Neurogenesis in the Adult Avian Song-Control System

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New neurons are added throughout the forebrain of adult birds. The song-control system is a model to investigate the addition of new long-projection neurons to a cortical circuit that regulates song, a learned sensorimotor behavior. Neuroblasts destined for the song nucleus HVC arise in the walls of the lateral ventricle, and wander through the pallium to reach HVC. The survival of new HVC neurons is supported by gonadally secreted testosterone and its downstream effectors including neurotrophins, vascularization, and electrical activity of postsynaptic neurons in nucleus RA (robust nucleus of the arcopallium). In seasonal species, the HVC→RA circuit degenerates in nonbreeding birds, and is reconstructed by the incorporation of new projection neurons in breeding birds. There is a functional linkage between the death of mature HVC neurons and the birth of new neurons. Various hypotheses for the function of adult neurogenesis in the song system can be proposed, but this remains an open question.

Song behavior in oscine birds is regulated by a network of pallial and striatal nuclei. The song-control system shows extensive plasticity in adults, including ongoing neurogenesis in several nuclei (Brenowitz 2008). The addition of new neurons to the adult brain of higher vertebrates was first suggested by the pioneering studies of Altman and Das (1965) and Kaplan and Hinds (1977). They reported that labeled cells were present in the dentate gyrus (DG) of rats following the injection of ^3H -thymidine. Their claims, however, met with skepticism and the neuronal identity of the new cells that they observed was called into question (Gross 2000). In an influential study, Rakic (1985) injected adult rhesus monkeys with ^3H -thymidine and reported that, “all neurons of the rhesus

monkey brain are generated during prenatal and early postnatal life.” The study of neuronal addition to the adult brain, was subsequently dropped for ~20 years in the face of the dogma that neurogenesis was largely completed by birth (Gross 2000). This prevailing view only started to be overturned when Nottebohm and colleagues published a series of studies showing that new cells are added to the cortical-like song nucleus HVC (Fig. 1) of adult canaries (*Serinus canarius*) (Goldman and Nottebohm 1983). These new cells have neuronal morphology, some of these cells fire action potentials in response to sound (Paton and Nottebohm 1984), receive synaptic input (Burd and Nottebohm 1985), may synapse on neurons in the efferent robust nucleus of the arcopallium (RA)

(Alvarez-Buylla et al. 1990), and express neuron-specific proteins (Barami et al. 1995). Together, these studies in songbirds showed that new neurons are born and incorporated into functional circuits in the brains of adults of higher vertebrates (Nottebohm 2004). This research on adult neurogenesis in songbirds stimulated investigators to re-examine this topic in mammals. It soon became clear that new neurons are added throughout life to the DG and olfactory bulb of mammals including humans (Cameron and Gould 1994; Gould et al. 1997,

Birds continue to be a productive model for the study of neurogenesis in the adult brain, as discussed below. In this article, we will focus on neurogenesis in the song-control system as this is the most intensively studied model in birds. (For a review of neurogenesis in the avian hippocampus [HC], see Barnea and Pravosudov

2011.) We will discuss the mechanisms of neurogenesis in the song system, intrinsic and extrinsic factors that influence neuronal addition, a linkage between cell death and neurogenesis, seasonal plasticity, and consider potential functions of adult neurogenesis.

WHY STUDY ADULT NEUROGENESIS IN BIRDS?

The birdsong system offers several advantages as a model:

1. Unlike mammals, in which substantial neurogenesis is primarily limited to two regions of the brain (HC and olfactory bulb), new neurons are added throughout most of the avian telencephalon, and this form of plasticity, therefore, seems to be a fundamental feature of the forebrain in birds (Alvarez-Buylla et al. 1994).
2. The song nuclei that incorporate new neurons in adult birds, HVC, and area X (a basal ganglia homolog) are dedicated to the regulation of song, a learned sensorimotor behavior. Placing adult neurogenesis in these regions into a functional context is, therefore, relatively straightforward (e.g., Brown et al. 1999; Gould et al. 1999b; Malberg et al. 2000; Morris 2006; Abrous and Wojtowicz 2008; Clelland et al. 2009; Konkel and Cohen 2009).
3. The level of ongoing neurogenesis in the avian brain is higher by at least an order of magnitude than that seen in mammalian brains. In birds, 0.1%–0.7% of all HVC neurons and 0.15%–0.37% of hippocampal neurons are newly recruited per day on average, depending on the species (Gahr et al. 2002). In contrast, it is estimated that 0.02% of total hippocampal granule cells in mature macaque monkeys, and 0.2% in 10-wk-old rats, are generated per day (Cameron and McKay 2001; Jabès et al. 2010). Using ^{14}C dating as a measure of postnatal cell proliferation in human brains, Spalding et al. (2013) estimated that 0.004% of neurons in the DG turn over each day. In mammals, “adult neurogenesis is not a mass phenomenon but appears to

make a qualitative rather than quantitative contribution” (Gage et al. 2008). The higher level of neuronal addition in birds makes it more tractable for experimental manipulation, and suggests that ongoing neurogenesis is a quantitative phenomenon in birds.

4. Neuronal addition to the mature DG is offset by cell death, and total neuron number does not change throughout adult life (Jabès et al. 2010). Seasonally breeding songbirds, however, show pronounced seasonal cycles of a large increase in neuronal number in HVC of breeding adults because of neurogenesis, followed by a decrease in nonbreeding birds owing to apoptosis (see below). This seasonal pattern facilitates relating changes in behavior to the addition or loss of neurons.
5. Most of the new neurons added to the adult HVC have long axons that project 4 mm or more to synapse on target cells in RA (Alvarez-Buylla et al. 1990; Scotto-Lomassese et al. 2007). New neurons added to the olfactory bulb in mammals, in contrast, are interneurons (Lledo et al. 2006; De Marchis et al. 2007), and those added to the DG are granule cells that synapse locally on the hippocampal hilus and CA3 (Toni et al. 2008). HVC, therefore, provides a unique model for investigating the incorporation of new neurons into long-range neural circuits, and facilitates manipulations of target cells to examine post-synaptic influences on the addition of new afferent neurons (e.g., Larson et al. 2013).
6. There are ~4000 species of songbirds. The species studied thus far all learn to sing, possess the same conserved network of hormone-sensitive brain nuclei that regulate song, and show adult neurogenesis in HVC and area X (Brenowitz 1997, 2008; Wilbrecht and Kirn 2004; Jarvis 2009; Barnea and Pravosudov 2011). There is much variation among taxa in whether song learning is restricted to juveniles or continues into adulthood, the number of songs learned, plasticity of adult song, and sexual patterns of song behavior (Beecher and Brenowitz 2005). This species diversity provides rich opportu-

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nities for comparative studies to test the role of neurogenesis in regulating different aspects of learned song behavior.

