Changes in the Social Environment Induce Neurogenic Plasticity Predominantly in Niches Residing in Sensory Structures of the Zebrafish Brain Independently of Cortisol Levels

Benjamin W. Lindsey, Vincent Tropepe

Department of Cell and Systems Biology, University of Toronto, Ontario M5S 3G5, Canada

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ABSTRACT: **The social environment is known to modulate adult neurogenesis. Studies in mammals and birds have shown a strong correlation between social isolation and decreases in neurogenesis, whereas time spent in an enriched environment has been shown to restore these deficits and enhance neurogenesis. These data suggest that there exists a common adaptive response among neurogenic niches to each extreme of the social environment. We sought to further test this hypothesis in zebrafish, a social species with distinct neurogenic niches within primary sensory structures and telencephalic nuclei of the brain. By examining stages of adult neurogenesis, including the proliferating stem/progenitor population, their surviving cohort, and the resulting newly differentiated neuronal population, we show that niches residing in sensory structures are most sensitive to changes in the social context, and that social isolation or novelty are both capable of decreasing the number of proliferating cells while increasing the number of newborn neurons within a single niche. Contrary to observations in rodents, we demonstrate that social novelty, a form of enrichment, does not consistently rescue deficits in cell proliferation following social isolation, and that cortisol levels do not negatively regulate changes in adult neurogenesis, but are correlated with the social context. We propose that enhancement or suppression of adult neurogenesis in the zebrafish brain under different social contexts depends largely on the type of niche (sensory or telencephalic), experience from the preceding social environment, and occurs independently of changes in cortisol levels.** © 2014 Wiley Periodicals, Inc. Develop Neurobiol 00: 000–000, 2014

Keywords: **adult neurogenesis; social environment; zebrafish; stem cell niche; cortisol**

INTRODUCTION

The social environment is known to regulate constitutive rates of adult neurogenesis in vertebrates, potentially influencing stem/progenitor proliferation, survival of the post-mitotic population, or the extent of neuronal differentiation (Lindsey and Tropepe, 2006; Kempermann, 2011; Maruska et al., 2012). By deconstructing the complexity of the social environment, however, it is clear that much of the information that an animal encodes encompasses both learned components that are processed in higher-order brain centers of the forebrain, such as the hippocampus and neocortex, as well as modality-specific sensory components that are processed foremost in corresponding primary sensory structures. What happens then to constitutive rates of adult neurogenesis when social animals venture outside of their home range, are separated from their social group, or undergo novel social interactions? How might

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Correspondence to: V. Tropepe (v.tropepe@utoronto.ca).

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environmental change activate neurogenic plasticity and alter the composition of the neurogenic niche in the adult vertebrate brain? To begin to address these questions, neurogenic niches residing within primary sensory structures of the brain must be compared with those localized to higher-order forebrain structures to determine if there are differential responses at the cellular level to deviations from a familiar social context.

Studies in birds and mammals have shown convincing evidence that acute postembryonic or adult social isolation is strongly correlated with decreases in cell proliferation and/or neuronal differentiation in hippocampal structures (Barnea et al., 2006; Lieberwirth et al., 2012), owing significantly to an increase in the stress-related hormone cortisol (Schoenfeld and Gould, 2011). The negative effect of isolation stress on cell proliferation in the dentate gyrus can also override the positive effects incurred by physical exercise in rats (Stranahan et al., 2006; Leasure and Decker, 2009), while stress-related decreases in hippocampal subgranular zone (SGZ) proliferation can be restored with short-term environmental enrichment (Veena et al., 2009). Interestingly, enrichment paradigms that elicit changes in adult hippocampal neurogenesis often have had little effect in the subependymal zone (SEZ) of rodents (Brown et al., 2003), implying that niches may be independently modulated by specific environmental contexts. Studies show that SEZ neurogenesis can be altered in response to olfactory enrichment (Rochefort et al., 2002), estrous induction (Smith et al., 2001), and male exposure (Fowler et al., 2002), although it is still unclear whether a situation such as social isolation can mediate changes in this niche. Few studies have successfully shown examples of parallel neurogenic plasticity in the SEZ and SGZ using the same methodology. Where observed, researchers have probed biologically relevant contexts including offspring recognition and mating pheromones (Mak et al., 2007; Mak and Weiss, 2010).

Our understanding of adult neurogenic plasticity in vertebrates is derived primarily from the cellular behavior of the SEZ and SGZ of mammals and the ventricular zone of birds (reviewed in Barnea and Pravosudov, 2011; Lieberwirth et al., 2012). Thus, there is a paucity of information concerning how other adult stem cell niches residing in different forebrain structures, or within sensory structures themselves, might be modulated in response to changes in the social environment. Comparative studies between different niches of a single species or between niches of different vertebrate groups have revealed common cellular features at the ultrastructural level (Doetsch et al., 1997; Alvarez-Buylla et al., 1998; Font et al., 2001; Seri et al., 2001; Lindsey et al., 2012; and reviewed in Garcia-Verdugo et al., 2002; Grandel and Brand, 2013) and in regards to the phenotype of stem/progenitor cells (Kaslin et al., 2009; Ganz et al., 2010; Ito et al., 2010; Marz et al., 2010). By uncovering the properties of neurogenic plasticity across unique adult stem cell niches, we may be able to expose overlapping cellular and molecular programs that control niche structure and its adaptive plasticity, and gain insight into novel functions of adult neurogenesis previously unknown.

Teleost fishes are tractable models to examine adult neurogenesis, owing to their abundant neurogenic compartments across the neuraxis and propensity for regeneration following injury (Zupanc et al., 2005; Adolf et al., 2006; Grandel et al., 2006; Chapouton et al., 2007; Becker and Becker, 2008; Kaslin et al., 2008; Kizil et al., 2012; Zupanc and Sirbulescu, 2011, 2013). Moreover, adult neurogenic niches appear to exist in both higher order telencephalic nuclei as well as primary sensory structures throughout the brain, facilitating comparisons of cellular and molecular modulation between these domains in response to changes in the social environment. Studies in cyprinids and cichlids have examined how brain growth patterns correlate with microhabitat and social organization (Brandstatter and Kotrschal, 1989, 1990; Kotrschal and Palzenberger, 1992; Huber et al., 1997; Kotrschal et al., 1998; Pollen et al., 2007). However, with the exception of recent studies investigating the effect of social hierarchies on adult neurogenesis in cichlids (Maruska et al., 2012, 2013), few studies have explored how changes in the social environment can specifically affect adult neural stem cell niches in the brain.

In this study, we tested how contrasting social contexts, such as isolation and novelty, can modulate constitutive rates of adult neurogenesis in niches located within primary sensory structures of the olfactory bulb, optic tectum, and vagal lobe compared with niches of the dorsal, ventral, and lateral zones of the telencephalon of the adult zebrafish brain. We additionally investigated the role of whole-body cortisol levels under different social contexts as a putative mechanism underscoring changes in the size of the stem/progenitor population. Finally, since zebrafish can be reared in chronic isolation, this further provided us the opportunity to examine how developmental social isolation compares with adult isolation. By comparing the properties of adult neurogenic plasticity between diverse niches in the zebrafish brain, we will broaden our appreciation of how specific social contexts can enhance or suppress

stages of adult neurogenesis in a brain-wide or niche-specific manner.

MATERIALS AND METHODS

Animals

Wildtype zebrafish (AB strain) of both sexes were randomly selected for all experiments and maintained on a 14 h light:10 h dark photoperiod at 28°C in our fish facility (Aquaneering, San Diego). Fish were fed a diet of granular food (ZM, Winchester, U.K.) and brine shrimp thrice daily, unless stated otherwise. Zebrafish were a minimum of 6-months old and a maximum of 11 months old before they were examined for changes in adult neurogenesis or cortisol levels in response to social isolation or social novelty. Animals were killed using an overdose of 0.4% Tricaine methanesulfonate diluted in tank water. Total body length (mm), weight (g), and sex of all fish were recorded, while brain length (mm) was additionally measured in animals used to study changes in cell proliferation and differentiation following treatment exposure. These parameters, along with the sample size of animals examined for changes in adult neurogenesis under each treatment condition are listed in Supporting Information Table S1. Handling procedures were done in accordance with the policies set forth by the University of Toronto and the Canadian Council for Animal Care (CCAC).

Social Isolation and Social Novelty Treatments

Developmental Isolation and Novelty. To examine the effects of being raised in isolation compared with a familiar social environment, zebrafish embryos were raised at 28°C until 5–6 days post fertilization (dpf) when their yolk is substantially depleted. Kin recognition between larvae is not yet established at this stage of development (Gerlach et al., 2008). Larval zebrafish from the same cohort were separated into individual tanks as groups of 6 animals (Gp, control) or isolates (Iso) and dividers placed between tanks to prevent visual cues from adjacent fish. Isolated and grouped zebrafish were fed a diet of rationed ZM granular fish food throughout development to ensure group-raised and isolate-raised fish received the same amount of food per fish. Upon reaching 6-months, half of the isolate-raised fish were tagged (Supporting Information Fig. S1 and see Elastomer tagging below) and exposed to social novelty (Iso-Nov) for an additional 2-wk to investigate whether any effects that occurred as a result of social isolation could be restored using this form of social enrichment (Fig. 2A). Social novelty consisted of a mixture of six age-matched, unfamiliar adult male and female zebrafish. For all developmental isolation groups (Gp, Iso, and Iso-Nov), following treatments animals received a single pulse of bromodeoxyuridine (BrdU; Sigma) and were killed 2-h or 4-wk after to examine changes in cell proliferation, the label-retaining $BrdU^+$ population, and neuronal differentiation. Comparisons were made between Gp:Iso and Iso:Iso-Nov. Since the control group (Gp) came from the same cohort of animals bred for all isolation and novelty treatments, it was additionally used as the control group for comparisons below.

Adult Isolation. To assess whether periods of adult social isolation could affect adult neurogenesis as commonly observed in the hippocampus of mammals, 6-month adult zebrafish were isolated for 2-wk (Gp-Iso 2-wk) in separate tanks and compared with the group-raised controls (Gp; Fig. 4A). Dividers were placed between tanks to prevent visual cues from adjacent fish and animals maintained as above. Following isolation treatments, animals were injected with BrdU and killed after 2-h or 4-wk to examine stages of adult neurogenesis. Comparisons were made between Gp:Gp-Iso 2-wk.

Adult Novelty and Isolation. To investigate the effect of adult social novelty in zebrafish raised in a social environment alongside familiar conspecifics, 6-month old animals were tagged and exposed to social novelty for 2-wk (Gp-Nov) before being pulsed with BrdU and killed for comparison with group-raised controls (Fig. 6A). To determine whether subsequent exposure to social isolation and social novelty, two extremes of the social environment, had a compound effect on rates of cell proliferation, survival, and differentiation, a separate set of 6-month old group-raised animals were isolated for 2-wk, and then exposed to social novelty for 2-wk (Gp-Iso-Nov). Thereafter, all animals were injected with BrdU and processed as above. Comparisons were made between Gp:Gp-Nov and Gp-Nov:Gp-Iso-Nov.

Elastomer Tagging

Nontoxic, Visible Implant Elastomer tags (Northwest Marine Technology, WA) were used to identify zebrafish during introduction into a novel social group of unfamiliar animals. Visible implant elastomer was prepared fresh according to the manufacturers' protocol the morning of tagging and kept on ice until application. Zebrafish were anaesthetized with 0.04% Tricaine diluted in facility water until movement slowed, then tagged with visible implant elastomer dorsolaterally beneath the skin, between black stripes using a 0.3 cc injecting syringe. Tags were ~3–5 mm long and easily visible (Supporting Information Fig. S1). Thereafter, animals were briefly placed in a recovery tank and respiration and swimming behavior monitored. After this time, tagged zebrafish were introduced into novel groups of adult zebrafish according to treatments.

BrdU Administration and Brain Processing

BrdU administration and tissue processing was performed as described in Lindsey et al. (2012). In brief, a single

10 mM bolus of BrdU (Sigma) was administered intraperitoneally at a volume of 50 µL/g body weight into anaesthetized fish to detect proliferating cells in the S-phase of the cell cycle. Following 2-h or 4-wk BrdU chase periods, animals were killed and transcardially perfused with ice-cold 1X-phosphate buffered saline (PBS; pH 7.4) and 4% paraformaldehyde, brains processed, and cryosectioned at 20 µm intervals for immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was used to detect $BrdU^+$ cells 2-h and 4-wk post-BrdU injection within neurogenic niches to identify changes in the proliferating population (2-h), BrdU-label-retaining population (4-wk; i.e., postmitotic) and proportion of cells that differentiated into a neuronal phenotype (4-wk), respectively. This marker was also used to detect changes in the proliferative population in response to different concentrations of hydrocortisone injections. Single labeling of $BrdU^+$ cells and double labelling of $BrdU^+$ cells with the neuronal marker HuCD were carried out as described in Lindsey et al. (2012). It was not a goal of our study to examine the phenotype of $BrdU^+/HuCD^-$ cells at 4-wk, although we expect that these populations would likely consist of a mixture of glial fates, some of which might include members of the BrdU-label-retaining pool. A separate cohort of group-raised controls (Gp) and developmentally raised isolates (Iso) were labelled with the nuclear counterstain Hoechst 33258 to compare changes in the density of cells within each niche under investigation to further validate stereological counting methods used here. All brain sections were mounted in 100% glycerol for visualization and imaging.

