CHAPTER 15

What is the adaptive role of neurogenesis in adult birds?

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Introduction

Neurogenesis in adult brains was widely thought to be a specialization of lower vertebrates related to life-long changes in body size (Birse et al., 1980). In mammals, it was accepted that neurogenesis is restricted to the olfactory epithelium, despite early reports of neurogenesis in the rodent hippocampus (Altman and Das, 1965; Kaplan and Hinds, 1977; Bayer et al., 1982). This dogma of rare adult neurogenesis was further challenged by the work of Goldman and Nottebohm (1983), who described extensive neurogenesis in the brain of adult canaries, a songbird species. Since then it has become obvious that adult neurogenesis is a general feature of the forebrain and hippocampus of birds and that adult neurogenesis also occurs in various mammalian brain areas such as the hippocampus, the striatum, and the cortex, even in primates and humans (for review see Alvarez-Buylla and Kirn, 1997; Eriksson et al., 1998; Goldman, 1998; Gould et al., 1999a; Kornack and Rakic, 1999; Kaplan, 2001). Thus, besides anatomical and biochemical plasticity of permanent neurons and neuronal networks, the loss and addition of neurons in adulthood might be a major adaptive mechanism used by vertebrates to cope with changing environments. Alternatively, neurogenesis might be a repair strategy to maintain neural networks. The present paper examines the functional relevance of adult neurogenesis. We focus on birds since most hypotheses on the functional role of adult neurogenesis have been put forward within the realm of the vocal and food-storing behaviors of birds (Alvarez-Buylla et al., 1990; Barnea and Nottebohm, 1994; Kirn et al., 1994; Patel et al., 1997; Scharff et al., 2000).

Dynamics of protracted neurogenesis and neuronal loss in the adult bird brain

Regional specificity of neuron recruitment in the adult avian brain

Within a species, the maturation of various brain divisions, such as the brainstem and the forebrain, follow different time scales, with evolutionarily older brain divisions leading more recent ones. Generally, bird brain size, mass, and cell numbers reach adult values between 1 (most songbirds) and 3 (most larger birds) months of post-hatching age. Brain circuits such as the vocal control system of songbirds show delayed development relative to the period of general brain growth and may attain an adult phenotype only after several years (e.g. after 2 years in Mynahs (Gracula religiosa intermedia) (Rausch, 1985)), related to the age at which a species enters reproduction. Clearly, the distinction between a period of ontogeny and adult life is entirely academic
in view of the continued dynamics of the adult vertebrate brain (Breedlove and Jordan, 2001). Here we define ‘adulthood’ as the species-specific age of reproductive maturity.

In adult birds, newly born neurons are integrated only in defined subparts of the forebrain (Fig. 1), particularly in the hippocampus, the striatum (the lobus paraolfactorius), and the doroventricular ridge (Goldman and Nottebohm, 1983; Alvarez-Buylla et al., 1994; Ling et al., 1997). In the doroventricular ridge, new neurons are found in the neostriatum caudale and intermedium, and in the hyperstriatum ventrale, accessorium and intercalatus superior (Goldman and Nottebohm, 1983; Alvarez-Buylla et al., 1994; Ling et al., 1997). From a comparative point of view, these avian areas with protracted neurogenesis are homologous to the hippocampus of mammals and the cortex of reptiles (avian hippocampus) and to the striatum of mammals and reptiles (avian lobus paraolfactorius). The areas of the doroventricular ridge of birds have no homology in mammals although they function as cortex-analogous areas. Because adult neurogenesis is found in widely unrelated avian orders, the Galliformes (Japanese quail (Coturnix c. japonica)), Columbiformes (ring dove (Streptopelia risoria)), Psittaciformes (parakeet (Melopsittacus undulatus)), and Passeriformes (songbirds: canary (Serinus canaria), starling (Sturnus vulgaris), zebra finch (Tae-niopterygia guttata), Bengalese finch (Lonchura striata domestica), white-crowned sparrow (Zonotrichia albicollis gambelii), and black-capped chickadee (Parus atricapillus)), neurogenesis in the adult forebrain appears to be a general feature of male and female birds (Goldman and Nottebohm, 1983; Alvarez-Buylla et al., 1994, 1997; Barnea and Nottebohm, 1994; Ling et al., 1997). From a functional point of view, the avian areas with protracted neurogenesis include the vocal control system and the hippocampus, areas involved in the control of learned behaviors. The hippocampus appears to play a major role in the formation of spatial memory during food-storing behavior (for review: Clayton, 1998) and the vocal control system for vocal learning and singing (for review: Marler, 1991; Nottebohm, 1991).