BASIC PROCESSES

Neurogenic Niches and Proliferation

Proliferative cells that give rise to new neurons and glia reside in “hotspots” in the walls of the lateral ventricle in the brain (Fig. 1) (Alvarez-Buylla et al. 1990, 2002; Vellema et al. 2010). Mapping studies in birds show that proliferating cells reside at the lateral and tectal ventricles in the adult canary (*S. canarius*) brain (Alvarez-Buylla et al. 1990; Vellema et al. 2010). Cell proliferation at the ventrolateral and the dorsomedial walls of the lateral ventricles account for 93% and 6% of the total number of proliferating cells, respectively (Alvarez-Buylla et al. 1990). Less than 1% of the total number of all cells proliferating in the canary brain arise at the tectal ventricle (Alvarez-Buylla et al. 1990). The proliferating cells in the ventricular zone (VZ) generate multiple cell types. Tissue explant cultures containing the ependyma of the lateral ventricle give rise to both radial glia and new neurons that differentiate and form synapses within 2 wk (Goldman 1990; Goldman et al. 1993, 1996). Neural stem-cell-derived radial glial cells are thought to serve as the neural progenitors in the adult bird brain, although this remains a topic of debate (Gray and Sanes 1992; Goldman et al. 1996; Alvarez-Buylla et al. 2002).

Migration of Neuroblasts

Proper migration and incorporation of new neurons is necessary for them to be integrated into functional neural circuits. In birds, the departure of neuroblasts and newly differentiated neurons from the neurogenic niche begins 1 to 4 d after birth (Fig. 2) (Alvarez-Buylla and Nottebohm 1988; Barami et al. 1994). These new cells must migrate over long distances to reach most of their destinations in the telencephalon. New HVC neurons migrate over 1 to 2 wk (Alvarez-Buylla and Nottebohm 1988) by one of two modes: radial or undirected wandering.

Neuroblasts with a classical bipolar migratory phenotype are generally associated with the fibers of ependymally derived radial glia, both in vitro (Goldman et al. 1993) and in vivo (Alvarez-Buylla and Nottebohm 1988; Scott et al. 2012). About 30% of new HVC neurons are associated with radial glia (Scott et al. 2012). Typical radial glial cells reside in the VZ at the lateral ventricles and project their processes mediolaterally (Alvarez-Buylla and Nottebohm 1988). Association of ³H-thymidine-positive cells with radial fibers suggests that these processes provide the scaffolding on which some neuroblasts migrate through the adult avian brain (Alvarez-Buylla and Nottebohm 1988). Radial glia also provide trophic support for the migrating neuroblasts. Insulin-like growth factor-1 (IGF-1) is expressed by radial glia cells and their fibers, and promotes the migration of neuroblasts (Jiang et al. 1998).

Most of the neuroblasts (~70%) that give rise to new HVC neurons adopt a multipolar

Figure 2. A timeline of the processes of avian neurogenesis and the proportion of new neurons that persist through each of these processes. The timeline is based on the work of Alvarez-Buylla and Nottebohm (1988), Barami et al. (1994), and Scott et al. (2012). The percentages of survival through the various processes of avian neurogenesis are based on the findings of Kirn et al. (1991, 1999), Barnea and Nottebohm (1994), Nottebohm et al. (1994), Scott and Lois (2007), and Walton et al. (2012). VZ, Ventricular zone.

phenotype and follow a wandering, tortuous route with frequent changes in direction rather than a straight path through HVC during migration (Scott et al. 2012). Movement is produced by translocation of the cell body along one of the processes that extends from the soma. Wandering may continue for several days, during which the cell may travel several hundred micrometers. These multipolar cells differentiate into neurons once they reach their destination (Scott et al. 2012).

It is unclear whether bipolar and multipolar neuroblasts represent different stages of migration of the same cells or distinct neuronal types. Neuroblasts may initially migrate away from the VZ along radial glial fibers, and then transition to wandering during the final stages of migration (Alvarez-Buylla and Nottebohm 1988; Scott et al. 2012). Alternatively, bipolar and multipolar neuroblasts may represent different neuronal types. Scott et al. (2012) suggest that this latter possibility is less likely, given the changes in phenotype, polarity, direction, and scaffolding observed in migrating neuroblasts in mammalian brains.

Postmigratory Maturation and Fate Specification

As early as 8 d following birth, new neurons that express neuronal markers but are not yet fully mature and functional can be found in HVC (Fig. 2) (Kirn et al. 1999). At the end of migration, these new neurons form close contact with the soma of mature HVC neurons (Burd and Nottebohm 1985). These new cells integrate into functional circuits, and form somatal contact with mature HVC interneurons and neurons that project to area X (HVC_X) and RA (HVC_{RA}) (Fig. 2) (Kirn et al. 1999; Scott et al. 2012). These cellular interactions may provide a “stop” signal that terminates migration (Scott et al. 2012). Together, the new and mature neurons form clusters that may represent a functional unit (Kirn et al. 1999). The neurons within these clusters appear to be connected by gap junctions (Gahr and Garcia-Segura 1996). This coupling may allow HVC_X neurons to entrain new HVC_{RA} neurons to produce the appropri-

ate motor pattern for song production (Alvarez-Buylla and Kim 1997).

Most or all of the new HVC neurons project to the afferent nucleus RA. It is unclear whether some neuroblasts differentiate into HVC interneurons (Scott and Lois 2007; Scotto-Lomas et al. 2007). By 2 wk of age, these neurons can form synapses on RA neurons as shown by the uptake and retrograde transport from RA of tract tracers (Kirn et al. 1999). Although capable of forming synapses by 2 wk, nearly half of the new HVC neurons that will ultimately project to RA have not yet formed synapses even by 30 d following birth in adult canaries and zebra finches (Kirn et al. 1999; Scott and Lois 2007). By 8 mo, all new HVC_{RA} neurons form synapses onto RA neurons in canaries (Kirn et al. 1999).

Survival of Newly Generated Neurons

Adult-born neurons in the avian brain persist for periods ranging from days to years. Between 2 and 3 wk following birth, roughly half of all postmigratory neurons in HVC die (Fig. 2) (Kirn et al. 1999). In the HC of the adult black-capped chickadee (*Parus atricapillus*), the number of ³H-thymidine-labeled neurons decreases between 6 and 10 wk after birth (Barnea and Nottebohm 1994). The new neurons that persist through the initial culling can survive months (Kirn et al. 1991; Nottebohm et al. 1994) to years (Barnea and Nottebohm 1994; Walton et al. 2012), depending on their time of birth, location of incorporation, and other factors discussed below (Fig. 2). Given that the number of newly generated HVC neurons does not decrease significantly between 9 mo and 4 yr in the zebra finch (*Taeniopygia guttata*) (Walton et al. 2012), it is possible that some adult-born neurons persist in HVC and other regions of the avian brain for the remainder of the bird's life.

