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Fish Neurogenesis in Context: Assessing Environmental Influences on Brain Plasticity within a Highly Labile Physiology and Morphology

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Key Words

Fish · Neurogenesis · Cell proliferation · Environment · Brain plasticity · Behavioral plasticity

Abstract

Fish have unusually high rates of brain cell proliferation and neurogenesis during adulthood, and the rates of these processes are greatly influenced by the environment. This high level of cell proliferation and its responsiveness to environmental change indicate that such plasticity might be a particularly important mechanism underlying behavioral plasticity in fish. However, as part of their highly labile physiology and morphology, fish also respond to the environment through processes that affect cell proliferation but that are not specific to behavioral change. For example, the environment has nonspecific influences on cell proliferation all over the body via its effect on body temperature and growth rate. In addition, some fish species also have an unusual capacity for sex change and somatic regeneration, and both of these processes likely involve widespread changes in cell proliferation. Thus, in evaluating the possible behavioral role of adult brain cell proliferation, it is important to distinguish regionally specific responses in behaviorally relevant brain nuclei from global proliferative changes across the whole brain or body. In this review, I first highlight how fish differ from other vertebrates, particularly birds and mammals, in ways that have a bearing on the interpretation of brain plasticity. I then summarize the known effects of the physical and social environment, sex change, and predators on brain cell proliferation and neurogenesis, with a particular emphasis on whether the effects are regionally specific. Finally, I review evidence that environmentally induced changes in brain cell proliferation and neurogenesis in fish are mediated by hormones and play a role in behavioral responses to the environment.

Introduction

Compared to mammals, fish have vastly greater rates of cell proliferation in the adult brain. For example, it is estimated that the brain of an electric fish, i.e. *Apteronotus leptorhynchus*, generates new cells at rates ~10–100 times greater than the brain of rodents [Zupanc and Horschke, 1995; Zupanc, 2006]. On top of this phylogenetic influence on proliferative capacity, the environment causes brain cell proliferation within individual fish to vary at almost the same magnitude. For example, within the brain of another electric fish, i.e. *Brachyhypopomus gauderio*, cell proliferation rates vary by 6- to 25-fold depend-

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ing on the physical and social environment [Dunlap et al., 2011b]. This high level of brain cell proliferation combined with the high degree of responsiveness to environmental change indicates that cell addition or turnover is a prominent process in brain plasticity and might be a particularly important mechanism in behavioral responses to environmental change in fish. However, an understanding of the behavioral function or adaptive significance of such brain plasticity in fish must be developed in the context of their broader biology, which is characterized by a greater lability than that of birds and mammals.

In this review, I first discuss several features of fish biology that make them different from other vertebrates, especially birds and mammals, and that have a bearing on understanding their brain plasticity. I then summarize the environmental influences on fish brain cell proliferation and neurogenesis and review evidence that these changes are regulated by hormones and have behavioral correlates.

Brain Cell Proliferation in Context

Most of what we know about environmental influences on adult neurogenesis comes from studies of mammals and birds [Barker et al., 2011; Opendak and Gould, 2015]. Plasticity in the brains of these animals happens in the context of a relatively stable physiology and adult morphology. Most mammals and birds are endotherms whose body temperatures are relatively warm and constant. Their body size and sex are fixed in adulthood, and they show little capacity for regeneration of new body parts following injury. This stable internal background makes it relatively simple to identify neurogenic changes that are tied to specific environmental changes and consequent behavioral responses. For example, given the observation in some birds that seasonal changes in neurogenesis in song nuclei correlate positively with seasonal song production, one can be fairly confident that seasonal changes in the environment (e.g. day length) have specific effects on the brain and vocal behavior [Kirn, 2010].

By contrast, fish are generally more labile and dynamic in their physiology and adult morphology. Most fish are ectotherms whose body temperature fluctuates according to the thermal environment. Most species grow continuously during adulthood (indeterminate growth), some species change sex or switch phenotypes as adults, and, in general, fish have a great capacity for regeneration [Maginnis, 2006], including in their central nervous system [Zupanc, 2006]. (Many of these features of fish biology are also shared by amphibians and reptiles, but in this review I focus only on fish.) With this high degree of background variability, it is more difficult to associate neurogenic changes with specific behavioral responses to the environment since such changes may result from the environment acting though indirect, nonspecific changes in body temperature or growth rate and sometimes sex change or injury. Below, I provide examples showing how fish's more labile biology has implications for the detection and interpretation of environmentally induced neurogenic changes that may drive behavioral change.

Temperature

As mentioned above, several authors have attempted to correlate seasonal changes in neurogenesis and behavior in diverse vertebrates [Barnea and Nottebohm, 1994; Vidal Pizarro et al., 2004; Maine et al., 2014; Sherry and MacDougall-Shackleton, 2015]. In endotherms, one can generally rule out the possibility that seasonal changes in adult neurogenesis are due to direct effects of temperature changes on the brain. However, in ectotherms, including fish, seasonal changes in the brain might be largely (or entirely) attributable to seasonal changes in temperature since mitotic rates depend on body temperature [Rieder and Cole, 2002; Radmilovich et al., 2003] and body temperature varies with the thermal environment. So, for example, while it may be tempting to link elevated neurogenesis in the spring to changes in reproductive behavior, such seasonal change may be due to overall warmer body temperatures that increase mitotic rates all over the body in the spring rather than a specific mechanism facilitating reproductive behavior.

Growth

Some authors have correlated population differences in brain cell proliferation with environmental differences in harshness [Chancellor et al., 2011] and climate [Galea et al., 1994]. In animals with determinate growth, such as most birds and mammals, population comparisons of neurogenesis are relatively simple since all adults, regardless of the population, grow very little, and population differences are thus likely an effect of the environment acting specifically on the brain rather than acting through differential overall growth rate among populations. However, in animals with indeterminate growth, such as most fish, populations may differ substantially in adult growth rate, and variation in brain cell proliferation could be a by-product of a more general environmentally (or genetically) related variation in the growth rate of the whole body.