The vocal control system of songbirds consists of interconnected areas in the fore-, mid-, and hind-brain that directly control the syrinx and indirectly control the respiratory muscles (Fig. 2A). The HVC (nucleus hyperstriatalis ventrale, pars caudale; also named higher vocal center) functions as a sensory-motor integration area that projects to the descending

![Fig. 1. The areas that frequently contain new neurons (black dots) in the adult avian brain depicted in schematic drawings of a medial (A) and lateral (B) sagittal plane. New neurons originate from the walls of the lateral ventricle (V). Note that new neurons are only found in certain parts of the forebrain but not in the midbrain or brainstem. Areas that contain new neurons are in the hippocampus (HP), in the lobus paraolfactorius (LPO), in the neostriatum (N) involving the neostriatum caudale (NC) and intermedium (NI), in the hyperstriatum ventrale (HV), accessorium (HA) and intercalatus superior. Further abbreviations: Cb, cerebellum; HD, hyperstriatum dorsale; PA, paleostriatum primitivum; ICo, nucleus intercollicularis; TEO, tectum opticum. (Based on data from: Goldman and Nottebohm, 1983; Alvarez-Buylla et al., 1994; Barnea and Nottebohm, 1994; Ling et al., 1997; and Gahr, unpublished.)

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(A) Medial view
(B) Lateral view

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motor control pathway of vocal production and contains a forebrain loop involved in auditory-motor feedback mechanisms for vocal learning and maintenance (Nottebohm et al., 1976; Bottjer et al., 1984; Scharff and Nottebohm, 1991; Yu and Margoliash, 1996; Wild, 1997; Brainard and Doupe, 2000).

Within a functionally defined neural system new neurons might occur only in certain areas within the same brain subdivision. Such an example is found in the vocal control system of songbirds. Among areas of the adult vocal control circuit, new neurons are found in the striatal nucleus Area X and in the neostriatal nucleus HVC (Goldman and Nottebohm, 1983; Alvarez-Buylla et al., 1994) (Fig. 2A). Other vocal control areas are located in the neostriatum such as l-MAN (lateral part of the nucleus magnocellularis anterioris), m-MAN (medial part of the nucleus magnocellularis anterioris) and NIF (nucleus interfacialis), and, even though new neurons are found throughout the entire caudorostral exten-

Fig. 2. In the vocal control system of songbirds (A) new neurons (black dots) occur in the HVC and Area X. The HVC is part of the descending motor control pathway of sound production (green arrows) and part of a forebrain loop (yellow arrows) involved in auditory-motor feedback and vocal learning. Vocal areas that express androgen receptors are in red, those that express androgen and estrogen receptors are in orange (HVC only), and those that express neither are in gray. The light-blue forebrain area is a neostriatal zone of high aromatase activity. (Data after Gahr and Metzdorf, 1997; Metzdorf et al., 1999; Fusani et al., 2000.) In B, the fate of new neurons (circles with bold rim) in the HVC is indicated in more detail. About half of the new HVC neurons are of the RA-projecting type (R) and half of the interneuron (I) type. The Area X-projecting HVC neurons (X) and certain subpopulations of the interneuron and RA-projecting type are not produced in adulthood. (Data after Paton et al., 1985, 1986; Alvarez-Buylla and Nottebohm, 1988; Nottebohm et al., 1994; and Scharff et al., 2000.) (C) Area X-projecting HVC neurons (red) and RA-projecting HVC neurons (green) in the HVC after retrograde labeling with red or green fluorescent beads. The yellow appearance of some neurons is not due to double-labeling, but due to superimposition of neurons in this 40-µm sagittal section of a male canary. Abbreviations: DLM, nucleus (n.) dorsolateralis thalami, pars medialis; DM, n. dorsomedialis of n. intercollicularis; HVC, n. hyperstriatalis ventrale pars caudale; l-MAN, lateral part of n. magnocellularis anterioris; m-MAN, medial part of n. magnocellularis anterioris; NIF, n. interfascialis; nXllts, n. nervi hypoglossi, pars tracheosyringealis; RA, n. robustus archistriatalis; nRAM, n. retroambigualis; rVRG, rostro-ventrale respiratory group; Uva, n. uvaeformis.
sion of the neostriatum, these areas do not recruit new neurons (Figs. 1 and 2A).