MOLECULAR AND PHYSIOLOGICAL MECHANISMS

Genomics

Gene regulatory networks that control different aspects of neurogenesis and cell death have

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Figure 3. A schematic of the factors ranging from gene expression to behavior that influence the birth and migration of neuroblasts and the addition of new neurons to an HVC. The solid black lines indicate factors that positively influence the given cell, factor, or process to which it points. The dashed lines represent changes in cell process indicated (e.g., cell divisions and migration). The dotted lines represent indirect routes of influence (i.e., through other genes, factors, etc.). The breakout panel to the right summarizes patterns of gene expression in different brain regions that positively influence component processes of neurogenesis. In the ventricular zone (VZ), the (+) indicates genes that promote proliferation, whereas the (-) indicates genes that promote exit from the cell cycle. DCX⁺, Doublecortin-positive migratory neuroblast; IN, interneuron; NPC, neural progenitor cell; NSC, neural stem cell; PN, projection neuron. ER, endoplasmic reticulum; VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor; GABA, γ -aminobutyric acid; RA, arcopallium; PAm, par-ambigialis; RAm, retroambigialis.

been identified through the sequencing and analysis of the zebra finch genome and microarray analyses (Fig. 3) (Warren et al. 2010; Thompson et al. 2012). Thompson et al. (2012) identified 132 genes in HVC cells in adult male white-crowned sparrows (*Zonotrichia leucophrys*), which changed in expression between breeding and nonbreeding physiological conditions when compared with gene expression in RA, a nonneurogenic nucleus (Alvarez-Buylla et al. 1994). In general, genes

that promote proliferation, angiogenesis, and neurite extension were up-regulated, whereas genes that support programmed cell death were down-regulated in HVC under breeding conditions. Specific genes that encode for neurotrophins known to promote neuronal migration, recruitment, and survival, including brain-derived neurotrophic factor (BDNF), IGF-1, and vascular endothelial growth factor (VEGF), were up-regulated in HVC cells under breeding conditions.

Interactions of Steroid Hormones and Neurotrophins

Steroid sex hormones secreted by the gonads and synthesized de novo in the brain influence the survival of adult-born HVC neurons. The effects of hormones on avian neurogenesis are mediated by the expression of neurotrophic factors. Treatment of adult female canaries with exogenous testosterone (T) increases the number of new neurons added to HVC (Rasika et al. 1994). This effect of T on neuronal addition requires BDNF (Rasika et al. 1999). T treatment increases both BDNF mRNA and protein in HVC cells (Rasika et al. 1999; Wissman and Brenowitz 2009). Intracerebral infusion of recombinant BDNF (rBDNF) into HVC increased the number of new neurons by the same amount as did T treatment, whereas infusion of a BDNF-blocking antibody prevented the T-induced increase in new neurons in HVC (Rasika et al. 1999). BDNF promotes neuronal addition to HVC during a discrete critical period. Infusion of rBDNF into HVC 14–20 d after the birth of new neurons increased the number of neurons that survived for at least 8 mo. Birds that were infused with BDNF either 4–10 or 24–30 d after birth, however, showed the typical die-off of 50% of new HVC neurons by 4 mo (Nottebohm et al. 1994; Alvarez-Borda et al. 2004). These observations suggest that T-induced expression of BDNF increases the survival of newly generated neurons in HVC, and that there is a sensitive period soon after new neurons first reach HVC when BDNF has this effect on neuronal survival.

The interaction between T and BDNF in adult neurogenesis is mediated by local angiogenesis in HVC. T treatment expands the microvasculature in HVC, including increases in the number of new endothelial cells, and the diameter, perimeter, and area of capillaries (Louissaint et al. 2002). In female canaries, this T treatment mediates these effects in HVC by up-regulating expression of both VEGF and its receptor *Quek1*/VEGF receptor 2 (R2) tyrosine kinase within 2 wk. Both the induction of VEGF and *Quek1*/VEGFR2 appear to be mediated by the metabolic conversion of T to 17 β -

estradiol (E_2). Treatment of cultured HVC endothelial cells with E_2 , but not the androgenic metabolite 5- α dihydrotestosterone (DHT), increased *Quek1*/VEGFR2 mRNA expression. Furthermore, systemic treatment of female canaries with E_2 -induced *Quek1*/VEGFR2 and increased the addition of new HVC neurons in vivo (Hidalgo et al. 1995; Louissaint et al. 2002).

BDNF expression by HVC endothelial cells lags VEGF expression by 1 wk or more. Inhibition of VEGFR2 in vivo in HVC prevented both increased vasculature and neuronal addition, whereas treatment of cultured endothelial cells with T, E_2 , or DHT increased production of BDNF protein (Louissaint et al. 2002). Together, these results suggest a serial activation pathway: T is aromatized to E_2 , which increases vascularization in HVC by increasing VEGFR2 expression in endothelial cells. The endothelial cells in the expanded vascular bed are then stimulated by T via VEGF binding to VEGFR2 to express BDNF. BDNF, in turn, increases the survival of new neurons as well as increasing song behavior (Louissaint et al. 2002).

Effects of Neural Activity

Activity within the circuit to which new neurons are added influences the incorporation and survival of these cells (Barnea and Pravosudov 2011). Singing behavior increases neuronal survival in HVC via increased BDNF mRNA and protein expression in the population of HVC_{RA} neurons to which new neurons are added (Li et al. 2000). BDNF expression correlates with the number of songs produced per unit time in male zebra finches and, as discussed above, this BDNF production supports neuronal survival. The number of new neurons added to the adult HVC of male canaries is correlated with individual differences in the average amount of song produced (Alvarez-Borda et al. 2002). Alternatively, deafening (i.e., loss of auditory neural activity) decreases neuronal addition to HVC and the auditory caudomedial nidopallium (NCM) in adult zebra finches (Wang et al. 1999; Pytte et al. 2010).

The addition of HVC_{RA} projection neurons is influenced by the electrical activity of target

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Figure 4. Postsynaptic activity influences the addition of new robust nucleus of the arcopallium (RA)-projecting neurons to HVC. Spontaneous activity of neurons in RA was decreased by unilateral infusions of muscimol (2.8 mg/ml) or increased by infusion of KCl (100 mM). Inhibiting activity decreased neuronal addition (Larson et al. 2014). Increasing activity increased neuronal addition to HVC (ipsilateral to KCl infusion, 312 ± 75 new neurons; contralateral to KCl infusion, 160 ± 23 new neurons). Asterisks indicate $p < 0.05$ in post hoc t -test following two-way ANOVA. BrdU, 5-bromo-2'-deoxyuridine; SD, short day; LD, long day; T, testosterone.

neurons in RA. The spontaneous activity of RA neurons is high in breeding sparrows and low in nonbreeding birds (Meitzen et al. 2007a,b, 2009a). Inhibiting activity in breeding-condition birds by infusing RA with the GABA_A receptor agonist muscimol decreased the number of new neurons in HVC of adult white-crowned sparrows by 56% (Fig. 4) (Larson et al. 2013). Increasing RA activity in nonbreeding condition birds by infusing KCl increased the number of new HVC neurons by 95% (Fig. 4) (TA Larson, TW Wang, and EA Brenowitz, unpubl.). These results are consistent with the observation that activity is required for the survival of new neurons in the mammalian olfactory bulb (Corotto et al. 1994; Rochefort et al. 2002) and DG (Tashiro et al. 2006).