Sex and Behavioral Morph

In birds and mammals, researchers have correlated sex differences in neurogenesis with sex differences in behavior [Nordeen and Nordeen, 1988; Galea et al., 1994; Falconer and Galea, 2003]. In these cases, it is commonly assumed that embryonic sex determination leads to sex-specific patterns of hormone secretion in the adult, which subsequently cause differential neurogenesis in the adult. Differences between males and females can be attributed to their genetic sex or adult hormone profile. However, in some teleosts, sex can change during adulthood as a response to changes in the social environment [Perry and Grober, 2003]. Thus, sex differences in neurogenesis could arise via a direct action of social stimuli on the brain or secondarily through gonadal differentiation and changes in hormone secretion. Moreover, some fish switch sex with age (sequential hermaphrodotism) [Zikopoulos et al., 2000; Frisch, 2004], so sex differences in neurogenesis might result from age-related changes rather than sex per se. Finally, in some species, males are polymorphic in morphology and behavior and can switch between morphs in response to change in the social environment [Oliveira et al., 2001; Maruska et al., 2012]. Often, behavioral changes are accompanied by changes in growth rate whereby fish that were formerly subordinate become both more aggressive and larger. So, neurogenic differences between behavioral morphs could be specific to behavioral change or secondary to changes in the overall growth rate.

Regeneration

The neurogenic response to somatic injury, such as that produced by sublethal encounters with predators, has received little attention. However, it is clear that such injury can lead to dramatic changes in behavior, and some authors have tied these behavioral effects to stress-induced changes in neurogenesis [Dimitrov et al., 2014]. In birds and mammals, which have relatively little capacity to regenerate, one can assess injury-induced changes in brain cell proliferation separately from changes in somatic cell proliferation since injury does not cause a prolonged period of cell proliferation at the wound site. However, in highly regenerative taxa, such as fish, changes in the brain following somatic injury occur within the context of elevated cell proliferation in the regenerating tissue. Thus, brain changes might result from the regenerative process rather than from the injury per se. For example, the response to injury might increase proliferative rates all over the body, including both the site of regeneration and the brain [Ilieş et al., 2014], through a generalized response, or it might inhibit brain cell proliferation if there is a trade-off between mitotic rates among tissues.

Many of the ambiguities raised above can be addressed by assessing the specificity of the environmental effect on brain regions. Global proliferative changes across the brain are more likely a consequence of nonspecific influences through body temperature, growth, sex change, or injury, while regionally specific changes in the brain are more likely responses to specific environmental changes and thereby more likely adaptive. Consequently, it is especially important in fish to assess brain cell proliferation in relation to the whole brain and to somatic growth. Below, I review environmental influences on brain cell proliferation and neurogenesis, with a particular emphasis on whether the effects are regionally specific within the brain. A summary of the studies is also presented in table 1.

Overview of Environmental Influences on Brain Cell Proliferation and Neurogenesis

Scope Environmental Influence

Just how plastic is the fish brain? The scope of environmental influences on brain cell proliferation was estimated quantitatively in studies of a temperate electric fish species, i.e. *B. gauderio,* examined in both field and captive environments during both the breeding and the nonbreeding seasons [Dunlap et al., 2011b]. Fish living in the wild during the breeding season had rates of brain cell proliferation that were ~6–25 times greater than those of isolated fish living in captivity with temperature and light regimes that mimicked the nonbreeding season. (Only the midbrain and hindbrain were examined.) The great majority of the variation in proliferation rates was expressed globally across the brain, with ~60% of the variation related to season and likely due to strong effects of seasonal changes in water temperature, and ~10% of the variation related to environmental complexity (field vs. captivity; the remaining 30% variation was unaccounted for).

A regionally specific effect on brain cell proliferation became evident in a third treatment group, in which fish were housed in social groups in large, outdoor, seminatural enclosures under conditions that promote reproductive activity. This treatment allowed us to distinguish the effect of social stimuli, reproductive behavior/physiology, and small-scale physical enrichment. Group interaction and physical enrichment had no global effects across the brain but rather had a regionally specific effect in brain

Table 1. Summary of environmental influences on brain cell proliferation and neurogenesis and its hormonal control in teleost fish

Species	Environmental stimuli	Comparison group	Brain regions examined	Cell marker	Cellular change	Hormonal association	Reference
Cyprinoformes *Danio rerio*	Isolation	Group housing	Sensory niches: OB, PGZ, LX; telencephalic niches: Dd, V, Dm	BrdU/ 2 h, 28-day survival	High CP in sensory niches, unaffected in the Telenceph; high NG in the Telenceph; generally unaffected in sensory niches	Cort changes by treatment, but not associated with CP or NG	Lindsey and Tropepe, 2014
	Social novelty	Constant social group	Same as above	Same as above	Low CP in most sensory niches and unaffected in the Telenceph; high NG in the PGZ and Dl	Same as above	Lindsey and Tropepe, 2014
	Chemostimulants	Controlled chemical exposure	Olfactory structures (OB, LX); visual structures (PGZ, TL)	Same as above	CP unaffected; high NG in olfactory but not visual regions	ND	Lindsey et al., 2014
	Bright light	Dim light	Same as above	Same as above	High CP in visual but not olfactory regions; NG unaffected	ND	Lindsey et al., 2014
	Enriched physical environment	Barren environment	Forebrain	PCNA	High CP	High Cort	von Krogh et al., 2010
Carassius auratus	Males exposed to female pheromones	No odor and nonpheromonal odor	Many forebrain, midbrain, and hindbrain regions	BrdU/ 4-hour survival	High CP only in the midbrain	High brain GnRH mRNA and plasma androstenedione	Chung-Davidson et al., 2008
Gymnotiformes *Apteronotus leptorhynchus*	Paired social interaction	Isolated	Midbrain PVZ	BrdU/ 4-day survival	High CP and NG	High Cort	Dunlap et al., 2006
	Novel social partners	Constant social partner	Same as above	Same as above	High CP	ND	Dunlap and Chung, 2012
	Electro-communication signal	Sinusoidal electrical stimulus	Same as above	Same as above	High CP and NG	ND	Dunlap et al., 2008
Brachyhypopomus gauderio	Breeding season in the wild	Nonbreeding season in the wild	Midbrain and hindbrain	BrdU/ 2-hour survival	High CP globally across the brain	High 11kT	Dunlap et al., 2011b
	Wild environment	Captive group housing	Same as above	BrdU/ 2-hour survival	Same as above	Same as above	Dunlap et al., 2011b
	Captive group housing	Captive isolation	Same as above	BrdU/ 2-hour survival	High CP only in regions associated with electrocommunication	Same as above	Dunlap et al., 2011b
	Predator simulation in captivity	Undisturbed in captivity	Forebrain (Dl, Dm, V) and midbrain PVZ	PCNA	Low CP in forebrain, no effect in midbrain	ND	Dunlap et al., in preparation
	Tail amputation in captivity	Intact tail in captivity	Same as above	PCNA	Same as above	ND	Dunlap et al., in preparation
Brachyhypopomus occidentalis	High predator exposure in the wild	Low predator exposure in the wild	Same as above	PCNA	Same as above	Cort not related to predators or CP	Dunlap et al., 2016
	Tail injury in the wild	Intact in the wild	Forebrain (Dl, Dm, V)	PCNA	Low CP in the forebrain	Same as above	Dunlap et al., 2016
Perciformes *Astatotilapia burtoni*	Subordinate	Dominant	Forebrain (V, POA), midbrain (NRL, Cpt TPp), Cerebel	BrdU/ 24-hour survival	Low CP in all regions	ND	Maruska et al., 2012
	Subordinate	Isolated	Same as above	Same as above	Low CP in all regions	ND	Maruska et al., 2012