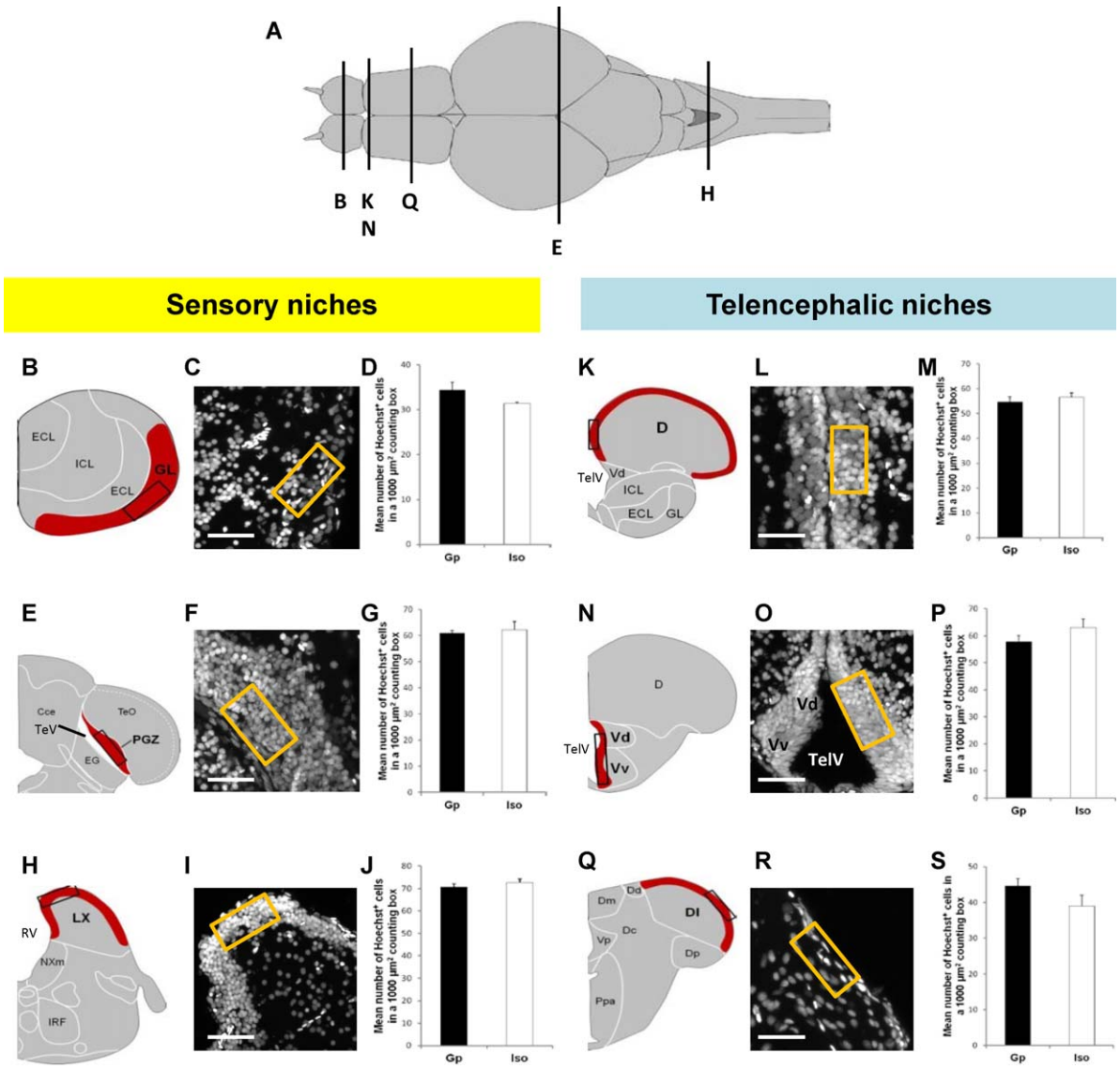

Figure 1

Adult Neurogenic Niches Investigated

Six neuroanatomically distinct neurogenic niches of the adult zebrafish brain were examined in this study within tightly demarcated rostrocaudal boundaries. We sought to determine how niches residing within primary sensory structures would be modulated compared with niches in higher-order domains of the telencephalon that receive secondary sensory input and which may be implicated in learning (Salas et al., 2006; Wullimann, 2009). Three niches were located within primary sensory processing structures [Fig. 1(A,B,E,H); red portion of schematic], and three niches located within nuclei of the telencephalon [Fig. 1(A,K,N,Q); red portion of schematic]. All neuroanatomical terminology and landmarks are in accordance with Wullimann et al. (1996). Here, niches residing in modality-specific, primary sensory processing structures are referred to as "sensory niches," and defined as neurogenic niches displaying constitutive levels of adult neurogenesis within a neuroanatomical subregion of adult primary sensory structures of known function. Sensory niches were located in the olfactory bulb that processes primary olfactory input (OB, 23–50×; primarily the glomerular layer, GL) [Fig. 1(B)], the caudal-most portion of the periventricular gray zone of the optic tectum, responsible for processing visual stimuli including movement, shape, and color (PGZ, 213–223x) [Fig. 1(E)], and in the vagal lobe situated in the hindbrain of cyprinids that receives primary taste stimuli (LX, 279–303) [Fig. 1(H)]. In the case of the PGZ, by dividing the tectum into rostral and caudal domains, we have previously found the most dense population of $BrdU^+$ cells to be localized within the caudal portion of this structure (data not shown), and for this reason, here we have limited our analysis to these boundaries. Telencephalic niches were located in the dorsal telencephalic area surrounding the perimeter of the pallium (D, 50–60×); [Fig. 1(K)], the dorsal (Vd) and ventral (Vv) zone of the ventral telencephalon lining the medial subpallium adjacent the telencephalic ventricle (VdVv, 60×) [Fig. 1(N)], and the lateral zone of the dorsal telencephalon (Dl, 70–107×) [Fig. 1(Q)].

Cell Counting and Imaging

Imaging was performed using a Leica TCS SP5 confocal microscope. Quantification of $BrdU^+$ cells to examine

Figure 1 Density counts in neurogenic niches compared between group-raised (Gp) and isolate-raised (Iso) animals. A: Schematic showing the top view of an adult zebrafish brain and the rostrocaudal position of cross-sections where sensory (B, E, H) and telencephalic (K, N, Q) niches reside. Notation corresponds to cross-sectional view of neurogenic niches in schematics below. B–D: Sensory niche of the olfactory bulbs (OB). B: Schematics showing cross-sectional view of OB. The niche of OB is primarily located in the glomerular layer (GL; red). ECL: external cellular layer; ICL: internal cellular layer. C: Representative image of Hoechst-labeled cells in GL of OB. D: Comparison of the number of $Hoechst^+$ cells between group and isolate-raised zebrafish in OB. E–G: Sensory niche of the periventricular gray zone (PGZ) of the optic tectum (TeO). E: Schematics showing cross-sectional view of PGZ within the TeO. The niche of the PGZ of the caudal optic tectum (red) is located adjacent the tectal ventricle (TeV). Cce: corpus cerebelli; EG: eminentia granularis. F: Representative image of Hoechst-labeled cells in PGZ. G: Comparison of the number of $Hoechst^+$ cells between group and isolate-raised zebrafish in PGZ. H–J: Sensory niche of the vagal lobe (LX) located in the hindbrain. H: The niche of LX is restricted to the perimeter of this structure (red), and medially lies adjacent the rhombencephalic ventricle (RV). NXm: vagal motor nucleus; IRF: inferior reticular formation. I: Representative image of Hoechst-labeled cells in LX. J: Comparison of the number of $Hoechst^+$ cells between group and isolate-raised zebrafish in LX. K–M: Telencephalic niche of the dorsal zone of the telencephalon (D, pallium). K: The niche of D surrounds the perimeter of the dorsal telencephalon (red). L: Representative image of Hoechst-labeled cells in D. M: Comparison of the number of $Hoechst^+$ cells between group and isolate-raised zebrafish in D. N–P: Telencephalic niche of the ventral telencephalon (VdVv, subpallium). N: The niche of VdVv is located adjacent the telencephalic ventricle (TelV) and consists of the dorsal (Vd) and ventral (Vv) zones of the ventral telencephalon (red). O: Representative image of Hoechst-labeled cells in VdVv. TelV: telencephalic ventricle. P: Comparison of the number of $Hoechst^+$ cells between group and isolate-raised zebrafish in VdVv. Q–S: Telencephalic niche of the lateral zone of the dorsal telencephalon (Dl). Q: The niche of Dl surrounds the perimeter of this structure (red) and is localized between the dorsal zone (Dd) and posterior zone (Dp) of the dorsal telencephalon. R: Representative image of Hoechst-labeled cells in Dl. S: Comparison of the number of $Hoechst^+$ cells between group and isolate-raised zebrafish in Dl. In B, E, H, K, N, and Q, black rectangles in schematics represent subregions of each niche examined for the size of the BrdU-label-retaining population and for the number of colabeled $BrdU^+/HuCD^+$ cells at 4-wk post-BrdU injection. In C, F, I, L, O, and R, dorsal is up. Orange boxes represent the location of 1000 μm^2 counting boxes in which the total number of $Hoechst^+$ cells where assessed for each niche. Orange counting boxes are located within a subregion of black rectangles shown in schematics. In D, G, J, M, P, and S, $p > 0.05$ (independent samples t-tests), therefore no significant differences are noted on graphs.

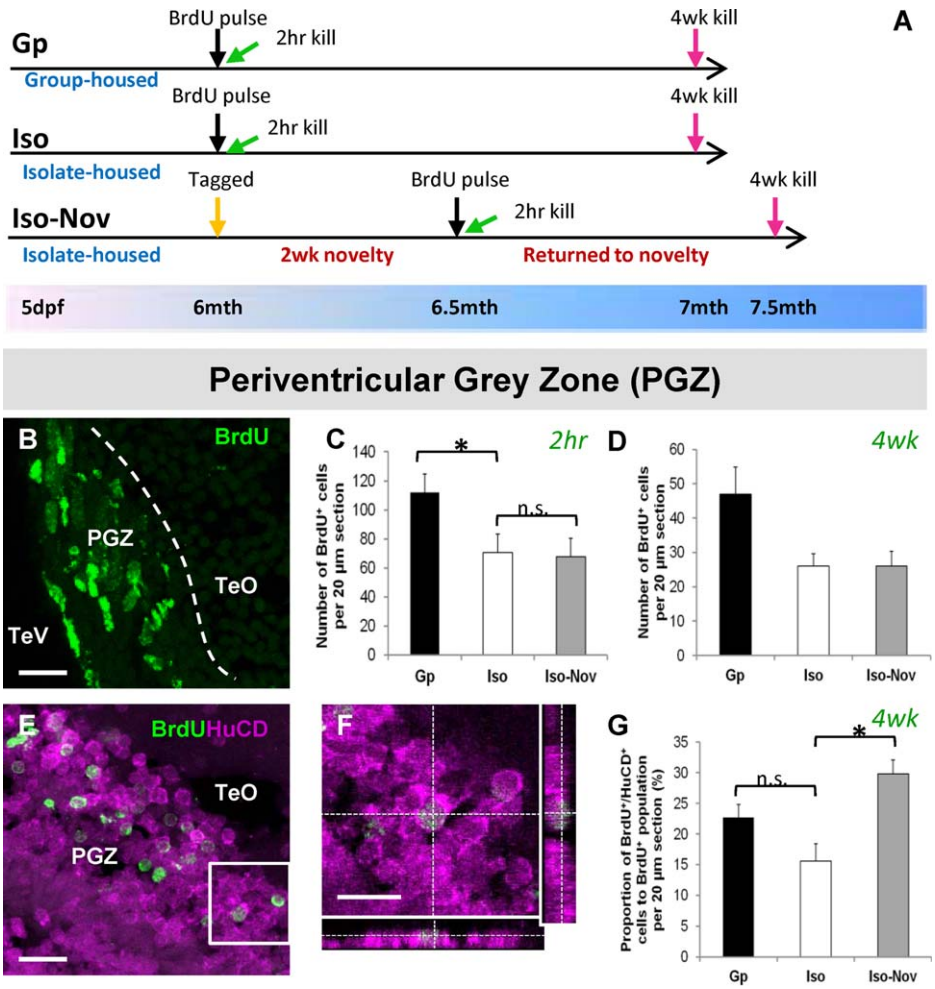

Figure 2 Effect of Developmental Isolation and Novelty treatments (A) on cell proliferation 2-h post-BrdU injection (2 h) and on the BrdU-label-retaining population and proportion of newly differentiated neurons 4-wk post-BrdU injection (4 wk) in the sensory niche of the periventricular gray zone (PGZ, B–G) of the optic tectum. A: Experimental design of zebrafish developmental isolation and novelty treatments. Gp: Raised in a familiar social group of 6 animals until 6-months. Iso: Raised in isolation until 6-months. Iso-Nov: Raised in isolation until 6-months then elastomer-tagged and introduced into a novel group of 6 unfamiliar, adult zebrafish for 2-wk (social novelty). dpf: days post-fertilization. Groups of animals to be examined for changes in neuronal differentiation 4-wk post-BrdU injection were maintained under the treatment condition until sacrifice. B: Representative image showing $BrdU^+$ cells localized within PGZ adjacent the tectal ventricle (TeV) and deep to the more superficial layers of the optic tectum proper (TeO). Hashed line represents the division between the PGZ and TeO. C: Number of $BrdU^+$ cells 2-h post-BrdU injection. D: BrdU-label-retaining population 4-wk post-BrdU injection. E and F: Representative image of $BrdU^+/HuCD^+$ newly differentiated neurons. White square depicts higher magnification shown in F. F: Orthogonal view confirming colabeling of $BrdU^+/HuCD^+$ cells. G: Proportion of $BrdU^+/HuCD^+$ cells 4-wk post-BrdU injection. In B, E, F, dorsal is up; scale bars = 10 µm. In C, D, G, statistical comparisons were performed between Gp:Iso and Iso:Iso-Nov only. Statistically significant comparisons are denoted by an asterisk (*), where $p < 0.05$ (independent samples t-tests). For clarity, on graphs where significance is present, comparisons that were not significant (n.s.) are also noted. Where no notation is present on graphs, this indicates that both comparisons were not significantly different.