The mechanism by which activity influences neuronal addition to HVC is not yet known. Activity-induced regulation of genes encoding molecules that promote survival of adult-born HVC neurons, axonal path finding, and/or synapse formation is likely to be important (Zhang

et al. 2001; Kay et al. 2011). Interestingly, activity-induced guidance molecules are seasonally regulated in RA neurons of white-crowned sparrows; microarray analysis of cDNA extracted from RA showed that the expression of axonal guidance cue genes, including netrin 4 and galectin, is increased in breeding-condition birds (Thompson et al. 2012). The retrograde transport of activity-induced trophic factors produced by target neurons that influence the survival of new HVC_{RA} neurons may be modulated by activity in RA. Microarray analysis also showed that the expression of proneurogenic genes, including IGF-1 and neuromodulin, is increased in RA of breeding-condition birds (Thompson et al. 2012). In HVC, the expression of mRNA for sex steroid receptors, which facilitate the retrograde transport toward the neuronal soma of trophic factors bound to their receptor, also increases during breeding conditions (Fusani et al. 2000; Jezierski and Sohrabji 2003; Fraley et al. 2010). Once transported back to the soma, trophic factors likely activate signaling cascades that promote the growth and survival of new neurons (Gottschalk et al. 1999; Yoshii and Constantine-Paton 2007; EA Brenowitz, K Lent, R Luche, et al., unpubl.). Inhibition of neural activity in RA may result in a failure of new HVC neurons to form synapses on RA neurons and/or a decreased production of activity-induced trophic factors in RA. The consequence would be a lack of retrograde transport of the trophic signals and, thus, a decrease in addition of adult-born neurons to HVC.

Cell Death

The addition of new neurons to HVC is functionally linked with the death of mature neurons. In adult male canaries, mature HVC neurons die when T levels decrease at the end of the breeding season. Within a few weeks, there is a peak in the addition of new neurons to HVC (Kirn et al. 1994). Laser photo-ablation of both HVC_X and HVC_{RA} projection neurons in adult male zebra finches increases the addition of new HVC_{RA} neurons, but not of new HVC_X neurons (Scharff et al. 2000). In adult

white-crowned sparrows, mature HVC neurons die seasonally through caspase-mediated apoptosis when T levels drop (Thompson and Brenowitz 2008). Reduction of this neuronal loss in HVC by infusion of a cocktail of caspase inhibitors reduces the number of new neurons added to HVC (Thompson and Brenowitz 2009). Within 2 d following the peak of cell death in HVC following the drop in T level, proliferation of neural progenitor cells in the VZ increases (Larson et al. 2014). These observations suggest that the death of mature neurons facilitates the addition of new neurons and, thus, apoptosis and neurogenesis may be causally linked. Consistent with this observation, in adult ring doves (*Streptopelia risoria*), lesion of the medial preoptic area of the hypothalamus (mPOA) induces neurogenesis in this region that does not normally add new neurons (Cheng et al. 2011). The molecular mechanisms linking cell death to neurogenesis are under investigation.

Age

Levels of neurogenesis decrease in the aging brain. In adult birds, the rates of neural progenitor cell proliferation (Larson et al. 2014), neuronal recruitment (Wilbrecht and Nottebohm 2004), and neuronal survival (Wang et al. 2002; Adar et al. 2008a) decrease with age across species. New neurons continue to be added to HVC, however, for as long as 11 yr of age in zebra finches (Walton et al. 2012).

Seasonality

Most species of birds breed seasonally. In essentially every seasonally breeding songbird species that has been examined, there are pronounced seasonal changes in the morphology, electrophysiology, and gene expression of song nuclei (Fig. 5) (reviewed by Tramontin and Brenowitz 2000; Brenowitz 2008). These neural changes are accompanied by changes in song

Figure 5. A model of the seasonal changes in physiology, morphology, and behavior of songbirds. As day length increases at the onset of the breeding season, plasma testosterone (T) levels increase. The increase in T drives an increase in neuronal number in HVC along with changes in gene expression, morphology, and physiology of HVC and robust nucleus of the arcopallium (RA). All of these changes in morphology and physiology permit the production of stereotyped song. As day length decreases at the onset of the nonbreeding season, T levels drop, HVC neurons die, and song degrades. This cycle repeats annually.

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rate and structure (Nottebohm and Nottebohm 1978; Smith et al. 1997). The seasonal changes in the song nuclei, all of which express androgen and/or estrogen receptors, are primarily regulated by changes in gonadal T and its metabolites (Brenowitz 2008).

Neuron number in HVC changes seasonally. In wild-caught song sparrows (*Melospiza melodia*), for example, neuron number in HVC increases from ~150,000 in the fall to 250,000 during the breeding season (Smith et al. 1997). This change in neuron number results from seasonal patterns of cell death and ongoing neurogenesis. At the end of the breeding season, circulating T levels decrease, which prompts an increase in the death of mature HVC neurons (Thompson et al. 2007; Thompson 2011). This death of mature HVC neurons during the transition from breeding to nonbreeding seasons creates “vacancies” for the addition of new neurons (Scharff et al. 2000; Nottebohm 2004; Thompson and Brenowitz 2009).

Field studies of wild birds show that growth of the song system occurs rapidly once plasma T levels first start to rise as day length increases in late winter, and precedes full seasonal reproductive development (Smith et al. 1997; Tramontin et al. 2001). In a laboratory study of captive white-crowned sparrows implanted with a systemic T implant and exposed to a long day photoperiod to mimic breeding conditions, HVC grew to its full breeding size and neuron number increased from 90,000 to 150,000 within 7 d. This addition of 60,000 neurons to an adult brain within such a short time period is unprecedented.

As discussed above, the local expression of BDNF increases the survival of new HVC neurons. In a cDNA microarray hybridization study, Thompson et al. (2012) found that the gene for BDNF is significantly up-regulated in HVC following the transition of white-crowned sparrows from nonbreeding to breeding conditions. In situ hybridization measurements confirmed that BDNF mRNA is expressed widely throughout HVC of sparrows exposed to breeding conditions, and that expression increased over the 7 d following exposure to systemic T (Fig. 3) (Wissman and Brenowitz 2009).