Table 1 (continued)

Species	Environmental stimuli	Comparison group	Brain regions examined	Cell marker	Cellular change	Hormonal association	Reference
Salmoniformes *Oncorhynchus kisutch* (juvenile)	Complex structure; variable water flow velocity	Simple structure; uniform water flow velocity	Forebrain (Dl, Dm)	BrdU/ 14-hour survival	Low CP in the Dm; no effect in the Dl	ND	Lema et al., 2005
Oncorhynchus mykiss	Mild social stress	Undisturbed	Forebrain, Hypothal, Cerebel, brain stem, optic lobes	PCNA	No difference	Small increase in Cort	Sorensen and Overli, in preparation
	Chronically subordinate	Socially dominant	Forebrain	BrdU/ 24-hour survival	Low CP across the forebrain	High Cort; low brainstem 5-HT	Sorensen et al., 2012
	Acute confinement	Undisturbed	Forebrain, Hypothal, Cerebel, optic tectum	PCNA, NeuroD, DCX mRNA	High CP in the Hypothal; no change in NG	High Cort	Johansen et al., 2012
	Long-term social stress	Undisturbed	Same as above	Same as above	Low CP in the Hypothal, Cerebel, and optic tectum; low NG in the Cerebel	High Cort	Johansen et al., 2012
Salmo salar	Enriched and dynamic structure	Simple structure	Forebrain	NeuroD mRNA	High NG	ND	Salvanes et al., 2013
	Smolts	Parr	POA	BrdU/ 70- to 120-day survival	High CRF NG	High thyroxine	Ebbeson et al., 2011
	Natural photoperiod promoting smoltification	Constant light conditions inhibiting smoltification	POA	BrdU/ 70-day survival	High CRF NG	Same as above	Ebbeson et al., 2011

BrdU = Bromodeoxyuridine; Cerebel = cerebellum; Cort = cortisol; CP = cell proliferation; Cpt = central posterior thalamic nucleus; Dd = dorsal zone of the dorsal telencephalon; Dl = dorsolateral telencephalon; Dm = dorsomedial telencephalon; 5-HT = 5-hydroxytryptamine; Hypothal = hypothalamus; 11kT = 11-ketotestosterone; LX = vagal lobe; ND = no data; NG = neurogenesis; NRL = nucleus of the natural recess; OB = olfactory bulb; PCNA = proliferating cell nuclear antigen; PGZ = periventricular grey zone; POA = preoptic area; PVZ = periventricular zone; Telenceph = telencephalon; TL = torus longitudinalis; TPp = periventricular nucleus of the posterior tuberculum; V = ventral telencephalon.

areas that participate in electrocommunication (both generation and reception of electric communication signals). Thus, the scale of the environmental variation paralleled the effect on the brain: large-scale variations in habitat complexity and seasonal temperature yielded a global, nonspecific proliferative response, while small-scale manipulation of the physical and social environment produced regionally specific responses.

Physical Environment

Studies of several fish species have demonstrated that brain cell proliferation is stimulated by changes in the physical environment. In zebrafish, i.e. *Danio rerio,* enhancement of the small captive environment with plastic plants and gravel increased forebrain cell proliferation [von Krogh et al., 2010] and, in Atlantic salmon *(Salmo salar)*, a similar enrichment that was also modified temporally increased the expression of NeuroD mRNA, a marker of early neuronal differentiation [Salvanes et al., 2013]. Neither of these studies distinguished between regions within the forebrain or in nonforebrain regions, so it is not clear whether the effect was specific. However, since fish in both treatments grew to the same body size, it appears that the effect of the environment was not a by-product of differential growth.

A study on juvenile Coho salmon, i.e. *Oncorhynchus kisutch,* showed a regionally specific effect of the physical environment within the forebrain [Lema et al., 2005]. Fish living in large enclosures that were structurally simple but with complex water flow regimes had greater rates

of cell proliferation in the dorsomedial telencephalon, but not in the dorsolateral telencephalon. These results demonstrated that structural complexity can sometimes inhibit brain cell proliferation or that hydrodynamic complexity has an overriding stimulatory effect in specific regions. Fish living in the more structurally complex environment also grew to a smaller body size, and so it is unclear whether this lower overall growth rate may also have contributed to the reduction of cell proliferation in certain brain regions.