changes in the size of the proliferative population at 2-h was done by counting all $BrdU^+$ cells in a minimum of every third section through the rostrocaudal range of each niche at 40× magnification [Fig. 1(B,E,H,K,N,Q); red portion of schematic]. To quantify the size of the BrdU-label-retaining population or proportion of $BrdU^+/HuCD^+$

newborn neurons 4-wk post BrdU injection, a subregion of each niche was analyzed from images taken at 100× magnification from two or more, nonadjacent, sections [Fig. 1(B,E,H,K,N,Q)] (black rectangles within red niches). During this analysis, all $BrdU^+$ cells and $BrdU^+/HuCD^+$ cells within a tissue section were counted within a single counting box positioned in a representative region of the niche, with the position of the counting box maintained across like-niches of different biological samples. Since BrdU-labeling is specific to the nucleus, whereas our neuronal marker HuCD is predominantly expressed cytoplasmically, colabelling of $BrdU^+/HuCD^+$ cells were confirmed in orthogonal view. To account for possible changes in the cell density of niches in animals raised in a familiar group compared to isolation over the first 6-months of development, Hoechst-labelled cells were imaged at 63× magnification in three nonadjacent tissue sections of each niche across biological replicates, and afterwards all cells within a 1000 μm^2 counting box of individual sections summed [Fig. 1(C,F,I,L,O,R); orange rectangles]. All cell counting was completed by quantifying the absolute number of cells present in 0.5–1 μm z-stacks using the optical disector principle (West, 1999; Geuna, 2005) with Leica Application Suite Advance Fluorescence Lite 2.3.0 proprietary software. For all analyses, the total number of cells within a niche or individual counting box was averaged across replicates of the same biological sample, and a final group mean across all biological samples obtained. Images shown are maximum projections or orthogonal views where appropriate and were adjusted for brightness and contrast using Adobe Photoshop 7.0 software.

Cortisol Assays

To determine whether changes in cortisol levels were correlated with zebrafish exposed to different treatment conditions, we measured whole-body cortisol concentrations (ng/g fish) in separate sets of animals: group-raised (Gp), isolate-raised (Iso), 1-h adult isolated (Gp-Iso 1-h), 2-wk adult isolated (Gp-Iso 2-wk), and group-raised fish exposed to novelty for 2-wk (Gp-Nov). Cortisol extractions and assays were performed as described by Cachat et al. (2010). In brief, zebrafish heads were removed, body weight measured, and tissue homogenized in 1 mL of ice-cold 1×-PBS. Five milliliters of diethyl ether (Sigma) was next added to glass centrifuge tubes, the tissue vortexed for 1 min and then centrifuged at 7,000 rcf thrice for 15 min. After each spin, the top organic layer containing cortisol (yellow) was removed and placed in a glass vial overnight at room temperature in a fumehood to allow ether to evaporate. Once evaporated, the next day cortisol samples were reconstituted in 1 mL of 1×-PBS overnight at 4°C. Thereafter, whole-body cortisol samples were assayed in triplicate using a Salimetrics Salivary Cortisol Enzyme Immunoassay Kit as per the manufacturers' instructions (Salimetrics, LLC, State College, PA). The optical density of samples was read at 450 nm using a SPECTRAmax PLUS Microplate Spectrophotometer (Molecular Devices, CA). The final concentration of whole-body cortisol (μg/dL) was determined by interpolation using a 4-parameter sigmoid minus curve fit and final cortisol levels normalized to body weight (ng/g).

Cortisol Injections

To directly test the effect of increased cortisol levels on the size of the proliferative population across sensory and integrative niches, we injected hydrocortisone intraperitoneally [Fig. 8(A)]. A stock solution of 10 mg/mL hydrocortisone (Sigma) was first made by diluting the solute in a 1:1 solution of 100% ethanol: chloroform. The stock solution was then diluted in 1×-PBS to yield three ascending hydrocortisone concentrations: 50, 175, and 350 ng. These concentrations were chosen since they were all within or above the range of cortisol levels shown to be stressful in zebrafish following exposure to various types of stressors (Pavlidis et al., 2011; Alderman and Vijahan, 2012; Dhanasiri et al., 2013). Controls included 1×-PBS alone, or 5 μL of 100% ethanol diluted in 1×-PBS (ETOH control) to account for possible effects of the diluent on levels of cell proliferation. Zebrafish were fasted 24-h prior to the first injection, and thereafter injected under anesthetic once a day at 10:00 over three consecutive days with hydrocortisone or vehicle at a volume of 50 μL/g body weight. The following day, animals were injected with a single bolus of 10 mM BrdU and killed 2-h later. Thereafter, their brains were perfused, fixed, and cryosectioned as described above in preparation for BrdU immunohistochemistry.

Statistical Analysis

Values are expressed as mean ± standard error of the mean. Pairs of means were compared using independent samples t-tests. In cases where the same treatment group was used for more than one comparison, we applied the Holm-Sidak corrections for family wise comparisons to reduce our chances of obtaining a Type I error and at the same time maintaining biologically meaningful differences across experiments (Abdi, 2010). In such instances, the corrected *p*-value is listed. For multiple comparisons between treatment groups, a one-way ANOVA was used and is noted in the text. Samples were considered significant at $p < 0.05$, and statistical analyses completed using SPSS Statistics 17.0 or Sigma Plot 11.0 and graphs made using Microsoft Excel 2003.

RESULTS

Relatively Stable Brain Growth in Zebrafish Exposed to Developmental Social Isolation and Social Novelty

Exposing animals to different environmental contexts, in particular during early development, can be

associated with changes in body and brain growth as a result of increased stress or changes in physiology. For instance, removal of the social environment during sensitive periods of development has been shown to potentially impact the development of neuronal connections and the speed of signal propagation (Berardi et al., 2000; Hensch, 2004; Makinodan et al., 2012). Since we examined a number of different social contexts, we first wanted to confirm that time spent by zebrafish in these environments did not produce any gross morphological changes that might affect downstream analyses. Therefore, upon sacrifice, we recorded the total body length (mm), rostrocaudal brain length using digital calipers (mm), and body weight (g), and performed statistical comparisons for each cohort of animals to be analyzed for changes in cell proliferation or differentiation under each treatment condition (Supporting Information Table S1). We found a significant decrease in body length ($p = 0.004$; Holm-Sidak correction) and body weight ($p = 0.004$; Holm-Sidak correction) of animals subjected to novelty following developmental social isolation (Iso-Nov) compared with those exposed to developmental isolation (Iso) alone. However, in all other cases exposure to the treatment conditions had little effect on growth parameters. Notably, there was no significant difference ($p > 0.05$) across any of the three growth parameters examined between group-raised and isolate-raised animals. In the above groups where a significant difference was observed, these differences in body length and weight could be explained by the ratio of males to females comprising each group (gender was randomly assigned), with females often being larger and typically gravid at the time of sacrifice. Alternatively, fish isolated for 6-months followed by exposure to novelty were likely at a disadvantage in competing for food for the duration of the exposure to the novel group and that may have resulted in a relatively acute reduction in body weight and length.

To further validate that different social contexts did not produce changes in the overall density of cells making up neurogenic niches within distinct neuroanatomical structures, we performed density counts using Hoechst nuclear labeling. We compared the total number of Hoechst-labeled cells in a 1000 μm^2 counting box in each of the six neurogenic niches (3 counting boxes/biological sample/niche) between animals raised in a familiar social environment (Gp; $N = 6$) and those raised in social isolation (Iso; $N = 5$). We reasoned that animals isolated for 6-months over the course of development should display the greatest change in cell density, if any, compared with group-raised controls, given the timing and duration of exposure. Our results showed consistently that no significant difference was present in the density of Hoechst-labeled cells across any of the six niches [Fig. 1(D,G,J,M,P,S); $p > 0.05$] between group- and isolate-raised animals. Our morphometric measurements indicate that our zebrafish husbandry protocol generally ensured that across treatment groups the animals were similar in size and that neurogenic niches in the brains had comparable cell density, at least in those brain regions under investigation. As such, stereological methods were used to quantify differences in cell proliferation or differentiation between treatment groups.

Reduced Neurogenesis After Developmental Social Isolation in Sensory Niches can be Partially Corrected by Subsequent Exposure to Social Novelty

Larval zebrafish can be raised successfully in isolation without the need for parental care, therefore we first addressed whether chronic isolation from a social environment over the first 6-months of life causes a long-term effect on neurogenesis in adulthood between group- and isolate-raised zebrafish [Fig. 2(A); Gp vs. Iso]. Within sensory niches, a significant decrease in the proliferative population was detected in isolated fish in PGZ [Fig. 2(B,C); $p = 0.039$] and OB [Fig. 3(A,B); $p = 0.034$] compared with group-raised controls, but was absent in LX (data not shown). By contrast, no significant difference in the number of cycling cells was noted within any telencephalic niches between group and isolated fish following a 2-h BrdU chase period [Fig. 3(G,H), *VdVv*; Supporting Information Fig. S2(A,D,) *Dl*; and data not shown; $p > 0.05$]. By quantifying the size of the BrdU-label-retaining population 4-wk post-BrdU-injection, we observed no significant difference in the number of post-mitotic $BrdU^+$ cells between group- and isolate-raised animals within any niche [Fig. 2(D), *PGZ*; Fig. 3(C,I,) *OB,VdVv*; Supporting Information Fig. S2(E), *Dl*; and data not shown; $p > 0.05$], although there was a clear trend toward a decrease in the PGZ [Fig. 2(D)]. Similar to the BrdU-label-retaining population, 4-wk post-BrdU injection showed no significant difference in the proportion of newborn neurons detected in sensory or telencephalic niches under these treatment [Fig. 2(E–G), *PGZ*; Fig. 3(D–F,J–L), *OB, VdVv*; Supporting Information Fig. S2(C,F), *Dl*; and data not shown; $p > 0.05$], nevertheless a marked decrease in the size of the differentiated neuronal population in Dl was observed.