When circulating T drops to basal levels at the end of the breeding season, mature HVC neurons lose the trophic support provided by T, BDNF, and the electrical activity of postsynaptic neurons in RA, and rapidly die (Rasika et al. 1994, 1999; Hidalgo et al. 1995; Brenowitz 2008; Thompson and Brenowitz 2010). Within 4 d of transferring white-crowned sparrows from breeding to nonbreeding conditions in the laboratory, 25% of HVC neurons die (Thompson et al. 2007). The number of new neurons present in HVC increases following the death of mature neurons (Alvarez-Buylla et al. 1990; Tramontin and Brenowitz 1999). In the absence of trophic support, however, most of these new HVC neurons in nonbreeding birds do not persist in the song-control circuit. When two cohorts of new neurons were labeled by injections of 5-bromo-2'-deoxyuridine (BrdU) and 5-ethynyl-2'-deoxyuridine (EdU) at different intervals, >50% of the adult-born neurons present after 1 m in nonbreeding conditions belonged to the younger cohort of new neurons (TA Larson, NM Thatra, D Hou, EA Brenowitz, unpubl.). This result indicates that, in nonbreeding birds, many new HVC neurons are transient and are replaced by younger neurons. The turnover of new neurons continues until T levels start to rise early in the next breeding season. On transition into breeding conditions, the first cohort of neurons survived at a much higher rate when compared with nonbreeding conditions. Once HVC neuronal number stabilized at its maximum growth, subsequently added new neurons (i.e., the second cohort of labeled neurons) persisted at levels similar to nonbreeding condition. These observations suggest that the trophic cascade initiated by the increase in circulating T levels in breeding birds increases the survival of new neurons added during the initial growth of HVC, but does not affect neuronal turnover once HVC neuronal number stabilizes.

The HVC to RA circuit in white-crowned sparrows degenerates and is reconstructed seasonally. Over 50% of the HVC_{RA} neurons die when sparrows transition from breeding to nonbreeding conditions (TA Larson, RE Cohen, MC Cole, et. al., unpubl.). Concomitant with

this loss of projection neurons, song becomes less stereotyped and less complete in structure. The HVC to RA circuit is regenerated early the next breeding season when new neurons are integrated into HVC and grow their axons to synapse on RA neurons. The number of newborn HVC_{RA} neurons increases by more than 300% in breeding birds. As the circuit is regenerated, sparrows are once again able to produce stereotyped and complete song. These data show that adult neurogenesis in the song system is restorative (*sensu* Gage et al. 2008).

SOCIALITY (AND STRESS)

Social setting may influence neuronal recruitment (reviewed in Gheusi et al. 2009). In the male zebra finch, a highly social species, social enrichment (i.e., group housing with male and female conspecifics) increases neuronal addition to HVC and other neurogenic regions of the brain including area X, NCM, and the HC, when compared with males housed alone or with a single female (Lipkind et al. 2002; Barnea et al. 2006; Adar et al. 2008a,b). The mechanism through which social enrichment promotes neuronal recruitment and survival in birds is unknown. Investigation of this topic should consider the possibility that what appears to be social enhancement of neurogenesis may actually reflect recovery from suppression of neurogenesis because of stress induced by housing birds in socially impoverished conditions. Stress-induced elevation of circulating glucocorticoid levels decreases the number of new neurons in the mammalian DG and the number of proliferating cells in the avian VZ (Westenbroek et al. 2004; Mirescu and Gould 2006; Katz et al. 2008). It is possible that elevated glucocorticoids caused by social stress could decrease neuronal proliferation and/or survival in birds.

FUNCTIONS OF ADULT NEUROGENESIS

Does the replacement of mature neurons with newborn neurons (i.e., neuronal turnover) in adult avian brains serve an adaptive function, and if so, what is it? As discussed below, various functional hypotheses can be proposed (Notte-

bohm 1985, 2002; Wilbrecht and Kirn 2004; Barnea and Pravosudov 2011). These hypotheses are not necessarily mutually exclusive, and neurogenesis is likely to serve different functions in different taxa and different brain regions. A constraint on a discussion of possible function(s) of adult neurogenesis is that no studies that experimentally manipulate levels of neurogenesis in avian brain regions have yet been successfully conducted, and existing tests of hypotheses are, therefore, largely limited to correlational observations. Correlations typically have weak explanatory power by their nature. It is easy to confound correlation and causation, and difficult to determine the direction of causal relationships. As a consequence, no single hypothesis has yet received unambiguous support (Barnea and Pravosudov 2011). Any evaluation of different functional hypotheses is limited by these considerations. There is an urgent need to develop pharmacological or genetic methods of manipulating neuronal addition to adult brain regions to allow experimental tests of functional hypotheses.

Neuronal Addition Is Vestigial

The null hypothesis is that adult neurogenesis has no function in the avian brain. From this perspective, neurogenesis can be viewed as a vestige of developmental plasticity (Wilbrecht and Kirn 2004). The observation that levels of neurogenesis seen in the adult brain are much lower than those seen in developing brains is consistent with this hypothesis. If developmental neurogenesis persists into adulthood, however, we might expect to observe uniformly low levels of new neurons present throughout the adult brain. Contrary to this prediction, however, neurogenesis is widespread throughout the adult telencephalon, but is conspicuously absent from specific forebrain regions, such as the song nuclei RA and lateral magnocellular nucleus of the anterior neostriatum (LMAN). Furthermore, there is little adult neurogenesis outside the telencephalon (Alvarez-Buylla et al. 1994). These observations suggest that neurogenesis can be selectively suppressed in adult brains and, therefore, that the presence

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of neurogenesis is likely the consequence of active maintenance rather than passive persistence.

Neuronal Turnover Allows Plasticity While Keeping Brain Size Small

Ongoing neuronal turnover in adult brains may represent a compromise between the need to continue to form and store new memories throughout the relatively long lifespans of songbirds (3 to <10 yr), and the pronounced size and energetic constraints on brain size imposed by the demands of flight (Calder and King 1974). Replacing mature neurons with more plastic new neurons may be an avian alternative to the encephalization widely observed among mammals, especially those with enhanced cognition or social organization (Shultz and Dunbar 2010; Boddy et al. 2012). If individual neurons are viewed as the structural units of memory storage (e.g., Kempermann 2008), then neuronal turnover may allow a bird to continually encode new information while keeping absolute brain size relatively small (Nottebohm 2002, 2004). As is often true of evolutionary scenarios, a definitive test of this hypothesis would be challenging to conduct.

Neuronal Turnover Enables Adult Birds to Learn to Produce New Songs

The first demonstration of adult neurogenesis in songbirds was in the canary, a species that develops new motor patterns of song as an adult (Goldman and Nottebohm 1983). Nottebohm (1989) hypothesized that the addition of new neurons to HVC provides plasticity for encoding the memory of new song programs. Consistent with this model, neurons are added to HVC of adult canaries at a higher rate in the fall, when changes to song structure are most pronounced, than in the spring breeding season, when song structure is stable (Kirn et al. 1994). Further support for this model comes from the observation that new neurons are added to HVC of juvenile zebra finches at a high level while they are actively learning song, and at a lower level when song learning is completed (Nordeen and

Nordeen 1988; Wilbrecht et al. 2002). This decrease in neuronal addition can be delayed by isolating juvenile birds from adult male song tutors, which extends the sensitive period for song learning (Wilbrecht 2003).