In Atlantic salmon *(S. salar),* the physical environment has a potent stimulatory effect on the neurogenesis of corticotropin-releasing factor (CRF)-containing neurons as part of a larger effect on smoltification [Ebbesson et al., 2011]. The transition from the smolt to parr stages as fish migrate from fresh to salt water is regulated by both day length and temperature, and it is accompanied by an elevated production of CRF neurons in the preoptic area of the midbrain. Housing fish under constant light conditions that inhibited smoltification also inhibited CRF neurogenesis. The specificity of this effect is unknown since the authors did not report data on other brain regions or the number of newborn cells in the preoptic area that did not express CRF.

Social Environment

Social influences on brain cell proliferation have received the most attention, but studies vary widely in the form of social stimuli that is presented. In electric fish, i.e. *A. leptorhynchus,* Dunlap et al. [2006, 2013] examined the effect of same-sex dyadic interaction (7 days) after a long period of social isolation. Fish housed in pairs had ~4 times more cell addition (cell birth plus 4 day survival) in the midbrain than fish that remained isolated. The effect was regionally specific, occurring in the periventricular zone that contributes cells to a brain region (the prepacemaker nucleus) functionally important in electrocommunication, but not in adjacent axial regions of the midbrain [Dunlap et al., 2006] or in the forebrain [Dunlap et al., in preparation]. This effect habituated after 14 days with the same social partner but was reversed and further enhanced by the sequential presentation of novel social partners [Dunlap and Chung, 2012]. This study demonstrated that social change, rather than the mere presence of a conspecific, is the effective stimulus for the enhancement of brain cell addition in a region-specific manner.

In zebrafish, i.e. *D. rerio,* Lindsey and Troupe [2014] transferred fish from long-term social groups into isolation to reduce social stimuli or into novel social groups to enhance social stimuli. They examined cell proliferation and neurogenesis in sensory and telencephalic niches. Social isolation inhibited cell proliferation in sensory regions, but it had no effect on telencephalic regions. Isolation had mixed effects on neurogenesis but generally had a stimulatory effect on the telencephalon and no effect on sensory niches. By contrast, social novelty generally suppressed cell proliferation in sensory niches, with little effect on the telencephalon and enhanced neurogenesis in one sensory and one telencephalic region. Overall, social stimuli appeared to exert some degree of regional specificity on the brain of zebrafish, but the effects were rather complex.

In the cichlid *Astatotilapia burtoni,* which can rapidly switch phenotypes according to the social context, Maruska et al. [2012] examined the influence of social rank on cell proliferation by comparing isolated fish in the dominant phenotype with group-housed fish that were subordinate, dominant, or switching from the subordinate to the dominant phenotype. Subordinate fish had a lower brain cell proliferation than dominant phenotypes (both those in social groups and those in isolation). However, the dominant phenotype in social groups and in isolation had equivalently high levels of cell proliferation, indicating that social interaction suppressed cell proliferation in subordinates but had no effect on dominants. Fish ascending in rank had levels intermediate between dominants and subordinates. These researchers examined 5 brain regions across the midbrain and forebrain and found equivalent responses in all regions, indicating that social interaction had a global effect across the brain. This suggests that the enhanced proliferation in the transition between behavioral morphs was likely due to broad generalized effects of the social environment on the growth of the whole body (or brain) rather than a mechanism contributing to changes in social behavior.

Social rank was also studied in rainbow trout, i.e. *Oncorhynchus mykiss.* Short-term social subordination (mild social stress) did not affect cell proliferation, but chronic subordination decreased forebrain cell proliferation [Sorensen et al., 2012]. Notably, in isolated fish, the density of proliferating cells was correlated positively with growth rate, indicating that forebrain cell dynamics are tied to body growth. However, in socially interacting fish, this correlation disappeared, suggesting that the influence of the social environment overrides the background connection between overall growth and brain cell proliferation. Regional specificity within the brain was not examined in this study, but similar long-term exposure to subordination reduced the cell proliferation (PCNA mRNA expression) in the hypothalamus, cere-

bellum, and optic tectum, but not in the telencephalon, and it reduced neurogenesis (NeuroD mRNA expression) only in the cerebellum [Johansen et al., 2012]. Researchers have proposed that the variability in the proliferative response to social interaction (negative effect vs. no effect) is part of a biphasic effect of stress on the brain, with the severity and duration of stress determining the direction and magnitude of the altered brain cell plasticity [Sorensen et al., 2013].

Sex Change

Some fish have the extraordinary capacity to change gonadal sex during adulthood and undergo a concomitant change in sexual behavior. Le Page et al. [2010] argued that this plasticity in sexual behavior is functionally linked to the extension of widespread neurogenesis beyond the embryonic period into adulthood. Moreover, cell proliferation rates in some fish are highly influenced by estrogens and their production via aromatization in neural progenitors (radial glia) suggesting that regulation of cell production may contribute to hormonally driven changes in adult sexual behavior.

Zikopoulos et al. [2000] examined brain cell proliferation in the sea bream, i.e. *Sparus aurata,* a sequential hermaphrodite that, between its second and third years of life, switches from male to female. During this period, it also increases in body size by about 3-fold. Given that many fish show an age-dependent decrease in proliferative rate [Tozzini et al., 2012; Edelmann et al., 2013; but see Traniello et al., 2014], one might expect that females, because they are older, would show less brain cell proliferation than males. However, females had about a 3 times greater density of proliferating cells than males in the dorsal hypothalamus, a region that regulates reproduction via gonadotropins. Sexes did not differ in proliferation rates in other brain regions (cerebellum, forebrain, and several midbrain areas). This regional specificity combined with the known function of the dorsal hypothalamus in reproduction suggests that these proliferative changes are associated causally with sex change.

Predation and Injury

Although many studies have documented the effect of predator stimuli on brain cell proliferation in laboratory rodents, little is known about their effect in other vertebrates, including fish. Even less is known about the effect of predators in natural environments, where prey can use a broader range of behaviors to avoid or evade predators. Predators add a component of 'complexity' to the environment and thus might promote brain cell proliferation or neurogenesis. Alternatively, predators might 'stress' their prey to the point of decreasing cell proliferation directly or decreasing behaviors (e.g. exploration and foraging) that otherwise promote cell proliferation.