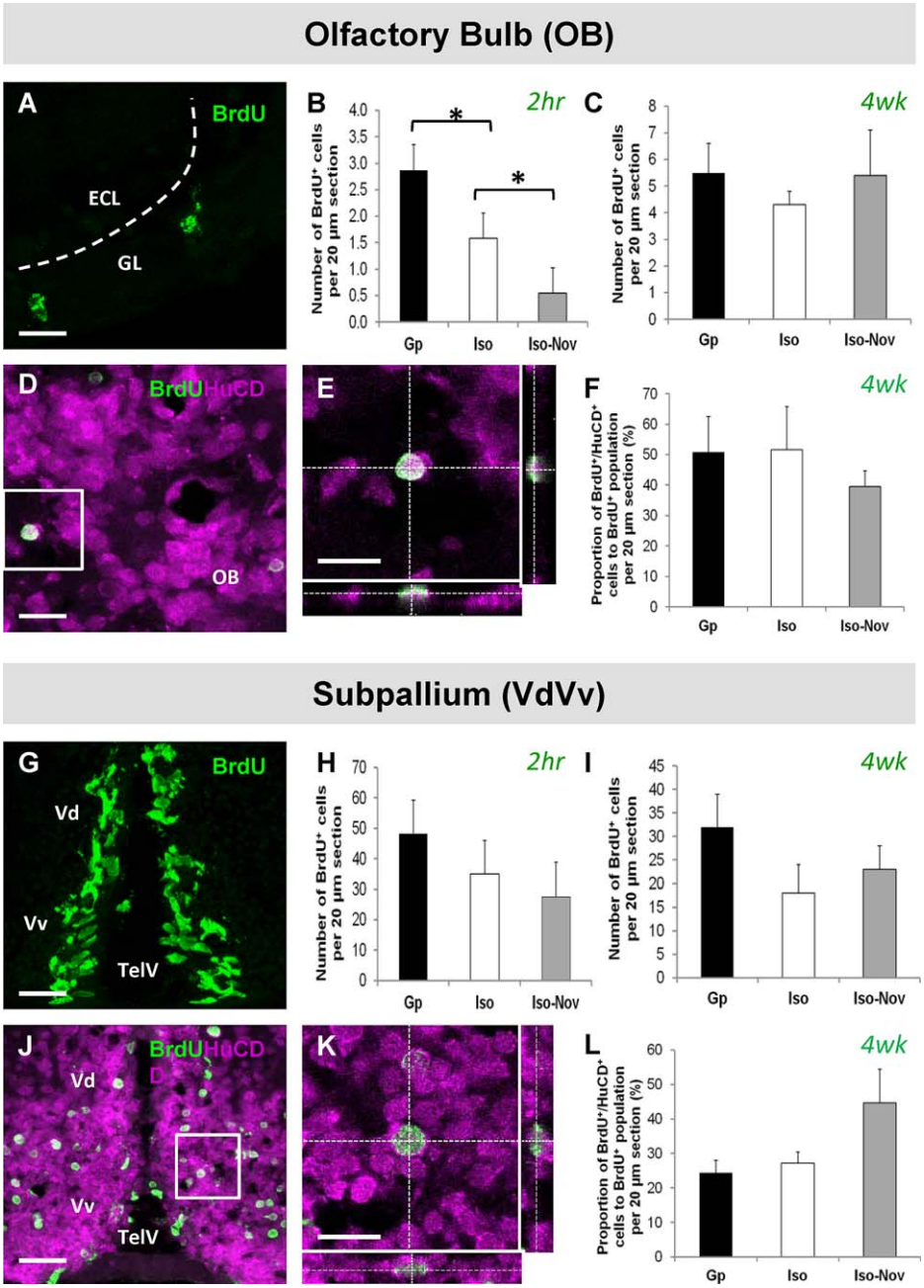

Figure 3 Effect of Developmental Isolation and Novelty treatments on cell proliferation 2-h post-BrdU injection (2 h) and on the BrdU-label-retaining population and proportion of newly differentiated neurons 4-wk post-BrdU injection (4 wk) in the sensory niche of the olfactory bulbs (OB, A–F) and telencephalic niche of the subpallium (VdVv, G–L). A and G: Representative images showing $BrdU^+$ cells localized primarily to the glomerular layer (GL) of OB (A) and within VdVv (G) adjacent the telencephalic ventricle (TelV). Hashed line in A represents the division between GL and the external cellular layer (ECL). B and H: Number of $BrdU^+$ cells 2-h post-BrdU injection. C and I: BrdU-label-retaining population 4-wk post-BrdU injection. D and J: Representative images of $BrdU^+/HuCD^+$ newly differentiated neurons. White squares depict higher magnification shown in E and K. E and K: Orthogonal view confirming colabeling of $BrdU^+/HuCD^+$ cells. F and L: Proportion of $BrdU^+/HuCD^+$ cells 4-wk post-BrdU injection in OB (F) and VdVv (L). In A, D, E, G, J, and K dorsal is up; scale bars = 10 µm. In B, C, F, H, I, and L, statistical comparisons were performed between Gp:Iso and Iso:Iso-Nov only. Statistically significant comparisons are denoted by an asterisk (*), where $p < 0.05$ (independent samples t-tests). Where no notation is present on graphs, this indicates that both comparisons were not significantly different.

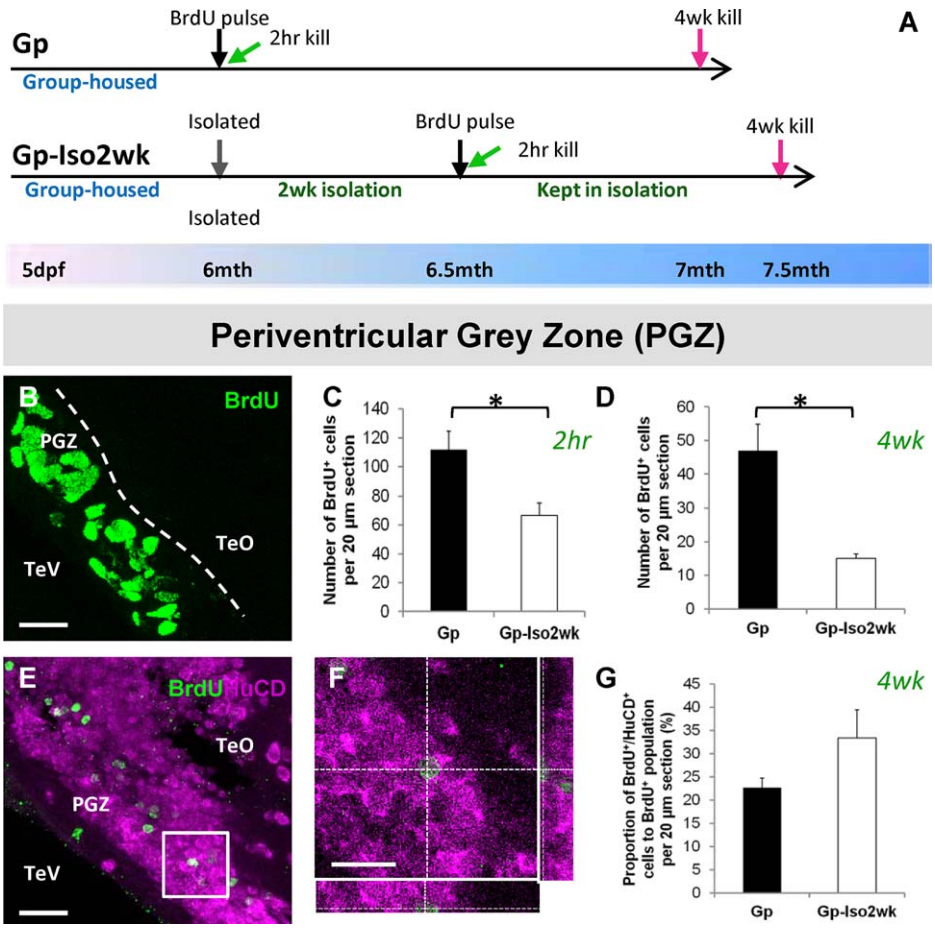

Figure 4 Effect of Adult Isolation treatments (A) on cell proliferation 2-h post-BrdU injection (2 h) and on the BrdU-label-retaining population and proportion of newly differentiated neurons 4-wk post-BrdU injection (4 wk) in the sensory niche of the periventricular gray zone (PGZ, B–G) of the optic tectum. A: Experimental design of adult isolation treatments. Gp: Raised in a familiar social group of 6 animals until 6-months. Gp-Iso2 wk: Isolated for 2-wk beginning at 6-months. dpf: days post fertilization. Groups of animals to be examined for changes in neuronal differentiation 4-wk post-BrdU injection were maintained under the treatment condition until sacrificed. B: Representative image showing $BrdU^+$ cells within PGZ (B) adjacent the tectal ventricle (TeV) and deep to the more superficial layers of the optic tectum proper (TeO). Hashed line represents the division between the PGZ and TeO. C: Number of $BrdU^+$ cells 2-h post-BrdU injection. D: BrdU-label-retaining population 4-wk post-BrdU injection. E and F: Representative image of $BrdU^+$/$HuCD^+$ newly differentiated neurons. White square depicts higher magnification shown in F. F: Orthogonal view confirming colabeling of $BrdU^+$/$HuCD^+$ cells. G: Proportion of $BrdU^+$/$HuCD^+$ cells 4-wk post-BrdU injection. In B, E, and F, dorsal is up; scale bars = 10 µm. In C, D, and G, statistical comparisons were performed between Gp:Gp-Iso2 wk. Statistically significant comparisons are denoted by an asterisk (*), where $p < 0.05$ (independent samples t-tests). Where no notation is present on graphs, this indicates that comparisons were not significantly different.

To next investigate whether we could restore decreases in cell proliferation observed following developmental social isolation, we exposed isolated zebrafish to a novel social group of adult zebrafish for 2-wk [Fig. 2(A); Iso vs. Iso-Nov]. We predicted that exposure to social novelty should be positively correlated with an increase in cell proliferation given that it is a typical component of an enriched environment (Baroncelli et al., 2010). To our surprise, compared with fish subjected to isolation only, isolated animals exposed to social novelty had significantly fewer proliferating cells in OB [Fig. 3(B); $p = 0.028$], with no further decrease detected elsewhere following a 2-h BrdU chase [Fig. 2(C), *PGZ*;

Fig. 3(H), *VdVv*; Supporting Information Fig. S2(D), *Dl*; and data not shown; $p > 0.05$]. At 4-wk, the size of the BrdU-label-retaining population between zebrafish isolated alone for 6-months or introduced to novelty thereafter displayed no significant difference [Fig. 2(D), *PGZ*; Fig. 3(C,I,) *OB, VdVv*; Supporting Information Fig. S2(E), *Dl*; and data not shown; $p > 0.05$]. Quantification of the proportion of newly differentiated neurons after a 4-wk chase period did however reveal a significant increase in the relative number of $BrdU^+/HuCD^+$ cells in both PGZ [Fig. 2(E–G); $p = 0.007$] and Dl [Supporting Information Fig. S2(B,C,F); $p = 0.001$], while no change was seen in the remaining niches [Fig. 3(D–F,J–L), *OB*, *VdVv*; and data not shown; $p > 0.05$]. Thus, a decrease in the number of proliferating progenitor cells was detected primarily in sensory niches after developmental isolation. Subsequent exposure to social novelty did not reverse the change in proliferation, but appeared to partially compensate for the reduced production by enhancing neuronal differentiation in the surviving postmitotic population.

Adult Social Isolation Suppresses Cell Proliferation and Survival in Sensory Niches but Enhances the Proportion of Newly Differentiated Neurons in the Telencephalon

To uncover how social isolation during adulthood might modify constitutive levels of adult neurogenesis, we raised zebrafish in a familiar social environment until 6-months and then isolated animals for 2-wk [Fig. 4(A); Gp vs. Gp-Iso 2 wk]. Compared with group-raised controls, a significant reduction in the size of the $BrdU^+$ population was noted after 2-wk isolation in the PGZ [Fig. 4(B,C); $p = 0.027$], similar to the effect of developmental social isolation [see Fig. 2(C)]. In the niche of LX, 2-wk isolation led to significantly fewer cycling cells [Fig. 5(A,B); $p = 0.021$], with no changes observed in other niches [Fig. 5(G,H), *D*; Supporting Information Fig. S2(G), *Dl*; Supporting Information Fig. S3(A,B,G,H), *OB, VdVv*; $p > 0.05$]. In line with the neurogenic response of PGZ and LX to changes in the cycling population at 2-h, survival of the BrdU-label-retaining population at 4-wk within these same niches further displayed a significant decrease in the number of $BrdU^+$ cells following 2-wk adult isolation compared with group-raised animals [Fig. 4(D), *PGZ*; $p = 0.003$, Holm-Sidak correction; Fig. 5(C), *LX*, $p = 0.003$, Holm-Sidak correction; Fig. 5(I), *D*; Supporting Information Fig. S2(H), *Dl*; Supporting Information Fig. S4(C,I), *OB, VdVv*; $p > 0.05$]. Four-weeks post-BrdU injection resulted in no change in the proportion of adult-born neurons in most niches [Fig. 4(E–G), *PGZ*; Fig. 5(D–F), *LX*; Supporting Information Fig. S2(I), *Dl*; Supporting Information Fig. S3(D–F,J–L), *OB, VdVv*; $p > 0.05$], although a significant increase in the pallium was observed [Fig. 5(J–L), *D*; $p = 0.005$]. These data suggest the possibility of a conserved mechanism inhibiting cell proliferation and survival during both long-term developmental isolation (i.e., 6-months) and short-term adult isolation (i.e., 2-wk) in sensory niches that acts independently from mechanisms regulating neuronal differentiation across niches.