Other observations, however, are inconsistent with this hypothesis. Even though the level of neuronal addition to HVC declines in zebra finches when song learning is completed, it does continue into adulthood after the birds have completed song learning. Like zebra finches, male song sparrows and white-crowned sparrows complete song learning in the first year of life, but continue to add new neurons to HVC and area X as adults (Tramontin and Brenowitz 1999; Thompson and Brenowitz 2009). As in canaries, wild song sparrows show higher levels of neuronal addition to HVC in the fall than in the spring, even though they retain the same songs throughout adulthood (Tramontin and Brenowitz 1999). The songs of both song sparrows and white-crowned sparrows, however, become variable in structure in the fall, as do those of canaries (Smith et al. 1995, 1997; Meitzen et al. 2009b). The loss of mature neurons that encode song, and addition of many new neurons that have not yet been “programmed” to produce the previously learned song, may contribute to the increased variability of song that sparrows sing during the fall. Together these observations suggest that, although neuronal turnover may be necessary or permissive for adult learning of new motor patterns of song, it is not sufficient to produce adult song learning.

Neuronal Turnover Enables Adult Birds to Learn to Recognize New Songs

Alternatively, neuronal addition may be a mechanism for acquiring new perceptual memories of song. New neurons are added to the auditory region NCM (Alvarez-Borda et al. 2002; Lipkind et al. 2002), as well as HVC and area X, each of which contributes to auditory discrimination of song (Brenowitz 1991; Scharff et al. 1998; Gentner et al. 2000). Both male and female birds learn to recognize the songs of individuals with whom they frequently interact; males can learn

the individually distinctive songs of their immediate territorial neighbors (e.g., Stoddard et al. 1991) and females can recognize the songs of their mates (e.g., O’Loghlen and Beecher 1999). As birds may have a new neighbor or mate each year, the ability to update auditory song memories is advantageous. Neuronal turnover in auditory-responsive regions, such as HVC, area X, and NCM may provide a cellular mechanism for this perceptual plasticity (Wilbrecht and Kirn 2004). Support for this hypothesis comes from the observation that neuron number in HVC of juvenile swamp sparrows increases more during the early sensory phase of song learning, when adult song is heard and memorized, than during the later sensorimotor phase, when a juvenile begins to sing (Nordeen et al. 1989). If auditory experience influences neuronal addition, then deafening birds should disrupt this process. A decrease in neuronal addition to HVC was observed on deafening in adult (Wang et al. 1999) but not juvenile (Wilbrecht et al. 2002) zebra finches. The disparity between these deafening studies is difficult to explain. A direct test of the hypothesis that neuronal addition enables perceptual learning has not yet been performed. The observation that a bird’s ability to form new auditory memories is impaired when neuronal addition is experimentally suppressed in adults would support this hypothesis.

Neuronal Turnover Is Necessary for Song Maintenance

Maintaining previously learned song in adults is an ongoing process, and the addition of new neurons to HVC and area X may provide cellular plasticity for continual updating of motor programs for song (Wilbrecht and Kirn 2004; Barnea and Pravosudov 2011). Disrupting auditory feedback can lead to degraded song structure in adult birds. Song begins to deteriorate within 1 wk of deafening in Bengalese finches (*Lonchura striata domestica*) and after 2–8 wk in zebra finches, depending on their age (Nordeen and Nordeen 1992; Woolley and Rubel 1997; Lombardino and Nottebohm 2000). Exposing adult zebra finches to delayed auditory feedback

similarly leads to song deterioration after several weeks (Leonardo and Kinishi 1999). Masking selected Bengalese finch song frequencies or disrupting auditory feedback in real time results in rapid changes in song structure (Sakata and Brainard 2006; Tumer and Brainard 2007). These observations indicate that birds need to hear themselves sing to maintain stereotyped song production as adults. Adult birds may compare ongoing auditory feedback from their song with a previously memorized sensory model of song (i.e., a “song template”). Alternatively, the song-control circuitry may generate an instructive signal that enables birds to adjust song as necessary to match a learned song production program (reviewed in Brainard 2008).

An essential component of error-correction models is that there is variability in song structure that can be exploited to allow birds to adjust song structure to more closely match a sensory template or learned motor program. The addition of many new HVC neurons that have not been programmed to produce a previously learned song may increase the variability of song and, thus, provide the raw material for error correction. The process of adjusting variable song to match a learned model may program new neurons to produce the appropriate premotor pattern. This function of adult neuronal turnover could be adaptive for the long-term maintenance of song in adults. A prediction of this hypothesis is that suppression of neuronal addition to HVC should result in a short-term decrease in song variability but a long-term progressive deviation of song structure away from the stereotyped version produced at the completion of juvenile song learning.

Alvarez-Borda et al. (2002) compared neuronal recruitment to HVC in gonadally intact and castrated male canaries that produced comparable amounts of song in the fall, when song becomes more variable. When matched for the amount of singing, the intact birds had ~4 times as many new RA-projecting neurons, and ~2.6 times as many total new HVC neurons as did the castrated birds. The investigators observed no difference between the intact and castrated birds in either the diversity of song syllables or song stereotypy, however, despite the pronounced

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differences in the addition of new neurons to HVC. These results do not support the hypothesis, although the manipulation of circulating testosterone levels is a considerable potential confound. A test of the hypothesis involving a more direct manipulation of neuronal addition to HVC is warranted.

Neuronal Turnover Enables Adult Birds to Replace Overexcited Neurons

Excessive activation of glutamate receptors by excitatory neurotransmission can result in cell death because of various factors, including disrupted intracellular calcium homeostasis, impaired organelle function, increased production of nitric oxide and free radicals, prolonged activation of proteases and kinases, and increased expression of proapoptotic transcription factors (Wang and Qin 2010). Neuronal addition to adult HVC may be an adaptation for replacing cells that are damaged by excitotoxicity and high metabolic demand resulting from prolonged activity (Wilbrecht and Kirn 2004).

During the breeding season, male birds can sing at extremely high rates and do so for several months consecutively. A territorial male red-eyed vireo, for example, may sing more than 20,000 times a day (www.birds.cornell.edu). Individual HVC_{RA} neurons fire at high rates in temporally sparse bursts of activity during singing (Hahn and Ball 1995; Fee et al. 2004), and these bursts can propagate through the HVC_{RA} neuronal population via local excitatory synapses (Mooney and Prather 2005). HVC_{RA} neurons stimulate excitatory postsynaptic potentials (EPSPs) via ionotropic glutamate receptors (Mooney and Prather 2005). Excitatory synaptic transmission between HVC neurons, and between HVC and RA neurons, also involves the activation of both AMPA/kainate and NMDA-type glutamate receptors (Dutar et al. 2000). Given the extraordinary high rate of song production, neuronal activation during the months-long breeding season should also be high. Supporting this assumption, the activity of cytochrome oxidase, an enzyme important in cellular oxidative metabolism, is higher in neurons of HVC, area X, and RA in breeding

birds than nonbreeding birds, indicating that the metabolic demands of these neurons increase when birds sing at high rates (Wennstrom et al. 2001).