We examined brain cell proliferation in an electric fish, i.e. *Brachyhypopomus occidentalis,* in natural populations in Panama that vary in predator density and the incidence of predator-induced tail injury [Dunlap et al., 2016]. Across all 6 populations, forebrain cell proliferation correlated negatively with predator pressure, with fish in high predator populations generating newborn cells at about half the rate of fish in low predator populations. Such population differences could arise from genetic divergence among populations or some other environmental variable (e.g. water composition or climate). However, even when comparing populations within a drainage, which are genetically and environmentally similar, fish in populations facing a greater predation pressure had lower forebrain cell proliferation than fish exposed to relatively few predators. Interestingly, this effect of predators was found at equivalent levels across 3 forebrain regions (i.e. the dorsolateral, dorsomedial, and ventral telencephalon), but it was not found at all in the midbrain. Thus, predators appear to exert specific effects across major brain regions but nonspecific effects within the forebrain.

In addition to this effect of predator density, we also found an additional effect of predator-induced tail injury, with injured fish producing newborn cells at a ~30% lower rate than intact fish. Subsequent laboratory studies on a congeneric fish, i.e. *B. gauderio,* showed that these relationships between predators and brain cell proliferation described in the field are likely causal. Experimental tail amputation decreased forebrain cell proliferation in a manner quantitatively similar to that found in naturally injured fish [Dunlap et al., in preparation]. In both field-captured fish and experimentally amputated fish, the tail had regrown to almost half of the amputated tail segment. Thus, it is not clear whether the injury or the process of regeneration causes the inhibition of forebrain cell proliferation.

Modality-Specific Environmental Influences

The studies described above examined the proliferative and neurogenic response to complex, multimodal stimuli. In complementary studies, researchers have asked how the brain responds to selected components of the physical and social world coming through single sensory modalities. Lindsey et al. [2014] found that zebrafish, i.e. *D. rerio,* showed proliferative and neurogenic responses that were specific to sensory modality, brain re-

gion, and neurogenic phase (i.e. proliferation vs. neuronal differentiation). Fish presented with chemostimulants had an elevated cell proliferation in olfactory brain regions, but not in visual brain regions, and neurogenesis was unaffected. Conversely, fish presented with light of different intensities showed an elevated neurogenesis in visual brain regions, but not in olfactory regions, and cell proliferation was not affected.

Unimodal components of the social environment can also have specific effects on brain regions contributing to social behavior. For example, in goldfish, i.e. *Carassius auratus,* female pheromones but not other odorants have a specific effect on the midbrain and not on other brain regions [Chung-Davidson et al., 2008]. Similarly, electric fish *(A. leptorhynchus)* presented with a real conspecific electric signal had an elevated cell addition in a midbrain proliferative zone. An artificial sinusoidal electric signal of identical frequency had no effect [Dunlap et al., 2008]. Thus, unimodal electric stimuli are sufficient to elevate cell addition, but fine-scale details of the electrocommunication signal (e.g. its waveform or amplitude modulation) are necessary stimulus components for the promotion of cell addition.

Interspecific comparisons also suggest modality-specific effects on brain cell proliferation. Within the killifish genus *Austrolebias,* a species *(A. affinis)* that relies most heavily on visual communication during courtship has an especially high rate of cell proliferation in visual areas of the brain (optic tectum and torus longitudinalis) and a low rate in the olfactory bulb, while another species that uses chemical signals *(A. reicherti)* in courtship shows the reverse pattern [Fernández et al., 2011]. Thus, during the evolution of this clade, it appears that brains are most plastic in regions that have the most crucial functions in sexual behavior.

Are Environmentally Induced Changes in Brain Cell Proliferation Mediated by Hormones?

Many studies have focused on glucocorticoids as physiological mediators linking environmental stimuli and brain cell proliferation; however, the results are rather inconsistent. In some cases, fish follow the pattern originally described in laboratory rodents, in which environmental stressors elevate plasma glucorticoids, which then suppress brain cell proliferation [Mirescu and Gould, 2006]. For example, when trout *(O. mykiss)* were exposed to chronic social stress, plasma cortisol levels increased and forebrain cell proliferation decreased [Sorensen et al., 2007, 2012, 2013]. Cortisol treatment in unstressed fish similarly decreased forebrain cell proliferation [Sorensen et al., 2011].

In other cases, enhanced cell proliferation or neurogenesis is associated positively with glucocortoid secretion. Electric fish *(A. leptorhynchus)* housed in conspecific pairs had increased plasma cortisol and midbrain cell addition compared to isolated fish [Dunlap et al., 2002, 2006]. Exogenous cortisol given to isolated fish increased cell addition, and pharmacological blockade of glucocorticoid receptors partially blocked the proliferative response to social interaction [Dunlap et al., 2006, 2011a]. Similarly, zebrafish exposed to an enhanced physical environment simultaneously displayed increased plasma cortisol and forebrain cell proliferation [von Krogh et al., 2010]. Interestingly, in trout *(O. mykiss),* short-term stress caused by an aggressive conspecific increased forebrain and hypothalamic cell proliferation, and genetic lines selected for high stress responsiveness showed elevated rates of forebrain (and hypothalamic) neurogenesis compared to those with low stress responsiveness [Johansen et al., 2012; Sorensen et al., 2013]. So, in fish, it appears that cortisol sometimes contributes positively to the environmental enhancement of cell proliferation or neurogenesis.

In yet other fish, brain cell proliferation is unrelated to plasma cortisol. In zebrafish, social manipulations (isolation and novelty) altered whole-body cortisol levels, but these changes were independent of proliferative or neurogenic responses to social stimuli, and exogenous cortisol did not affect the brain cell proliferation or neurogenesis [Lindsey and Tropepe, 2014]. In free-living electric fish *(B. occidentalis),* capture stress significantly elevated cortisol without affecting cell proliferation [Dunlap et al., 2016]. Moreover, cortisol levels among populations varied by over 2-fold, but there was no relationship between this cortisol variation and predator pressure or brain cell proliferation across populations or across individuals.