Adult Short-Term Social Novelty can Produce the Same Effects as Social Isolation While the Combination of Isolation and Novelty Leads to Bidirectional Responses in Sensory Niches

To examine how the presence of a novel social group might modulate neurogenic plasticity in fish housed with familiar conspecifics over development [Fig. 6(A); Gp vs. Gp-Nov], 6-month old group-raised animals were tagged and exposed to social novelty for 2-wk. A consistent, but nonsignificant, decrease in the number of proliferative cells was observed between group controls and animals exposed to social novelty after 2-h BrdU chase periods across sensory niches [Fig. 6(B,C), *PGZ*; Fig. 7(A,B), *LX*; Supporting Information Fig. S4(A,B), *OB*; $p > 0.05$], whereas the size of the proliferating population remained relatively unaltered in telencephalic niches [Fig. 7(G,H), *D*; Supporting Information Fig. S2(J), *Dl*; Supporting Information Fig. S4(G,H), *VdVv*, $p > 0.05$]. A similar trend was observed in the size of the BrdU-label-retaining population 4-wk post-BrdU injection in sensory niches of PGZ and LX between animals raised in groups or further subjected to novelty at adulthood [Fig. 6(D), *PGZ*; Fig. 7(C), *LX*; $p > 0.05$]. Of the remaining $BrdU^+$ post-mitotic population at 4-wk, a significant decrease in the proportion of newly generated neurons was detected in LX with introduction to social novelty [Fig. 7(D–F); $p = 0.046$], with no changes seen elsewhere [Fig. 6(E–G), *PGZ*; Fig. 7(J–L), *D*; Supporting Information Fig. S2(L), *Dl*; Supporting Information Fig. S4(D–F,J–L), *OB, VdVv*; $p > 0.05$].

To test how the combination of social novelty and social isolation would act on constitutive levels of adult neurogenesis in zebrafish first raised in a social environment, we designed an experiment where animals were isolated for 2-wk before being exposed to social novelty for an additional 2-wk period

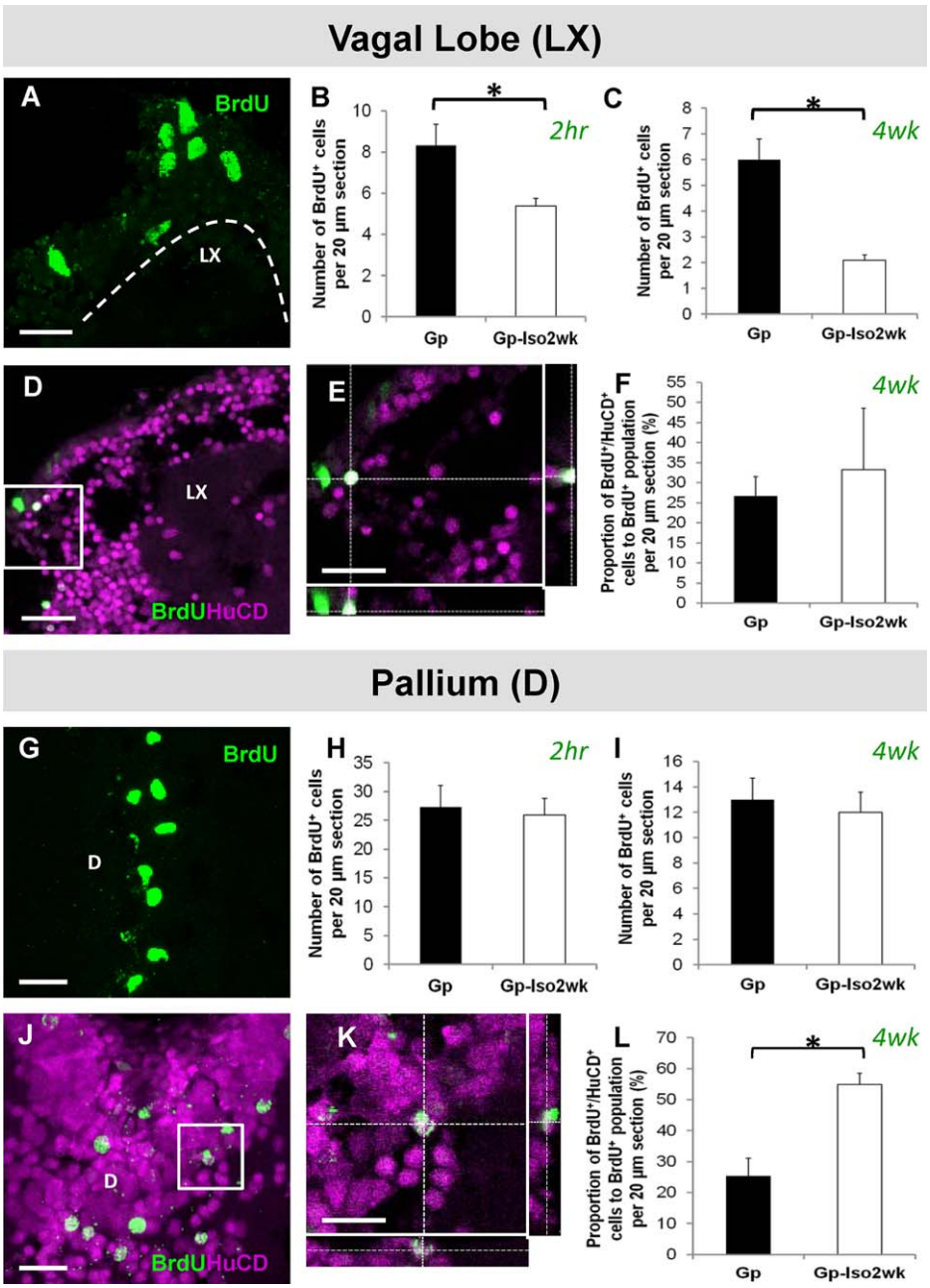

Figure 5 Effect of Adult Isolation treatments on cell proliferation 2-h post-BrdU injection (2 h) and on the BrdU-label-retaining population and proportion of newly differentiated neurons 4-wk post-BrdU injection (4 wk) in the sensory niche of the vagal lobe (LX, A–F) and telencephalic niche of the pallium (D, G–L). A and G: Representative images showing $BrdU^+$ cells localized within LX (A) and at the dorsal midline of D (G). Hashed line in A demarcates the ventral border of the niche of LX. B and H: Number of $BrdU^+$ cells 2-h post-BrdU injection. C and I: BrdU-label-retaining population 4-wk post-BrdU injection. D and J: Representative images of $BrdU^+/HuCD^+$ newly differentiated neurons. White squares depict higher magnification shown in E and K. E and K: Orthogonal view confirming colabeling of $BrdU^+/HuCD^+$ cells. G: Proportion of $BrdU^+/HuCD^+$ cells 4-wk post-BrdU injection. In A, D, E, G, J, and K, dorsal is up; scale bars = 10 µm. In B, D, F, H, I and L, statistical comparisons were performed between Gp:Gp-Iso2 wk. Statistically significant comparisons are denoted by an asterisk (*), where $p < 0.05$ (independent samples t-tests). Where no notation is present on graphs, this indicates that comparisons were not significantly different.

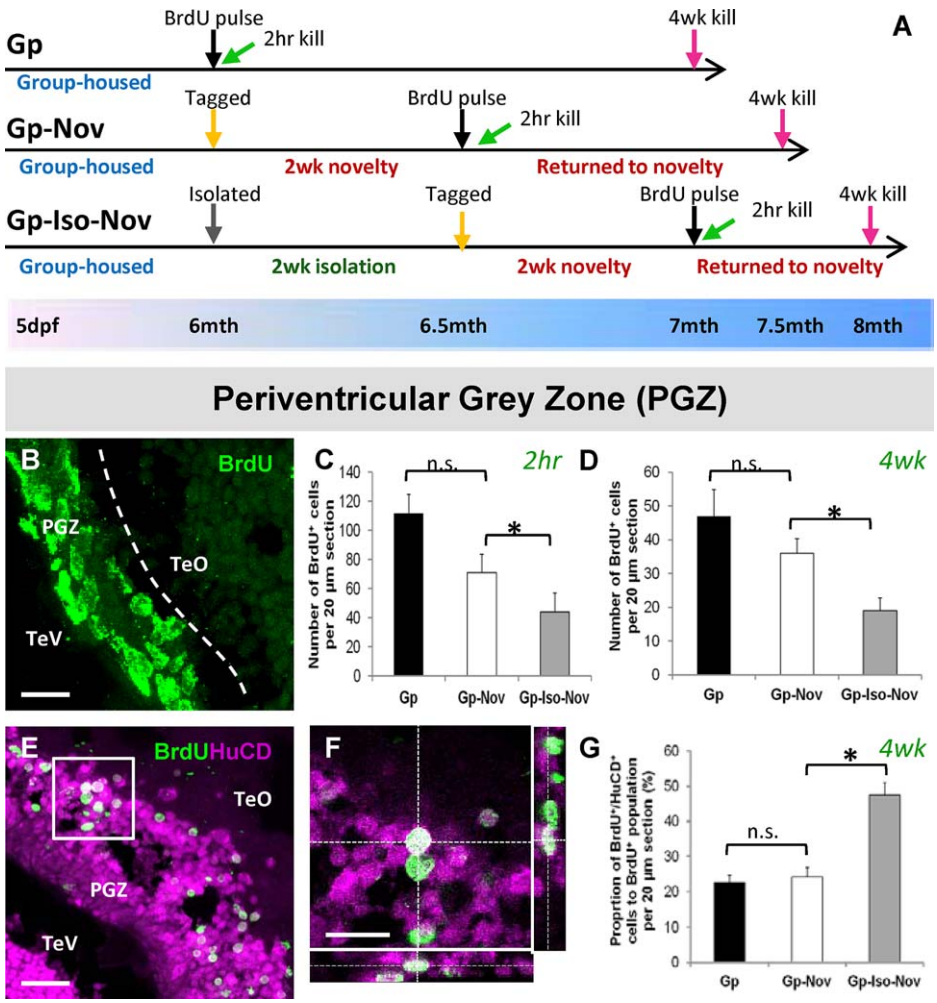

Figure 6 Effect of Adult Novelty and Isolation treatments (A) on cell proliferation 2-h post-BrdU injection (2 h) and on the BrdU-label-retaining population and proportion of newly differentiated neurons 4-wk post-BrdU injection (4 wk) in the sensory niche of the periventricular gray zone (PGZ, B–G) of the optic tectum. A: Experimental design of adult novelty and isolation treatments. Gp: Raised in a familiar social group of 6 animals until 6-months. Gp-Nov: Tagged and introduced into a novel group of 6 unfamiliar, adult zebrafish for 2-wk beginning at 6-months (social novelty). Gp-Iso-Nov: Isolated for 2-wk beginning at 6-months then tagged and introduced into a novel group of 6 unfamiliar, adult zebrafish for an additional 2-wk (social novelty). dpf: days post fertilization. Groups of animals to be examined for changes in neuronal differentiation 4-wk post-BrdU injection were maintained under the treatment condition until sacrificed. B: Representative image showing $BrdU^+$ cells within PGZ (B) adjacent the tectal ventricle (TeV) and deep to the more superficial layers of the optic tectum proper (TeO). Hashed line represents the division between the PGZ and TeO. C, Number of $BrdU^+$ cells 2-h post-BrdU injection. D: BrdU-label-retaining population 4-wk post-BrdU injection. E and F: Representative image of $BrdU^+/HuCD^+$ newly differentiated neurons. White square depicts higher magnification shown in F. F: Orthogonal view confirming colabeling of $BrdU^+/HuCD^+$ cells. G: Proportion of $BrdU^+/HuCD^+$ cells 4-wk post-BrdU injection. In B, E, and F, dorsal is up; scale bars = 10 µm. In C, D, and G, statistical comparisons were performed between Gp:Gp-Nov and Gp-Nov:Gp-Iso-Nov only. Statistically significant comparisons are denoted by an asterisk (*), where $p < 0.05$ (independent samples t-tests). For clarity, on graphs where significance is present, comparisons that were not significant (n.s.) are also noted.

