that are decorated with SV2⁺ synaptic contacts (Fig. 7F). Clonally marked cells also express the mGlu2 at 100 dpl, suggesting that newly generated neurons at the lesion site make contacts with other neurons and are integrated into the neuronal circuitry. How well normal neuronal circuitry is re-established is unknown and needs further testing, e.g. by electrophysiological analysis.

Conclusion

Our study demonstrates that adult zebrafish can regenerate severe traumatic stab lesions in the telencephalon efficiently, and that they do so via a neural stem/progenitor cell based process. Using a conditional genetic lineage-tracing strategy, we identify a *her4.1*-positive ventricular RG progenitor population as a major population reacting to injury. Many newly generated mature neurons that are maintained for long survival times derive from this *her4.1*-positive progenitor population. Therefore, further analysis of the *her4.1*-positive RG will help to understand the molecular mechanisms involved in compensatory proliferation and regenerative neurogenesis in vertebrates. To this end, efforts have already been started to analyze the transcriptome of *her4.1*-expressing RG after stab lesion injury. Understanding this ability to generate long-term persisting neurons after lesion of the adult brain is of fundamental importance and could help to develop new therapeutic strategies for the diseased or injured human brain.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

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