Studies on smoltification in salmon *(S. salar)* showed that thyroid hormone can play a role in environmentally induced changes in neurogenesis [Ebbesson et al., 2011]. Endogenous thyroxine levels rose during the natural smolt-to-parr transition and the accompanying period of elevated CRF neurogenesis. Thyroxine treatment in fish that otherwise would not undergo smoltification also increased CRF neurogenesis. Thyroxine is part of a network of endocrine changes that regulate smoltification, so it might act directly to promote CRF neurogenesis or indirectly via other hormones (e.g. cortisol, growth hormone, and prolactin).

Are Adult Brain Cell Proliferation and Neurogenesis Related to Behavior?

Much of the current interest in adult neurogenesis centers on whether this form of brain plasticity is a possible mechanism of environmentally induced behavioral plasticity [Glasper et al., 2012; Opendak and Gould, 2015]. In mammals, researchers have developed methods to experimentally manipulate neurogenesis to determine the causal role of adult-born neurons in behavioral change [Aimone et al., 2014]. However, because such methods are not yet available for fish studies, we can only assess this idea through correlations between neurogenesis and behavior.

Studies in juvenile salmon *(S. salar)* indicate that enhanced forebrain neurogenesis in response to an enriched physical environment may play a role in spatial learning. Fish living in a complex and dynamic spatial structure showed elevated forebrain neurogenesis (NeuroD mRNA) while simultaneously improving in their spatial learning ability [Salvanes et al., 2013]. Since the teleost forebrain is known to regulate spatial orientation and contains the likely homolog of the mammalian and avian hippocampus, it appears that fish, like other vertebrates, may use neurogenesis as a mechanism underlying spatial learning.

Environmentally induced changes in forebrain cell proliferation also correlate with change in 'boldness' and foraging behavior. Among socially interacting trout, subordinate fish, which also showed low rates of cell proliferation, hesitated to approach food and ate less during feeding periods compared to dominant and isolated fish [Sorensen et al., 2012]. Similarly, salmon [Salvanes et al., 2013] and zebrafish [von Krogh et al., 2010] raised in simple environments that inhibited forebrain cell proliferation were more tentative in venturing out of shelters or seeking food than fish raised in a complex environment that promoted cell proliferation. These negative correlations between forebrain cell proliferation and boldness parallel those found in rodents, where an experimental reduction of hippocampal neurogenesis inhibits exploratory behavior and induces anxiety behavior [Revest et al., 2009]. Given that predator exposure also inhibits forebrain cell proliferation in fish [Dunlap et al., 2016], it appears that suppression of cell proliferation may promote a suite of cautious behaviors that make them adaptively more wary in threatening environments.

Other evidence for a behavioral role of neurogenesis comes from electric fish *(A. leptorhynchus)* social behavior [Dunlap et al., 2013]. Fish exposed to long-term social interaction showed potentiation of an aggressive signaling behavior termed chirping. Coinciding with this behavioral change was an elevation in cell addition in the proliferative zone that contributes cells to the nearby brain region (prepacemaker nucleus) controlling chirping. About 60% of these neurons differentiate into neurons and some eventually reside in the prepacemaker nucleus. Many experimental treatments that alter this cell addition, including social novelty [Dunlap and Chung, 2012], glucocorticoid manipulations [Dunlap et al., 2006, 2011a], and unimodal electrocommunication stimuli [Dunlap et al., 2008], simultaneously alter the chirping behavior. These strong correlations suggest that cell addition to the prepacemaker nucleus contributes to changes in chirping behavior, perhaps enabling fish to recalibrate their aggressive electric signaling in response to changes in the social environment. However, it is possible that neurogenic changes are the consequence rather than the cause of behavioral changes, with chirping behavior driving prepacemaker cell addition via an activity-dependent mechanism [Dunlap et al., 2013].

In mammals, exercise has a potent stimulatory effect on neurogenesis. In fish, several authors have assessed whether environmental changes, such as social interaction or structural enrichment, that alter neurogenesis might exert their effects by altering locomotion. So far, this does not appear to hold true. The elevated cell proliferation in zebrafish was accompanied by less locomotor activity [von Krogh et al., 2010], and socially induced cell addition in electric fish [Dunlap et al., 2011a] was unrelated to swimming behavior.

The discussion above has emphasized making connections between specific environmental changes and behaviors with region-specific cell proliferation. However, it is possible that an elevated level of global cell proliferation caused by environmentally induced changes in physiology (e.g. body temperature or growth rate) may nonetheless have important behavioral consequences by providing the raw material for using neurogenesis as a mechanism of behavioral plasticity. By analogy, just as the generalized overproduction of synapses in early brain development enables subsequent experience to selectively sustain or eliminate specific neuronal connections, the abundant and widespread production of cells in the adult fish brain may enable them to tie major life transitions (e.g. seasonal reproduction, migration, injury, or sex change) to behavioral changes. Regardless of whether new cells originate in a global or regionally specific pattern, they could equally participate in behavioral change if they incorporate into neural circuits regulating behavior.

Future Directions

As argued above, environmental regulation of cell proliferation in fish happens in the context of a temperature-dependent physiology and indeterminate growth, two important features that influence cell proliferation all over the body, and in the context of overall high proliferative rates across the brain. To assess the behavioral significance of neurogenic responses, future studies should include one or more control regions of the brain to evaluate the specificity of the environmental effect. In addition, the context of specific environmental effects will be clearer if researchers also control for or report the water temperature and, when possible, include information on body growth rates.

Although most fish species grow indeterminately, some show determinate growth. For example, even within the genus *Danio, D. aequipinnatus* grows continuously while *D. rerio* stops growing early in adulthood [Biga and Goetz, 2006]. Comparing such species could help to clarify how environmental regulation of cell proliferation operates within the overall context of continuous growth of the body and brain.

Many studies have demonstrated environmentally induced variation in fish brain size [Chapman and Hulen, 2001; Pollen et al., 2007; Gonda et al., 2012, 2013; DePasquale et al., 2016]. In many cases, the effect is regionally specific within the brain and evident in experimental manipulations, population comparisons, and interspecific comparisons. Future studies can now address how such environmentally induced variation in brain size is generated through differential regulation of adult brain cell proliferation.