Within a defined brain area, neurogenesis might concern only particular neuron populations. This is evident in the HVC of the canary and zebra finch. There are three types of HVC neurons that differ according to their projection properties: those projecting to the RA (nucleus robustus archistriatalis), those projecting to Area X, and non-projecting interneurons (Fig. 2B,C). New neurons are only found in the RA-projecting and the local HVC neuronal populations (Paton et al., 1985; Alvarez-Buylla and Nottebohm, 1988) (Fig. 2B). About 50% of the new neurons appear to differentiate into either phenotype (Nottebohm et al., 1994; Scharff et al., 2000). Among the HVC interneurons, only a subpopulation not expressing GABA appears to be recruited in adulthood (Paton et al., 1986). Among the RA-projecting HVC neurons, too, a subpopulation (between 25 and 50%) appears to be permanent. This has been deduced from the finding that RA-projecting HVC neurons of adult canaries which accumulate the retrograde tracer horseradish peroxidase, are not born in adulthood (Paton et al., 1985).

Thus, in the avian forebrain, there are area-specific mechanisms for the recruitment of migratory neurons and for the postmigratory differentiation of such neurons.

**The amount of neuron recruitment in the adult avian brain**

Quantitative data are only available for the vocal control nucleus HVC and the hippocampus. From cell birthdating techniques using bromodeoxy-uridine (BrdU) and tritiated-methyl-thymidine, thymidine analogues that are incorporated into DNA during S-phase of mitosis, it appears that the incorporation of new neurons in the HVC shows important species differences. For the entire neuron population of HVC, values between 0.1 and 0.74% new neurons per day (of DNA labeling) are reported for canaries, between 0.1 and 0.2% new neurons for the zebra finch, and of about 0.4% for the Bengalese finch (Alvarez-Buylla and Nottebohm, 1988; Kirn et al., 1994; Rasika et al., 1994; Scott et al., 2000). These numbers might be higher within particular neuronal subpopulations of the HVC, but in general are in the above range, 0.3–1% new RA-projecting HVC neurons in canaries, 0.09% in the zebra finch, and 0.29% in the Bengalese finch (Rasika et al., 1994; Scott et al., 2000). In the hippocampus, between 0.15% and 0.37% (per day of DNA-marker injection) of all neurons are newly recruited (Barnea and Nottebohm, 1994). These numbers of new neurons seem to vary seasonally (Barnea and Nottebohm, 1994; Kirn et al., 1994) but might also be influenced considerably by interstudy differences in the criteria for counting labeled cells (see Kirn et al., 1994; Nottebohm et al., 1994; Rasika et al., 1994; Scott et al., 2000).

Quantitative data on neuron recruitment per day of DNA-marker injections might suffer both from over- and underestimation due to technical problems of cell birthdating. Although it is expected that the DNA-markers label only dividing cells that are in the S-phase within 90 min after systemic injection (Alvarez-Buylla et al., 1990), sequestration or storage effects of the DNA-markers are possible. This is suggested by in-ovo injections of tritiated thymidine and subsequent cell counting (Alvarez-Buylla et al., 1994). On the other hand, injections over several days but with different postinjection survival times result in similar numbers of thymidine-labeled HVC neurons (Scharff et al., 2000). However, no pulse-chase control experiments in neuronal birthdating have been carried out to rule out the possibility of overestimation completely. False counts due to DNA repair are unlikely with the DNA-labeling procedures commonly utilized (Palmer et al., 2000). Underestimation might result from the postulated brief period of DNA-marker incorporation following injections. Intra- and interspecies variations in the duration of the S-phase during mitosis or possible circadian mitotic activity appear, however, not to influence the quantification of mitogenic activity due to a long S-phase of avian progenitor cells. Similarly, injections once or twice per day followed by similar survival times give the same range of labeling in the HVC (e.g. Kirn et al., 1994; Scharff et al., 2000). Thus, although it is not entirely clear if the numbers reported above reflect neuron production exclusively at the time of DNA-marker injections or reflect neuron numbers generated during the entire time window that ends with removal of the brains, the quantitative estimates of neurogenesis per day of DNA-marker injections seem reasonable.
A second technical caveat for quantifying neurogenesis concerns the neural identity of the newly generated cells in the adult brain, since ongoing production of glial and endothelial cells is as evident in birds as it is in mammals. Nottebohm and colleagues elegantly showed that some of the cells generated in adulthood are neurons, since they are electrically active (Paton and Nottebohm, 1984), form synapses (Burd and Nottebohm, 1985) or develop axons (Alvarez-Buylla et al., 1988, 1990; Rasika et al., 1994). Neuronal identity is, however, deduced in some studies from uncertain morphological features such as soma size or the structure of the cell nucleus (e.g. Kirn et al., 1994, 1999). This might confound other seasonal and hormone-dependent brain changes with recruitment of new neurons, since substantial numbers of new glia and endothelial cells occur in HVC after testosterone or estrogen treatment (Goldman and Nottebohm, 1983; Hidalgo et al., 1995).