This hypothesis is further supported by several other observations. About 50% of the HVC_{RA} neurons in adult male Gambel's white-crowned sparrows die rapidly when testosterone levels decrease at the end of the breeding season (Thompson et al. 2007; Thompson and Brenowitz 2008, 2010; TA Larson, RE Cohen, MC Cole, et. al., unpubl.). This burst of neuronal death coincides with a reduction in trophic support provided both by testosterone and its downstream effectors as discussed above. HVC_{RA} neurons and HVC interneurons that have been highly active during the preceding months may be especially prone to apoptosis from the cumulative detrimental effects of excitotoxicity and metabolic challenge in the absence of trophic support.

Other observations, however, do not support this hypothesis. Area X neurons also show high spontaneous and song-related activity, and receive glutamatergic inputs (Ding et al. 2003), but the rate of neuronal addition does not differ seasonally (Thompson and Brenowitz 2005). The neurons of RA also show high levels of activity, both spontaneous and evoked, during the breeding season (Fee et al. 2004; Meitzen et al. 2007b), and contain both AMPA/kainate and NMDA-type glutamate receptors (Wada et al. 2004). New neurons are not added to the adult RA, however. Motor neurons in nXII_{ts} that innervate the muscles of the vocal organ are presumably driven at a high rate during the months of frequent song production in the breeding season, and contain NMDA receptors (Sturdy et al. 2003), but this nucleus also does not add neurons in adults. Together, these observations suggest that the anatomical distribution of neuronal addition in adult brains is not related to either the presence of ionotropic glutamatergic receptors or metabolic demand in an obvious manner (Nottebohm 2002).

A prediction of this hypothesis is that the death of mature neurons, and the addition of new neurons, would be reduced by protecting HVC neurons from glutamate-mediated excito-

toxicity. Thus, this hypothesis could be tested by manipulations that protect HVC from metabolic damage, such as infusing HVC with exogenous acetoacetate, an energy substrate (Massieu et al. 2003), or overexpression of endogenous Sirt 3, a nicotinamide adenine nucleotide-dependent deacetylase (Kim et al. 2011), with a concomitant reduction in HVC neuronal death.

Adult-Born Neurons Replace Mature Neurons Weakened by Activity-Related DNA Damage

Neuronal activity, even at moderate levels, such as that involved in exploratory activity of mice, can result in DNA double-strand breaks (DSBs) in neuronal genomes (Suberbielle et al. 2013). Activity-related DSBs are exacerbated in J20 mice that express human amyloid- β precursor protein, and blocking extrasynaptic NMDA-type glutamate receptors containing the NR2B subunit prevents amyloid- β DSBs in neuronal cultures (Suberbielle et al. 2013). Amyloid- β precursor protein is present in HVC neurons, and is expressed at higher levels in breeding-condition birds (Thompson et al. 2012). As discussed above, birds sing at very high rates over the months of the breeding season, and activity in HVC neurons is expected to be high during this period. Excitatory synaptic transmission between HVC neurons involve the activation of NMDA-type glutamate receptors (Dutar et al. 2000), which contain the NR2B subunit (Singh et al. 2000, 2003). Together, these observations raise the possibility that HVC neurons may experience high levels of DNA DSBs. Prolonged and recurrent DNA damage may make these neurons more susceptible to cell death when the trophic support provided by T and its downstream effectors is withdrawn at the end of the breeding season. Neuronal addition to adult HVC may be an adaptation to replace mature neurons that have experienced high levels of DNA damage during the prolonged period of high song-related activity in breeding birds. As with the activity-based hypothesis, this hypothesis predicts that there should be a relationship between song production rate, DNA DSBs in HVC neurons, and neuronal turnover in HVC.

Neuronal Addition to HVC Is an Example of Performance-Associated Hypertrophy

The sustained peak performance of a seasonally predictable behavioral or physiological task is often preceded by growth of the organs and/or tissues involved in performance of the task (Piersma and Lindstrom 1997). For example, the size of the gonads and other reproductive structures increases dramatically in preparation for the annual breeding season, and these organs regress when the breeding season is terminated. Preparatory changes, such as these are stimulated by seasonal environmental cues and mediated by neural and endocrine signaling mechanisms. The maintenance of fully grown organ systems and tissues is energetically expensive, and these systems therefore, regress when peak performance is not required (Gaunt et al. 1990).

In applying the principle of performance-associated hypertrophy to seasonal cycles of neuronal turnover in HVC, one can make the following predictions. (1) Song performance should be maximal during the breeding season. This prediction is supported by data from canaries, white-crowned sparrows, and song sparrows among many other species. As described above, males of these species sing more stereotyped songs, and sing more frequently, during the breeding season. (2) Growth of the song nuclei should precede behavioral changes. In the study of Tramontin et al. (2000), the increase in neuron number in HVC observed in male white-crowned sparrows in breeding condition occurred within 7 d of exposure to a long day photoperiod and high T, whereas song stereotypy increased between 7 and 20 d (Tramontin et al. 2000). (3) Energetic costs of maintaining a fully developed song system throughout the nonbreeding season outweigh those associated with regrowing the song system each spring. The relative metabolic costs of maintaining and regrowing the song system each year are not yet known. The activity of cytochrome oxidase, an enzymatic marker of cellular metabolic capacity, increases considerably in HVC, RA, and area X of breeding-condition white-crowned sparrows (Wennstrom et al. 2001). This result suggests

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that the song system does impose a greater metabolic cost in its fully grown state than when regressed.

Changes in neuronal turnover in HVC, and plasticity of the song system in general, may be an adaptation to reduce the energetic costs imposed by the song system in the fall and winter. Outside the breeding season, males do not need to produce frequent, stereotyped song for mate attraction or territorial defense. In the fall and winter, birds may experience the energetic stress of migration, increased thermoregulatory demands, and decreased food availability. Songbirds are relatively small animals with large-surface-area-to-volume ratios and are, therefore, particularly subject to energetic constraints (Calder and King 1974). Given that the brain requires large amounts of energy to maintain signaling activities (see Ames 2000), regression of the song system outside the breeding season reduces the energetic costs imposed by the song nuclei. On balance, the reduced energy required by a regressed song system throughout the fall and winter may outweigh the energy required to regrow it the following spring.

Neuronal Turnover in HVC Occurs When Trophic Support Is Withdrawn

Most of the preceding hypotheses posit that neuronal turnover in HVC is functionally related to seasonal changes in motor or sensory aspects of song behavior. Plasticity in HVC may not be directly related to song, however. It may occur as a passive consequence of the sensitivity of HVC neurons to testosterone, its steroid metabolites, downstream trophic factors, such as BDNF, and signaling cascades that regulate phosphorylation of proteins related to cell proliferation, growth, and survival (Hidalgo et al. 1995; Dittrich et al. 1999; Hartog et al. 2009; Wissman and Brenowitz 2009; Chen et al. 2013; EA Brenowitz, unpubl.).