Cell proliferation also occurs in the context of cell death. Very little is presently known about the environmental regulation of apoptosis in fish brains, but clearly the cellular composition of the brain is an equal product of cell birth and cell death. Future studies should clarify whether and when the environment shapes brain structures by differentially regulating cell birth and death or by altering the neuronal turnover through modification of cell birth and death in the same direction.

References

Aimone JB, Li Y, Lee SW, Clemenson GD, Deng W, Gage FH (2014): Regulation and function of adult neurogenesis: from genes to cognition. Physiol Rev 94:991–1026.

Barker JM, Boonstra R, Wojtowicz JM (2011): From pattern to purpose: how comparative studies contribute to understanding the function of adult neurogenesis. Eur J Neurosci 34: 963–977.

Barnea A, Nottebohm F (1994): Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. Proc Natl Acad Sci USA 91:11217–11221.

Biga PR, Goetz FW (2006): Zebrafish and giant *Danio* as models for muscle growth: determinate vs. indeterminate growth as determined by morphometric analysis. Am J Physiol Regul Integr Comp Physiol 291:R1327–R1337.

Chancellor LV, Roth TC, LaDage LD, Pravosudov VV (2011): The effect of environmental harshness on neurogenesis: a large-scale comparison. Dev Neurobiol 71:246–252.

Chapman LJ, Hulen KG (2001): Implications of hypoxia for the brain size and gill morphometry of mormyrid fishes. J Zool 254:461–472.

Chung-Davidson YW, Rees CB, Bryan MB, Li W (2008): Neurogenic and neuroendocrine effects of goldfish pheromones. J Neurosci 28: 14492–14499.

DePasquale C, Neuberger T, Hirrlinger AM, Braithwaite VA (2016): The influence of complex and threatening environments in early life on brain size and behaviour. Proc Roy Sci B DOI: 10.1098/rspb.2015.2564 .

Dimitrov EL, Tsuda MC, Cameron HA, Usdin TB (2014): Anxiety- and depression-like behavior and impaired neurogenesis evoked by peripheral neuropathy persist following resolution of prolonged tactile hypersensitivity. J Neurosci 34:12304–12312.

Dunlap KD, Castellano JF, Prendaj E (2006): Social interaction and cortisol treatment increase cell addition and radial glia fiber density in the diencephalic periventricular zone of adult electric fish, *Apteronotus leptorhynchus.* Horm Behav 50:10–17.

Dunlap KD, Chung M (2012): Social novelty enhances brain cell proliferation, cell survival and chirp production in an electric fish, *Apteronotus leptorhynchus.* Dev Neurobiol 73: 326–332.

Dunlap KD, Chung M, Castellano JF (2013): Influence of long-term social interaction on chirping behavior, steroid levels and neurogenesis in weakly electric fish. J Exp Biol 216: 2434–2441.

Dunlap KD, Jashari D, Pappas KM (2011a): Glucocorticoid receptor blockade inhibits brain cell addition and aggressive signaling in electric fish, *Apteronotus leptorhynchus.* Horm Behav 60:275–283.

Dunlap KD, McCarthy EA, Jashari D (2008): Electrocommunication signals alone are sufficient to increase neurogenesis in the brain of adult electric fish, *Apteronotus leptorhynchus.* Dev Neurobiol 68:1420–1428.

Dunlap KD, Pelczar PL, Knapp R (2002): Social interactions and cortisol treatment increase the production of aggressive electrocommunication signals in male electric fish, *Apteronotus leptorhynchus.* Horm Behav 42:97–108.

Dunlap KD, Silva AC, Chung M (2011b): Environmental complexity, seasonality and brain cell proliferation in a weakly electric fish, *Brachyhypopomus gauderio.* J Exp Biol 214: 794–805.

Dunlap KD, Tran A, Ragazzi MA, Krahe R, Salazar VL (2016): Predators inhibit brain cell proliferation in natural populations of electric fish, *Brachyhypopomus occidentalis.* Proc Biol Sci 283:20152113.

Ebbesson L, Nilsen T, Helvik J, Tronci V, Stefansson S (2011): Corticotropin-releasing factor neurogenesis during midlife development in salmon: genetic, environmental and thyroid hormone regulation. J Neuroendocrinol 23: 733–741.

Edelmann K, Glashauser L, Sprungala S, Hesl B, Fritschle M, Ninkovic J, Godinho L, Chapouton P (2013): Increased radial glia quiescence, decreased reactivation upon injury and unaltered neuroblast behavior underlie decreased neurogenesis in the aging zebrafish telencephalon. J Comp Neurol 521:3099–3115.

Falconer EM, Galea LA (2003): Sex differences in cell proliferation, cell death and defensive behavior following acute predator odor stress in adult rats. Brain Res 975:22–36.

Fernández A, Rosillo J, Casanova G, Olivera-Bravo S (2011): Proliferation zones in the brain of adult fish *Austrolebias* (cyprinodontiform: Rivulidae): a comparative study. Neuroscience 189:12–24.

Frisch A (2004): Sex-change and gonadal steroids in sequentially-hermaphroditic teleost fish. Rev Fish Biol Fish 14:481–499.

Galea L, Kavaliers M, Ossenkopp K, Innes D, Hargreaves E (1994): Sexually dimorphic spatial learning varies seasonally in two populations of deer mice. Brain Res 635:18–26.

Glasper ER, Schoenfeld TJ, Gould E (2012): Adult neurogenesis: optimizing hippocampal function to suit the environment. Behav Brain Res 227:380–383.

Gonda A, Herczeg G, Merilä J (2013): Evolutionary ecology of intraspecific brain size variation: a review. Ecol Evol 3:2751–2764.

Gonda A, Valimaki K, Herczeg G, Merila J (2012): Brain development and predation: plastic responses depend on evolutionary history. Biol Lett 8:249–252.

Ilieş I, Sîrbulescu RF, Zupanc GK (2014): Indeterminate body growth and lack of gonadal decline in the brown ghost knifefish *(Apteronotus leptorhynchus),* an organism exhibiting negligible brain senescence. Can J Zool 92: 947–953.