Nevertheless, protracted neurogenesis in the avian forebrain is well established and has been instrumental in understanding the underlying mechanisms of adult neurogenesis in general. It has also prompted a re-evaluation of neurogenesis in the mammalian brain.

Mechanisms of avian neurogenesis

Pluripotent progenitors that underlie neurogenesis in birds are located in the ventricular/subventricular zone of the lateral ventricle, both in embryonic and adult life (Goldman and Nottebohm, 1983; Alvarez-Buylla et al., 1990; Goldman et al., 1993). Such cells occur in higher abundance in particular zones of the ventricular wall, called hot spots (Alvarez-Buylla et al., 1990) (Fig. 1). The most active hot spot is found in the rostroventral part of the lateral ventricle. Protracted neurogenesis, then, requires the production of neuronal precursors, the migration of such precursor neurons out of the ventricular wall into the adjacent forebrain tissue, the recruitment of these neurons into brain areas, and their final differentiation. Most knowledge of these events was uncovered through the establishment of long-term explant cultures of the neostriatal ventricular/subventricular zone of songbirds by Goldman and colleagues (for a review see Goldman, 1998) (Fig. 3).

Stem cell mitogenesis

In cultures of the adult neostriatal ventricular/subventricular zone there is a frequent association of new neurons, ciliated ependymal cells, and ependymally derived radial cells (Goldman et al., 1993). Cell-lineage studies with a retroviral vector introduced into the neostriatal proliferating zone of adult zebra finches showed co-derived clones of neurons and radial cells (Goldman et al., 1996b). These results suggest a pluripotent progenitor cell in the adult avian brain capable of generating both neurons and their radial guide cells together. This progenitor of radial cells and neurons might be the radial cells themselves (Alvarez-Buylla et al., 1990; Gray and Sanes, 1992). Further, astrocytes might maintain progenitor function in birds as shown recently for the proliferating subventricular layer of the mammalian hippocampus (Doetsch et al., 1999; Laywell et al., 2000; Alvarez-Buylla et al., 2001; Seri et al., 2001). About 30% of cells born in the adult brain are thought to develop into neurons (Alvarez-Buylla and Nottebohm, 1988). Factors that control the mitotic activity of the progenitors in birds are not known. Gonadal steroids, testosterone or estrogens, do not modulate mitotic activity in the ventricular/subventricular zone (Goldman and Nottebohm, 1983; Brouwn et al., 1993; Rasika et al., 1994). Although thyroid hormones appear to decrease mitotic activity in the ventricular/subventricular zone of adult canaries (Arai and Saito, 1995), these data might not be functionally significant due to the exposure of the animals to very high dosages of thyroxine.

Neuronal departure from the proliferating zone

Neuronal migration into the adult songbird parenchyma occurs along a system of guiding fibers that originate from radial guide cells of the ventricular epithelium (Goldman and Nottebohm, 1983; Alvarez-Buylla and Nottebohm, 1988; Goldman et al., 1993; Alvarez-Buylla et al., 1998). The fibers of the radial cells extend from their ependymal cell bodies widely throughout the avian forebrain (Alvarez-Buylla et al., 1988), providing a scaffold upon which new neurons migrate and integrate into parenchymal areas distant from the ventricular proliferating zones.
Area-specific factors that attract or repel growing fibers of radial cells might define those brain areas that potentially recruit new neurons. Alternatively, radial cell fibers might invade all forebrain areas but only certain areas provide signals to arrest migratory neurons. How the young neurons find their final position once they depart from the radial cell fibers is not known. New cells originating from as yet unknown parts of the lateral ventricle reach HVC within 8–10 days after birth (Kirn et al., 1999).