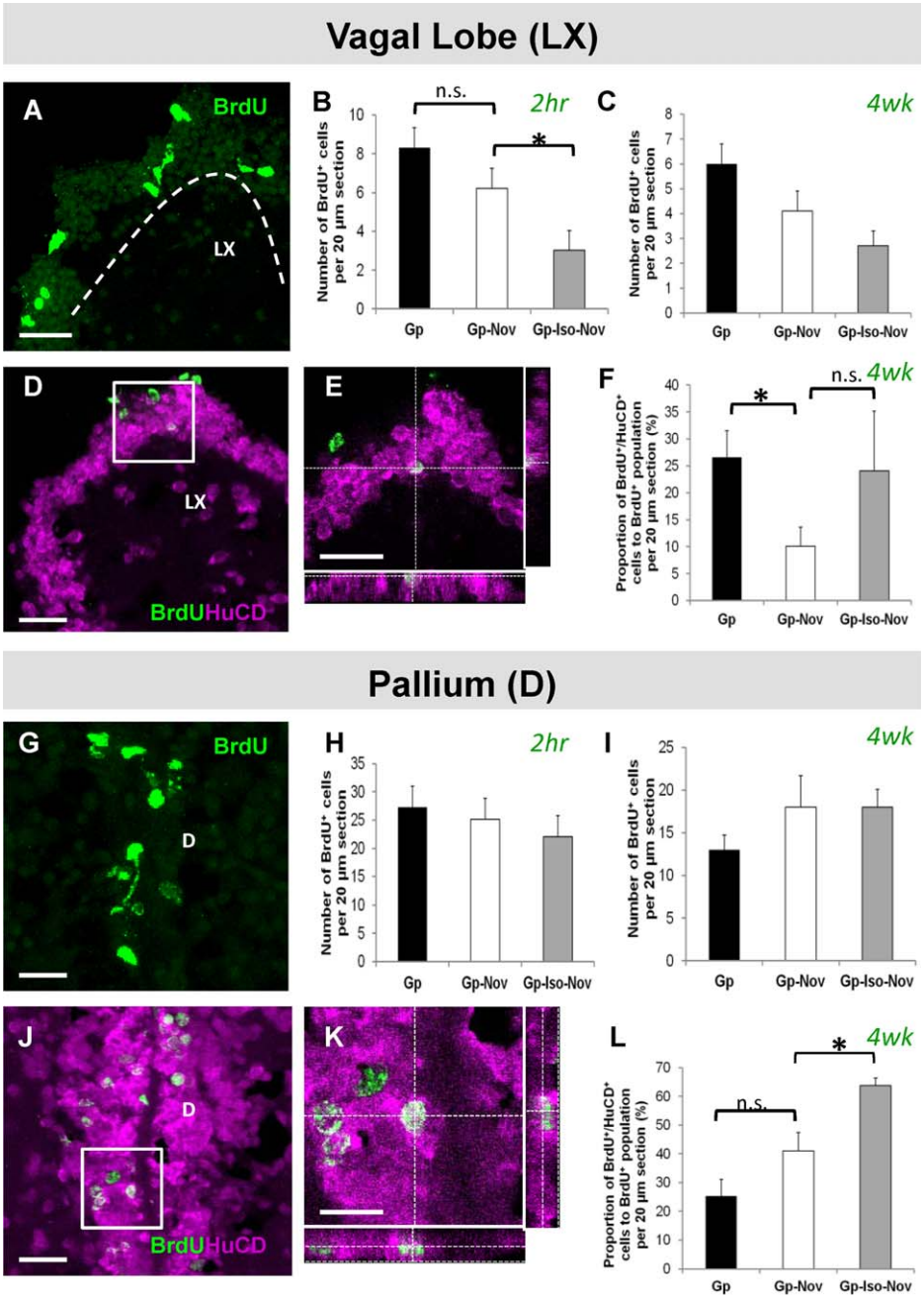

Figure 7 Effect of Adult Novelty and Isolation treatments on cell proliferation 2-h post-BrdU injection (2 h) and on the BrdU-label-retaining population and proportion of newly differentiated neurons 4-wk post-BrdU injection (4 wk) in the sensory niche of the vagal lobe (LX, A–F) and telencephalic niche of the pallium (D, G–L). A and G: Representative images showing $BrdU^+$ cells localized within LX (A) and at the dorsal midline of D (G). Hashed line in A demarcates the ventral border of the niche of LX. B and H: Number of $BrdU^+$ cells 2-h post-BrdU injection. C and I: BrdU-label-retaining population 4-wk post-BrdU injection. D and J: Representative images of $BrdU^+/HuCD^+$ newly differentiated neurons. White squares depict higher magnification shown in E and K. E and K: Orthogonal view confirming colabeling of $BrdU^+/HuCD^+$ cells. G: Proportion of $BrdU^+/HuCD^+$ cells 4-wk post-BrdU injection. In A, D, E, G, J and K, dorsal is up; scale bars = 10 µm. In B, D, F, H, I and L, statistical comparisons were performed between Gp:Gp-Nov and Gp-Nov:Gp-Iso-Nov only. Statistically significant comparisons are denoted by an asterisk (*), where $p < 0.05$ (independent samples t-tests). For clarity, on graphs where significance is present, comparisons that were not significant (n.s.) are also noted. Where no notation is present on graphs, this indicates that both comparisons were not significantly different.

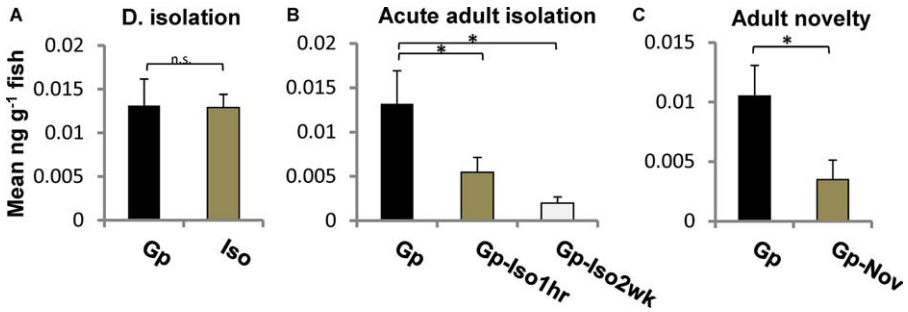

Figure 8 Relative whole-body cortisol measurements in adult zebrafish following exposure to different social contexts. A: Cortisol levels compared between 6-month group-raised (Gp) and 6-month isolate-raised (Iso) fish. D. isolation: Developmental isolation. B: Cortisol levels compared between animals raised to adulthood in a group environment (Gp), or raised to adulthood in a group and exposed to 1-h (Gp-Iso 1 h), or 2-wk (Gp-Iso 2 wk) social isolation. Statistical comparisons were performed between Gp:Gp-Iso1 h and Gp:Gp-Iso2 wk only. C: Cortisol levels compared between zebrafish raised in a group environment (Gp) or group-raised followed by exposure to a novel group of adult fish for a duration of 2-wk (Gp-Nov). All cortisol values are normalized to the averaged body weight of animals and represented in ng/g. Statistically significant comparisons are denoted by an asterisk (*), where $p < 0.05$ (independent samples t-tests). Comparisons that were not significant are denoted by n.s.

[Fig. 6(A)] (Gp-Nov vs. Gp-Iso-Nov). Our results showed that only neurogenic niches localized in sensory structures of the PGZ and LX had significantly fewer $BrdU^+$ cells following a 2-h chase period [Fig. 6(C); *PGZ*; $p = 0.043$; Fig. 7(B), *LX*; $p = 0.0001$; Fig. 7(H), *D*; Supporting Information Fig. S2(J), *Dl*; Supporting Information Fig. S4(B,H), *OB, VdVv*; $p > 0.05$]. At 4-wk, only the PGZ displayed a significant decrease in the BrdU-label-retaining population in animals exposed to isolation and novelty compared with novelty alone at adulthood [Fig. 6(D), *PGZ*; $p = 0.009$; Fig. 7(C,I), *LX, D*; Supporting Information Fig. S2(K), *Dl*; Supporting Information Fig. S4(C,I), *OB, VdVv*; $p > 0.05$]. However, a significantly greater number of newly differentiated neurons within the niches of PGZ and D were seen following exposure to the combination of isolation and novelty [Fig. 6(E,F), *PGZ*; $p = 0.001$; Fig. 7(J–L), *D*; $p = 0.021$]. Nonetheless, a trend towards an increase in the population size of differentiated neurons was consistently observed across all niches examined [Fig. 7(F), *LX*; Supporting Information Fig. S2(L), *Dl*; Supporting Information Fig. S4(F,L), *OB, VdVv*; $p > 0.05$]. Therefore, subjecting animals first raised in a familiar group environment to a novel group of unfamiliar fish can in some cases, such as in the niche of LX, reduce levels of adult neurogenesis similar to adult isolation. In contrast, exposing adult zebrafish to a combination of social isolation and novelty appears to more robustly modulate stages of adult neurogenesis in sensory niches by uncoupling the direction (i.e., increase or decrease) of the neurogenic response.

Physiological Levels of Cortisol in Animals Raised Developmentally in a Familiar Social Group or in Isolation are Similar, but Decrease After Social Isolation and Novelty in Adulthood, and Occur Independently from Changes in the Size of the Proliferative Population

Studies have implicated increased cortisol levels as a mechanism responsible for decreases in levels of adult neurogenesis with isolation in mammals (Schoenfeld and Gould, 2011; Lieberwirth et al., 2012). However, the manner by which cortisol levels regulate stages of neurogenesis under different social contexts in mammals and other vertebrate models of neurogenesis remain poorly understood. To explore whether elevated cortisol levels could account for the trend in lower rates of cell proliferation in sensory niches of OB, PGZ, and LX with developmental and adult isolation, and novelty, we assayed whole-body cortisol levels. We found that zebrafish reared in a familiar group environment ($N = 8$, Gp) alongside conspecifics or isolated for 6-months developmentally ($N = 8$, Iso) showed nearly identical cortisol levels [Fig. 8(A); $p = 0.942$]. However, animals first raised to adulthood in a group before being exposed to 1-h [Fig. 8(B); $N = 12$; $p = 0.048$] and 2-wk ($N = 10$, Gp-Iso 2 wk; $p = 0.004$) adult isolation, or introduced into a novel social group for 2-wk [Fig. 8(C); $N = 8$, Gp-Nov; $p = 0.04$) displayed significantly lower levels of cortisol compared with group-raised controls.

To more directly test the relationship between cortisol levels and cell proliferation within neurogenic

niches we next injected zebrafish with three different concentrations of hydrocortisone (CORT: 50, 175, and 350 ng) or vehicle (1×-PBS) and counted the number of $BrdU^+$ cells after a 2-h chase period [Fig. 9(A)]. Since there was no significant difference in the number of $BrdU^+$ cells across all six neurogenic niches between fish exposed to our 1×-PBS control and ETOH control (data not shown), these data were merged ($N = 9$) for comparisons with treatment groups. For treatment groups, the resulting total concentrations of hydrocortisone per gram fish were ∼13 ng/g ($N = 8$), ∼50 ng/g ($N = 8$), and ∼100 ng/g ($N = 8$), all well above physiological baseline levels of endogenous cortisol reported for zebrafish (Alderman and Vijahan, 2012; Dhanasiri et al., 2013). Of those animals injected with hydrocortisone, only three lethalities were observed over the 3-day injection period. Contrary to our hypothesis, by performing one-way ANOVA statistical tests we detected no significant between-group effect within niches residing in sensory structures [Fig. 9(B–E), *OB, PGZ*, and data not shown; $p > 0.05$], nor within the telencephalon [Fig. 9(F–I), *D, VdVv*, and data not shown; $p > 0.05$]. This led us to consider whether the already heightened levels of endogenously circulating cortisol present in the group-raised fish used for the analyses [see Fig. 8(A–C)] (Gp) were masking any effect on cell proliferation following hydrocortisone injections.

To address this, we used our 2-wk adult isolation paradigm in conjunction with hydrocortisone injections of 350 ng/mL (or 1×-PBS vehicle; $N = 7$) to investigate whether the social context played a role in regulating the effect of hydrocortisone injections on the proliferative population within neurogenic niches. We predicted that by injecting high levels of exogenous hydrocortisone into fish with low physiological levels of endogenous cortisol [see Fig. 8(B); Gp-Iso 2 wk], we could more readily detect changes in the number of cycling cells. Following three days of consecutive injections, the resulting concentration of hydrocortisone per gram fish was∼ 62 ng/g ($N = 8$). Surprisingly, we found that 2-wk of social isolation preceding cortisol injections at levels 4-fold greater