Increased circulating testosterone early in the breeding season initiates a trophic cascade that supports the survival of new neurons added to HVC (Rasika et al. 1994, 1999). As HVC grows, it provides transsynaptic trophic

support to neurons in its efferent targets RA and area X (Brenowitz and Lent 2001, 2002; Meitzen et al. 2007a; Brenowitz 2008). At the end of the breeding season, circulating T decreases to basal levels and the trophic cascade is terminated. As trophic support is withdrawn, both mature and new HVC neurons undergo apoptosis (Thompson et al. 2007; Thompson and Brenowitz 2008, 2009, 2010; Larson et al. 2014). As neurons are lost from the HVC→RA projection, song becomes shorter and more variable in structure. However, as noted above, birds do not need to produce stereotyped song to attract mates or defend territories during the fall and winter, and there is, consequently, no ecological cost to degradation of either the song circuits or song behavior. New neurons enter HVC in nonbreeding birds, but most die within weeks as long as plasma T levels remain low, as discussed above (Larson et al. 2014).

In this model, changes in song behavior are a passive consequence of, rather than the driving force behind, plasticity of the song-control circuits. This model clarifies several inconsistencies with the above models. (1) The model suggests that neuronal turnover in HVC could occur regardless of whether or not adult birds develop new songs. Seasonal patterns of neuronal addition to adult HVC are observed both in species that do and do not learn new songs. (2) This model suggests that neuronal turnover could occur independent of neural activity. As discussed above, neurons in the song nuclei RA and nXII_{ts} show high levels of activity when song is produced frequently during the breeding season, but new neurons are not added to these regions in adult birds. (3) This model also suggests that neuronal turnover is not just a consequence of cell death from excitotoxicity. HVC, RA, area X, and nXII_{ts} all contain neurons with glutamate receptors, but adult-born neurons are only added to HVC and area X, and neuronal addition is only seasonal in HVC. To the extent that this model resolves some of the observations that are inconsistent with the other hypotheses presented above, it may provide the most parsimonious explanation of neuronal turnover in the adult HVC.

Neuronal Turnover in the Hippocampus Enables Adult Birds to Form New Spatial Memories

The HC in birds is thought to play an important role in the formation of spatial memories in contexts including food hoarding, navigation, and migration (for review, see Barnea and Pravosudov 2011). Neuronal turnover in the adult HC may provide plasticity for encoding new spatial memories. New neurons added to the HC at different times may allow anatomical separation of memories formed at the different times (i.e., pattern separation [Clelland et al. 2009]). Hoshoo and Sherry (2007) measured food-caching behavior and neuronal addition to the HC in captive black-capped chickadees at four times of year. They found that food caching was greatest in October, and that neuronal recruitment to HC 1 wk after BrdU injection reached a peak in January. Using a 6-wk post-BrdU survival time, Barnea and Nottebohm (1994) found neuronal addition to HC to be greatest in semicaptive black-capped chickadees in October. Providing juvenile marsh tits the opportunity to cache food increased cell proliferation in the VZ of the HC, compared with matched-age tits that were not allowed to cache food (Patel et al. 1997). Captive adult mountain chickadees allowed to store food had more new doublecortin-expressing (DCX⁺) neurons in HC than birds deprived of this experience; both captive groups, however, had lower numbers of DCX⁺ neurons in HC than did wild chickadees (LaDage et al. 2010). Together, these studies suggest that neuronal addition to HC varies seasonally, roughly correlates with the time of greatest food caching and retrieval, and is influenced by experience in storing food.

Birds in many taxonomic groups are able to navigate over long distances. In some species, such as geese, juveniles learn the migratory route from their parents. Some birds migrate to the same destination with great accuracy, as in males that return to the same territory site each year. Other birds, such as homing pigeons are able to navigate over hundreds of miles to return to a home site. Successful navigation re-

quires that birds learn the location of their home site with reference to various cues, such as geographic landmarks, magnetic fields, polarization of sunlight, and olfactory cues (reviewed in Bingman and Able 2002; Bingman et al. 2003, 2005). Hippocampal-dependent spatial learning is essential for such long-distance migration and navigation. Lesion of the HC in homing pigeons disrupts navigation based on landmarks and the formation of spatial maps (Bingman et al. 2003, 2005). The role of the HC in migration and navigation raises the hypothesis that neuronal addition provides plasticity for encoding spatial memories necessary for long-distance orientation. Migration may take several weeks and ongoing neuronal addition may enable pattern separation of the different spatial memories that must be stored during the successive stages of these journeys. LaDage et al. (2011) reported that there are more DCX⁺ adult-born neurons in the HC of a migratory subspecies of white-crowned sparrow than a nonmigratory subspecies. A potential confound of this study, however, is that migratory birds experience intense and prolonged physical exercise, which could influence one or more components of neuronal addition to the HC (Kempermann et al. 2010).

SUMMARY

The avian song-control system offers several advantages as a model for studying adult neurogenesis. These advantages include: (1) the dedication of the neurogenic nuclei HVC and area X to the control of learned song; (2) the incorporation of new long-range projection neurons into a premotor circuit; (3) quantitative levels of neuronal addition; (4) the role of neurogenesis in large-scale changes in neuron number in HVC and the regeneration of the HVC→RA circuit in seasonally breeding birds; and (5) rich opportunities for comparative studies to test the role of neurogenesis in regulating different aspects of learned song.

Proliferative cells that give rise to new neurons reside in “hotspots” in the walls of the lateral ventricle. Most of the neuroblasts that give rise to new HVC neurons adopt a multipolar

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phenotype and follow a wandering, tortuous route from the VZ to HVC. Steroid sex hormones secreted by the gonads support the survival of adult-born HVC neurons. These hormone effects are mediated by the expression of genes that encode trophic factors, neurite extension, and vascularization. The survival of new neurons is also increased by song-induced activation of HVC cells and the electrical activity of postsynaptic neurons in RA. There is a functional linkage between the death of mature HVC neurons and the birth of new neurons.

Most birds breed seasonally and there is pronounced seasonal plasticity of the song-control circuits. Increased circulating levels of T early in the breeding season initiate a trophic cascade that supports the survival of new neurons, total neuron number in HVC increases dramatically, and the HVC→RA circuit is regenerated. At the end of the breeding season, plasma T levels decrease, the trophic cascade is terminated, mature and new HVC neurons undergo apoptosis, and the HVC→RA circuit degenerates.

It is unclear what the adaptive function of neurogenesis is in the adult song-control system. Various functional hypotheses can be proposed, but none has received unambiguous support. A constraint is that no study has yet directly manipulated levels of neurogenesis. There is an urgent need to develop pharmacological or genetic methods of manipulating neuronal addition to adult brain regions to allow definitive tests of functional hypotheses.

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