Johansen IB, Sorensen C, Sandvik GK, Nilsson GE, Hoglund E, Bakken M, Overli O (2012): Neural plasticity is affected by stress and heritable variation in stress coping style. Comp Biochem Physiol Part D Genomics Proteomics 7:161–171.

Kirn JR (2010): The relationship of neurogenesis and growth of brain regions to song learning. Brain Lang 115:29–44.

Lema SC, Hodges MJ, Marchetti MP, Nevitt GA (2005): Proliferation zones in the salmon telencephalon and evidence for environmental influence on proliferation rate. Comp Biochem Physiol A Mol Integr Physiol 141:327–335.

Le Page Y, Diotel N, Vaillant C, Pellegrini E, Anglade I, Mérot Y, Kah O (2010): Aromatase, brain sexualization and plasticity: the fish paradigm. Eur J Neurosci 32:2105–2115.

Lindsey BW, Di Donato S, Kaslin J, Tropepe V (2014): Sensory-specific modulation of adult neurogenesis in sensory structures is associated with the type of stem cell present in the neurogenic niche of the zebrafish brain. Eur J Neurosci 40:3591–3607.

Lindsey BW, Tropepe V (2014): Changes in the social environment induce neurogenic plasticity predominantly in niches residing in sensory structures of the zebrafish brain independently of cortisol levels. Dev Neurobiol 74:1053–1077.

Maginnis TL (2006): The costs of autotomy and regeneration in animals: a review and framework for future research. Behav Ecol 17:857–872.

Maine AR, Powers SD, Lutterschmidt DI (2014): Seasonal variation in cell proliferation and cell migration in the brain of adult red-sided garter snakes *(Thamnophis sirtalis parietalis).* Brain Behav Evol 84:181–196.

Maruska KP, Carpenter RE, Fernald RD (2012): Characterization of cell proliferation throughout the brain of the African cichlid fish *Astatotilapia burtoni* and its regulation by social status. J Comp Neurol 520:3471–3491.

Mirescu C, Gould E (2006): Stress and adult neurogenesis. Hippocampus 16:233–238.

Nordeen EJ, Nordeen KW (1988): Sex and regional differences in the incorporation of neurons born during song learning in zebra finches. J Neurosci 8:2869–2874.

Oliveira RF, Canario AV, Grober MS (2001): Male sexual polymorphism, alternative reproductive tactics, and androgens in combtooth blennies (Pisces: Blenniidae). Horm Behav 40:266–275.

Opendak M, Gould E (2015): Adult neurogenesis: a substrate for experience-dependent change. Trends Cogn Sci 19:151–161.

Perry AN, Grober MS (2003): A model for social control of sex change: Interactions of behavior, neuropeptides, glucocorticoids, and sex steroids. Horm Behav 43:31–38.

Pollen AA, Dobberfuhl AP, Scace J, Igulu MM, Renn SC, Shumway CA, Hofmann HA (2007): Environmental complexity and social organization sculpt the brain in Lake Tanganyikan cichlid fish. Brain Behav Evol 70:21–39.

Radmilovich M, Fernandez A, Trujillo-Cenoz O (2003): Environment temperature affects cell proliferation in the spinal cord and brain of juvenile turtles. J Exp Biol 206:3085–3093.

Revest J, Dupret D, Koehl M, Funk-Reiter C, Grosjean N, Piazza P, Abrous D (2009): Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Mol Psychiatry 14: 959–967.

Rieder CL, Cole RW (2002): Cold-shock and the mammalian cell cycle. Cell Cycle 1:169–175.

Salvanes AG, Moberg O, Ebbesson LO, Nilsen TO, Jensen KH, Braithwaite VA (2013): Environmental enrichment promotes neural plasticity and cognitive ability in fish. Proc Biol Sci 280:20131331.

Sherry DF, MacDougall-Shackleton SA (2015): Seasonal change in the avian hippocampus. Front Neuroendocrinol 37:158–167.

Sorensen C, Bohlin LC, Overli O, Nilsson GE (2011): Cortisol reduces cell proliferation in the telencephalon of rainbow trout *(Oncorhynchus mykiss).* Physiol Behav 102:518–523.

Sorensen C, Johansen IB, Overli O (2013): Neural plasticity and stress coping in teleost fishes. Gen Comp Endocrinol 181:25–34.

Sorensen C, Nilsson GE, Summers CH, Overli O (2012): Social stress reduces forebrain cell proliferation in rainbow trout *(Oncorhynchus mykiss).* Behav Brain Res 227:311–318.

Sorensen C, Overli O, Summers CH, Nilsson GE (2007): Social regulation of neurogenesis in teleosts. Brain Behav Evol 70:239–246.

Tozzini ET, Baumgart M, Battistoni G, Cellerino A (2012): Adult neurogenesis in the short-lived teleost *Nothobranchius furzeri:* localization of neurogenic niches, molecular characterization and effects of aging. Aging Cell 11: 241–251.

Traniello IM, Sîrbulescu RF, Ilieş I, Zupanc GK (2014): Age-related changes in stem cell dynamics, neurogenesis, apoptosis, and gliosis in the adult brain: a novel teleost fish model of negligible senescence. Dev Neurobiol 74: 514–530.

Vidal Pizarro I, Swain GP, Selzer ME (2004): Cell proliferation in the lamprey central nervous system. J Comp Neurol 469:298–310.

von Krogh K, Sorensen C, Nilsson GE, Overli O (2010): Forebrain cell proliferation, behavior, and physiology of zebrafish, *Danio rerio,* kept in enriched or barren environments. Physiol Behav 101:32–39.

Zikopoulos B, Kentouri M, Dermon CR (2000): Proliferation zones in the adult brain of a sequential hermaphrodite teleost species *(Sparus aurata).* Brain Behav Evol 56:310–322.

Zupanc GK (2006): Neurogenesis and neuronal regeneration in the adult fish brain. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 192:649–670.

Zupanc GK, Horschke I (1995): Proliferation zones in the brain of adult gymnotiform fish: a quantitative mapping study. J Comp Neurol 353:213–233.