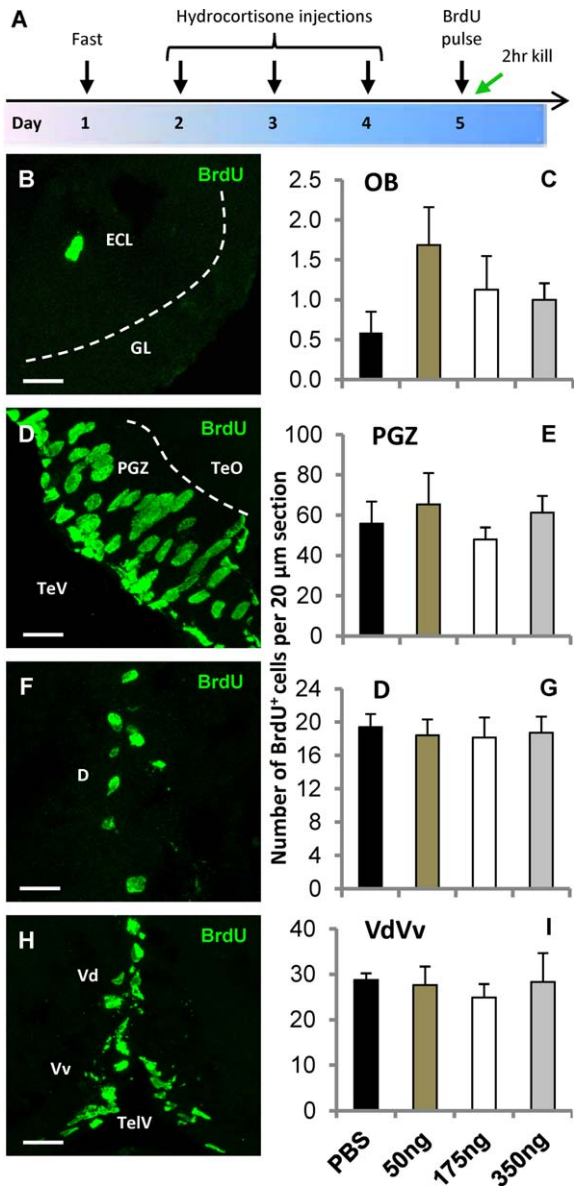

Figure 9 Effect of hydrocortisone injections in group-raised adult zebrafish on the number of $BrdU^+$ cells within sensory (OB, PGZ) and telencephalic niches (D, VdVv). A: Timeline of experimental protocol performed over a 5-day period. Animals were injected with PBS control, or 50, 175, or 350 ng of hydrocortisone over 3 consecutive days and thereafter received a 2-h pulse-chase of BrdU before being killed and processed for immunohistochemistry. B and C: Representative image of $BrdU^+$ cells in OB (B) and number of $BrdU^+$ counted between treatments (C). In B, ECL: external cellular layer; GL: glomerular layer. Hashed line represents the division between the GL and ECL. D–E: Representative image of $BrdU^+$ cells in PGZ (D) and number of $BrdU^+$ cells counted between treatments (E). In D, TeO: optic tectum proper; TeV: tectal ventricle. Hashed line represents the division between TeO and PGZ. F and G: Representative image of $BrdU^+$ cells in D (F) and number of $BrdU^+$ cells counted between treatments (G). H and I: Representative image of $BrdU^+$ cells in VdVv (H) and number of $BrdU^+$ cells counted between treatments (I). TelV: telencephalic ventricle. In B, D, F, and H dorsal is up; scale bars = 10 µm. In C, E, G, and I, statistical comparisons were performed between treatment groups using a one-way ANOVA. Since no significant group effect was present for any niche between the four treatments ($p > 0.05$), no statistical significance is denoted on graphs.

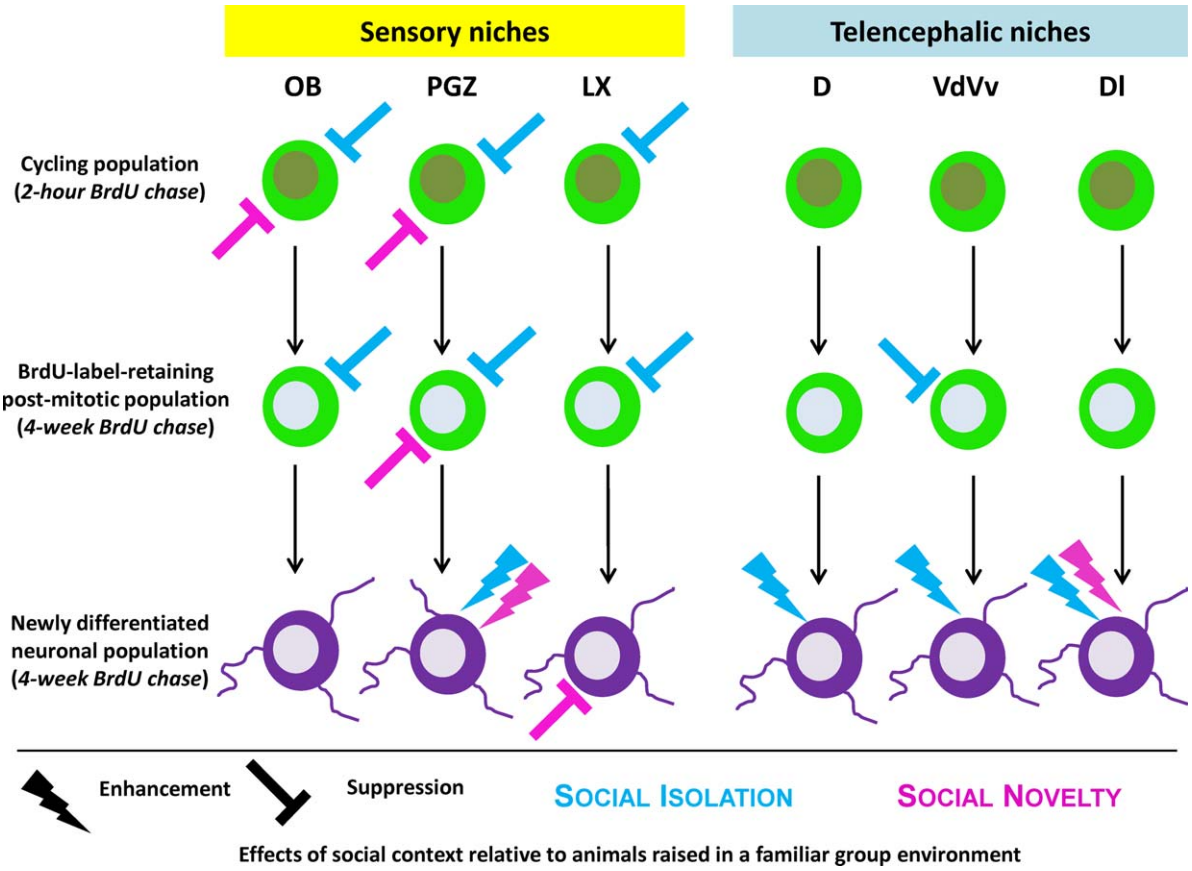

Figure 10 Model summarizing the effect of different social contexts (isolation, blue; novelty, pink) relative to group-raised animals within niches residing in sensory (OB, PGZ, and LX) and telencephalic (D, VdVv, Dl) structures at three stages of adult neurogenesis: the cycling stem/progenitor population 2-h post-BrdU injection; the BrdU-label-retaining post-mitotic population 4-wk post-BrdU injection; the newly differentiated neuronal population 4-wk post-BrdU injection. Enhancement and suppression refer to an increase or decrease, respectively, in the number of cells making up the population of interest at a given stage. The schematic represents both statistically significant differences as well as major trends observed across all data sets.

than those reported for acute stress in zebrafish (Alderman and Vijahan, 2012) still produced no significant difference in the number of $BrdU^+$ cells within niches compared with animals injected with vehicle only (data not shown; $p > 0.05$).

Collectively, our cortisol experiments show that cortisol levels do not correlate with rates of cell proliferation within sensory niches 2-h post-BrdU injection, but rather that cortisol levels are associated with the social environment or changes in the social context of the individual.

DISCUSSION

Environmental enrichment enhances neurogenesis in the adult vertebrate brain, whereas environmental deprivation suppresses it. We investigated the extent of neurogenic plasticity of six distinct neurogenic niches in response to social change in the adult zebrafish brain and whether these changes correlated with physiological levels of cortisol. By exposing animals to social isolation, social novelty, or a combination of the two, we show that neurogenic plasticity within the adult brain functions in a nonuniform, niche-specific manner, in which different niches adapt independently and distinct stages of neurogenesis can be differentially modulated (Fig. 10). Unexpectedly, we show that social isolation or social novelty are both capable of decreasing the number of proliferating cells while increasing the number of newborn neurons within a single niche, challenging the assumption that social deprivation and social novelty, a form of enrichment, have diametrically opposite effects on adult neurogenesis. This was most pronounced in

sensory niches of the brain, suggesting that niches residing in primary sensory structures are more sensitive to changes in the social environment compared with telencephalic niches. Contrary to observations in rodent studies, social enrichment does not consistently rescue deficits in cell proliferation following social isolation. Furthermore, zebrafish isolated during development or adulthood do not display elevated cortisol levels compared with group-raised animals, but rather that this hormone correlates with the social context and acts independently from changes in adult neurogenesis.

Neurogenic Niches in Primary Sensory Structures Are Most Sensitive to Changes in the Social Environment

By comparing adult neurogenic compartments located within primary sensory and telencephalic structures of the adult zebrafish brain, we clearly show that sensory neurogenic niches more readily adapt to changes in the social context. In line with this, neither isolation nor novelty conditions led to changes in the $BrdU^+$ population after 2-h chase periods in telencephalic niches. Still, we demonstrate that developmental and adult social isolation in zebrafish generally produces a decrease in the stem/progenitor pool in sensory niches, consistent with reports in the mammalian hippocampus (Lieberwirth et al., 2012). Zebrafish rely heavily on visual, olfactory, and taste cues for numerous behaviors (Gerlach et al., 2008), and this may partially explain the bias in modulation of sensory niches. Whether this implies that the production of new neurons in sensory structures may in some way serve a functional role in sensory processing is unknown, but given that these niches are differentially modulated by aspects of the social environment indicate that these sensory structures likely do not only undergo continuous, passive growth with age along with their amphibian and reptilian relatives (reviewed in Kaslin et al., 2008), but might also adapt to fulfill a functional role. However, the limited neurogenic plasticity observed in telencephalic niches analyzed in our study may have been caused by our experimental design that relied on manipulating the degree of sensory/social stimulation available to animals, rather than explicitly addressing any form of learning, which would be predicted to engage plasticity inherent in the telencephalon. These findings raise the interesting question of whether differences in neural circuit activity between primary sensory and telencephalic brain regions might, in part, contribute to the manner by which the neurogenesis in these niches is modulated under different environmental contexts.

Although neuroanatomical evidence in zebrafish is still sparse, studies in other cyprinids show that a number of sensory structures and telencephalic nuclei both receive and process one or more sensory modalities and may further act as integration centers to process, relay, and encode sensory and higher-order information. Thus, it may be the degree to which sensory and telencephalic structures fulfill each role (sensory processing vs. input integration) that determines why some niches are modulated while others are not. For example, only a subset of the superficial layers of the optic tectum receive afferents from retinal ganglion cells (Venegas et al., 1974; Meek, 1983), whereas other layers integrate modalities arising as secondary input from the lateral line, auditory systems, and torus longitudinalis (Meek, 1983; Gibbs and Northmore, 1998). In the vagal lobe, outer layers are innervated by vagal input of the intraoral system with deeper layers dedicated to processing motor input (Morita and Finger, 1985; Hayama and Caprio, 1989; Nieuwenhuys, 2011b). In the telencephalon, secondary efferent connections from the olfactory bulb project into the pallium and subpallium of the telencephalon (Friedrich, 2013), with the subpallium proposed as a functional homologue to the striatum/septum of mammals (Wullimann and Rink, 2002; Wullimann, 2009; Nieuwenhuys, 2011a). Moreover, reciprocal secondary retinal input is believe to reach the lateral aspect of the dorsal telencephalon, with this region debated as the hippocampal homologue to mammals (Broglio et al., 2005; Salas et al., 2006; Lau et al., 2011; Nieuwenhuys, 2011a).

Despite the potential of sensory and integrative processing occurring within structures housing sensory and telencephalic neurogenic niches, niches residing within primary sensory domains have a greater chance to be influenced foremost by sensory input. One hypothesis may be that the combination of primary sensory and secondary input from other modalities processed in the olfactory bulbs, optic tectum, and vagal lobe may more readily elicit changes in adult neurogenesis compared with telencephalic niches that only receive second or third order sensory input and may further necessitate more direct forms of learning to obtain a cellular response within the niche.

Social Isolation and Social Novelty Are Both Capable of Producing the Same Effect Across Stages of Adult Neurogenesis

What constitutes a stressful or enriching environment is largely determined by the life history of a species.

Environmental enrichment typically encompasses consistent multisensory/cognitive stimulation consisting of social stimuli, novel objects, and the opportunity for physical activity and exploration (Baroncelli et al., 2010). We predict that a social grouping of zebrafish encompasses many of these aspects of enrichment. In contrast, social deprivation involves the removal of such stimuli, with animals restricted to an isolated and/or barren environment with little or no social contact. Environmental enrichment and deprivation are known to produce structural changes at the level of neural networks and neuronal populations during sensitive periods of development, and indeed experience-driven environmental plasticity can impinge on the composition and function of neurogenic niches in adulthood (Spolidoro et al., 2009). It has been proposed that the complex brains of mammals maintain greater levels of neurogenic plasticity within the hippocampus to allow adult stem/progenitor cells within this niche to more readily adapt in response to changes in the natural environment, endowing animals with the flexibility to thrive under these circumstances (Kempermann, 2012). Our data, along with other reports (Maruska et al., 2012, 2013), suggests that adaptive neurogenic plasticity might be a common feature among vertebrates, still we know little about the cellular behavior of stem/progenitor cells in non-mammalian taxa under conditions of environmental enrichment or deprivation.

One hypothesis we tested was whether decreases in cell proliferation in the niches of animals isolated developmentally for 6-months could be restored by introduction into a novel social group (i.e., enrichment) for 2-wk thereafter. Despite PGZ and OB both displaying a significant change between group-raised and isolated animals, only in OB was a significant difference between isolated animals and those introduced into social novelty further observed [see Fig. 3(B)]. Congruent with olfactory sensory deprivation studies in mice using occlusion paradigms (Mandairon et al., 2006), developmental isolation in zebrafish resulted in a decrease in the number of $BrdU^+$ cells, but to our surprise this effect was exacerbated by social novelty (not rescued), a form of enrichment predicted to restore such decreases. This exemplifies how two seemingly opposing social contexts (isolation and novelty) can impose the same directional change on a single neurogenic niche. Experiments in the adult zebrafish telencephalon have demonstrated that the 'social isolation context' also plays a role, and that zebrafish socially isolated in a barren environment compared with an enriched environment (rocks, flora) have fewer $PCNA^+$ cells (von Krogh et al., 2010). Work in the electric fish has more recently shown that fish paired with multiple different partners over a 14-day period is required to increase rates of proliferation (Dunlap and Chung, 2013). Therefore, we must consider that continuously housing isolates with a single social group may have prevented the ability to restore proliferation rates due to habituation to the novel stimulus.

Since the presence of resident stem/progenitor cells within the olfactory bulb of vertebrates still remains controversial, our olfactory bulb data must be interpreted with caution. Earlier studies in rodents have shown evidence *in vitro* that cells isolated from the rostral extension of the rostral migratory stream projecting into the olfactory bulbs, are multipotent and self-renewing (Gritti et al., 2002), however *in vivo* evidence is still lacking. It is commonly accepted that in mammals (Lois and Alvarez-Buylla, 1994; Lois et al., 1996), and more recently in zebrafish (Byrd and Brunjes, 1995, 1998, 2001; Adolf et al., 2006; Grandel et al., 2006; Kishimoto et al., 2011), that the majority of stem/progenitor cells that populate the olfactory bulb arise from the SEZ or subpallial niche, respectively. Therefore, changes observed in the size of the proliferative population with developmental social isolation and novelty in the olfactory bulb may reflect modulation of a small number of resident stem/progenitor cells as well as a larger secondary pool of stem-like cells emigrating from the subpallial niche. Whether $BrdU^+$ cells within the olfactory bulb are truly resident or even neurogenic is still unclear, however. Another possibility is that the $BrdU^+$ cells detected within the olfactory bulb after a 2-h pulse of BrdU were in fact born earlier in the subpallium, and had migrated into the bulb prior to our BrdU injections. Thus, the progressively smaller $BrdU^+$ population with isolation, followed by isolation and novelty [see Fig. 3(B)], could be interpreted as a slowing of cell migration from the subpallium, a slowing of the cell cycle, or increased apoptosis in either the local or distal stem/progenitor populations. Subsequent studies resolving the contribution of these two populations to adult olfactory bulb neurogenesis in the adult zebrafish will be essential to better clarify how different social contexts impinge on cells within this niche.

The Social Context Independently Regulates the Stem/Progenitor Population Separately from the Proportion of Newborn Neurons

An intriguing finding of our study was that the size of the stem/progenitor pool could be regulated independently from the resulting number of newly

differentiated neurons under specific social contexts. It is interesting that patterns across a number of niches examined here show that irrespective of the social context (i.e., isolation, novelty, both) rates of cell proliferation are diminished while the number of newborn neurons generated is elevated. Earlier studies in the adult goldfish have demonstrated that consistent visual input to the optic tectum is required to maintain constitutive rates of cell proliferation in the tectal germinal zone, and that permanent or temporary denervation lead to a reduction in the number of cells incorporating [^{3}H] thymidine (Raymond et al., 1983). However, how changes in visual input can further modulate *de novo* neurogenesis are unknown. Data from the PGZ provides a particularly poignant example of this under treatments where zebrafish were raised as groups to maturity, then introduced into novelty, or a combination of isolation and novelty (see Fig. 6). Here, the proliferative pool at 2-h and the BrdU-label-retaining population at 4-wk showed a marked decrease in animals exposed to both isolation and novelty, compared with only novelty at adulthood. But from the smaller postmitotic population at 4-wk, a significantly larger proportion of differentiated neurons were generated. Thus, we illustrate that stages in the process of adult neurogenesis need not always occur in a linear fashion (i.e., each stage increasing or decreasing accordingly), but that within distinct niches the social context can give rise to bidirectional modulation.

One mechanism for the lower rate of cell division observed in the PGZ could be that neuroepithelial-like stem/progenitor cells residing in the dorsomedial margin (Alunni et al., 2010; Ito et al., 2010) are more susceptible to changes in environmental stimuli compared with glial-like stem/progenitor cells in the pallial and subpallial niches of the zebrafish telencephalon (Ganz et al., 2010). This is further supported by our observations within the lateral zone of the dorsal telencephalon (Dl) of the zebrafish, that also demonstrated little adaptive responses with exposure to isolation or novelty, and are composed of morphologically similar cell types as the pallium and subpallium (Lindsey et al., 2012). In evaluating these data, we must also consider the possibility that the behavior of the stem cell niche and its neuronal output within the caudal aspect of the PGZ may be different if all BrdU$^+$ cells were counted across the entire rostrocaudal axis of this structure, in which case would also include a second populations of stem/progenitor cells shown to have more classical glial features (Ito et al., 2010).

The ability to uncouple the direction of change at the starting (i.e., cell proliferation) and endpoints (i.e., neuronal differentiation) of adult neurogenesis under similar conditions might also be influenced by the experience of an animal from a previous social environment. For instance, animals raised in isolation lack the experience of sensory stimuli from social interactions, and upon exposure to social novelty new sensory neurons are born to process this information, regardless of the reduced state of the progenitor population [see Fig. 2(G)]. By contrast, group-raised animals have increased competition for food, partners, territory, and social status (Maruska et al., 2012), which increase anxiety and suppress neuronal differentiation (Sah et al., 2012; Diniz et al., 2013). With exposure to isolation during adulthood such as in the case of the pallium [see Fig. 5(L)], this may provide a reprieve from the challenges of the social context, resulting in increased neuronal differentiation. Our data support the notion that stage-specific modulation of adult neurogenesis following a shift to a new environmental context, are likely relative to the preceding social context of the animal.

Physiological Relationship of Cortisol Under Different Social Contexts and Its Impact on Stem/Progenitor Cell Proliferation During Adult Neurogenesis

Most forms of stress inhibit adult neurogenesis by lowering the rate of cell proliferation within neurogenic niches (Schoenfeld and Gould, 2011); however, we now know that even in mammals exceptions exist and that regulation of the stress axis and how it impinges on stages of adult neurogenesis is more complicated than once thought. Rewarding experiences such as running, sexual experience, and enrichment, some of which themselves are coupled with increased cortisol, can protect against the inhibitory effects of increased glucocorticoids on adult neurogenesis (Schoenfeld and Gould, 2013). Running, for example, elevates stress hormone levels, while at the same time increases cell proliferation and induces neuronal differentiation in the dentate gyrus of mice (Snyder et al., 2009) and rats (Stranahan et al., 2006). However, in a separate study, mice housed in isolation or social groups and allowed access to a running wheel have shown similar increases in the number of BrdU$^+$ and doublecortin$^+$ cells in the hippocampus compared with their respective sedentary controls, with no corresponding increase in cortisol levels (Kannangara et al., 2009). Here, we predicted that zebrafish subjected to 6-months of developmental, or 2-wk social isolation would be under chronic stress (long-term, continuous exposure; Lupien and McEwen, 1997), while animals exposed to novelty in the form of an unfamiliar social group would be under a 'non-stressful' condition. Our results show clearly

that changes in the complex social environment of zebrafish (i.e., isolation or novelty) are associated with distinct physiological changes in cortisol levels that occur independently of changes in proliferation rates within neurogenic niches.

By assaying endogenous whole-body cortisol levels in group-raised zebrafish subjected to social change via a shift to acute (1-h) or long-term social isolation (2-wk) or novelty (2-wk) beginning at 6-months, we show that changes in the social environment are consistently associated with significantly lower cortisol levels compared with grouped controls; refuting our prediction [see Fig. 8(B,C)]. These data reveal that the physiological response to cortisol can be triggered similarly under contexts perceived as deprived or enriched, unlike the situation in mammals where social isolation is often correlated with elevated cortisol levels and reduced hippocampal neurogenesis (Rizzi et al., 2007; Perez et al., 2012). Studies examining social status in zebrafish (Filby et al., 2010; Dahlbom et al., 2011; Pavlidis et al., 2011) and African cichlids (Maruska et al., 2012, 2013) have proposed that elevated cortisol levels in grouped animals may be explained by social competition for food and mates, and dominant or subordinate status, that together could increase anxiety- or vigilance-related behaviors in fish. Within populations of African cichlids, holding a dominant position is associated with elevated cortisol levels and proliferation rates, while the inverse is true in subordinates (Maruska et al., 2012, 2013). By contrast, subordinate, not dominant status within zebrafish hierarchies have been correlated with elevated levels of plasma cortisol (Filby et al., 2010), implying that the impact of social status on activation of the hypothalamus-pituitary-interrenal (HPI) axis may differ between taxonomically distinct Families. While not directly examined in our study, it is likely that group-raised controls formed established social hierarchies over a 6-month period. Of our groups of six fish, most would be of subordinate status, and thus the probability of transferring a subordinate compared with a dominant into a social isolation or novelty context would be much greater. Our data suggest that removal of stressors associated with social status in zebrafish during adulthood correlate, at least temporarily with decreases in cortisol, and either a decrease or no change in the proliferative population in sensory and telencephalic niches, respectively.

Given that zebrafish do not depend on parental care for development, we additionally posited whether long-term social isolation beginning at 5 dpf and continuing until 6-months might be correlated with lower cortisol levels compared with short-term isolation during adulthood. Unexpectedly, we found that long-term developmental social isolation had nearly identical cortisol levels as group-raised controls [see Fig. 8(A)], further substantiating that cortisol levels respond in accordance with the social context and are dissociated from the neurogenic response, as illustrated by significantly greater rates of cell proliferation in group-raised animals compared with isolates in PGZ and OB [see Figs. 2(C) and 3(B)]. This view is additionally supported by our hydrocortisone injection experiments that displayed no effect on the size of the proliferative population within neurogenic niches. We note, however, that our results are not consistent with earlier observations in the dentate gyrus of mammals that show decreases in rates of cell proliferation following direct injections of synthetic cortisol (Gould et al., 1992; Cameron and Gould, 1994). One possibility for this may be that the stress of the injection procedure itself (i.e., hydrocortisone or vehicle) might have led to a widespread decrease in the number of $BrdU^+$ cells within niches and therefore differences between control and hydrocortisone groups went undetected. This appears unlikely however, since although group-raised animals did display decreases in the number of $BrdU^+$ cells compared with noninjected wildtype animals that were used to examine stages of adult neurogenesis, the number of $BrdU^+$ cells in most niches following 2-wk adult social isolation (injected vs. noninjected) were nearly identical.

One physiological mechanism that may have blocked the effect of hydrocortisone on the stem/progenitor population in zebrafish is upregulation of type 2, 11β-hydroxysteroid dehydrogenase (Hsd11b2) that converts active glucocorticoids to their inactive derivatives (Alderman and Vijayan, 2012). Not only does the spatial distribution of *hsd11b2* mRNA overlap with adult neurogenic niches in the zebrafish brain, but acute stress is sufficient to upregulate Hsd11b2 activity, implying a role in negative feedback regulation of cortisol following activation of the teleost HPI axis. At present, it remains unclear as to why such a disparity exists between cortisol levels following long-term developmental social isolation compared with isolation at adulthood. A possible explanation may be that long-term deprivation of stimuli is equally as stressful as long-term subordinate status within a social hierarchy.

CONCLUSIONS

Using adult zebrafish, we have investigated how the social context can differentially shape neuronal

production in niches residing within sensory and telencephalic structures and whether cortisol is involved in regulating these changes. Our study leads to the following major observations: (1) there is a robust negative effect of social change on the proliferation of stem/progenitor cells and the survival of the BrdU-label-retaining population, primarily in sensory regions. In contrast, social change can have a positive effect on neuronal differentiation in both sensory and telencephalic niches of the brain; (2) within a single neurogenic niche, the same change in social context can uncouple (i.e., increase or decrease) distinct stages of adult neurogenesis; (3) experience from a preceding social context is associated with how cortisol levels will respond under a new social environment; (4) social enrichment does not consistently rescue deficits in cell proliferation following isolation, and cortisol levels correlate with changes in the social context independently of the neurogenic response and are not sufficient to drive changes in adult neurogenesis. Collectively, our findings demonstrate region-specific adaptive neurogenic plasticity in the adult zebrafish brain in response to social change.

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