The departure of new neurons from the proliferating ventricular zone might be induced by permissive signals as indicated by changes in protein expression of young cells prior to departure (Goldman, 1998). HU proteins, a protein family known to be expressed specifically by neurons throughout the adult
central nervous system (Brashear et al., 1991), appear in prospective new neurons within hours of their parental cell division (Barami et al., 1995). Such cells persist in the proliferating zone at least 4 days before initiating migration. This indicates a time window during which neuronal differentiation and initial migration is regulated in the adult brain (Barami et al., 1995) and suggest that adult neurogenesis is not just a by-product of ongoing gliogenesis. One factor that restricts neuronal departure from the adult subventricular/ventricular zone is the cell adhesion molecule N-cadherin (Barami et al., 1994). It is heavily expressed by adult ependymal and subependymal cells but shows little parenchymal expression. Furthermore, although cells containing both Hu proteins and N-cadherin are frequently found within the proliferating zone of adult canaries, N-cadherin expression is completely down-regulated before a new HU-positive cell departs from the zone. The role of N-cadherin for initiating cell migration is experimentally supported by explant culture work; the addition of antibodies directed against N-cadherin to cultures of the proliferating zone accelerates the migration of neuronal cell bodies from these explants (Barami et al., 1994). In contrast, antibodies directed against a variety of other surface proteins exhibit no such effect on emigration (Barami et al., 1994). These observations suggest that the onset of neuronal migration depends on (unknown) signals that induce a preceding down-regulation of N-cadherin in newly born cells.

**Survival of migratory and recruited new neurons**

The recruitment of new neurons could depend on their intrinsic life-span, trophic factors that affect survival during migration, factors that arrest the migration, and postmigratory survival factors. Detailed studies on survival during migration or subsequent to recruitment have been carried out only in the caudal neostriatum including the HVC of songbirds. Songbirds (oscine passerines) include about 50% of all bird species (Sibley and Ahlquist, 1990). Based on comparative knowledge of forebrain chemistry we comment on the generality of the events deduced from songbirds for non-songbirds.

In songbirds, migratory neurons that depart from the caudomedial zone of the lateral ventricle travel through areas of high estrogen receptor densities and estrogen-producing enzyme (aromatase) concentration (Schlinger and Arnold, 1991; Gahr et al., 1993; Metzdorf et al., 1999; Gahr, 2000) (Fig. 3). Further, insulin-like growth factors are expressed in the radial-guide cells of the new neurons and in neostriatal astrocytes (Jiang et al., 1998). Recruitment and subsequent survival of new cells in the HVC might be sensitive to a variety of environmental information. This environmental input to HVC is electrical in the case of auditory (Doupe and Konishi, 1991) and visual (Bischof and Engelage, 1985) stimuli, and hormonal in case of gonadal steroids (androgens and estrogens) (Gahr et al., 1987, 1993; Gahr and Metzdorf, 1997; Bernard et al., 1999; Metzdorf et al., 1999) and melatonin (Gahr and Kosar, 1996; Bentley and Ball, 2000). Further, the HVC contains neurons that produce factors known to be neurotrophic, such as brain-derived nerve growth factor (BDNF) (Dittrich et al., 1999), insulin-like growth factor 2 (IGF-2) (Holzenberger et al., 1997), and retinoic acid (Denisenko-Nehrbass et al., 2000). Thus, environmental information including sociosexual interactions could affect neuron recruitment in the HVC directly (sensory input) or indirectly (hormonal input) via alterations in the local expression of trophic factors and their receptors. This is supported by the reduced recruitment of new neurons in the HVC of zebra finches deafened as adults (Wang et al., 1999) and the relation between the number of new forebrain neurons and the complexity of the housing environment of adult finches (Lapkind et al., 2002).

**Seasonality, steroid hormones and neuronal recruitment and survival**

The recruitment and survival of new neurons in the hippocampus of chickadees and in the HVC of domesticated canaries fluctuates seasonally (Barnea and Nottebohm, 1994; Kirn et al., 1994). The most likely candidate for the regulation of seasonal fluctuations is the availability of gonadal steroid hormones, testosterone and its estrogenic metabolite 17β-estradiol. Gonadal testosterone production follows a seasonal pattern in male canaries (Nottebohm et al., 1987; Leitner et al., 2001a) while estrogens are derived from testosterone in the caudal neostriatum (Schlinger and Arnold, 1991; Metzdorf et al., 1999).
and hippocampus (Metzdorf et al., 1999; Saldanha et al., 2000) through high activity of local aromatases. Aromatases are testosterone-sensitive in the caudal neostriatum (Fusani et al., 2001). Testosterone and estrogens might act on the HVC or hippocampus via androgen- and estrogen-receptors, respectively (Gahr et al., 1993; Gahr and Metzdorf, 1997; Metzdorf et al., 1999). X-projecting HVC neurons mainly express estrogen receptors, interneurons might contain one or the other, and RA-projecting HVC neurons frequently contain androgen receptors (Gahr, 1990a,b; Johnson and Bottjer, 1997). Furthermore, the expression level of these receptors per HVC neuron appears to undergo some seasonal variation (Fusani et al., 2000).

For the discussion of seasonality and hormones in relation to neuronal recruitment in the adult HVC, we need to consider the following observations: (1) New HVC neurons are recruited all year round with an increased recruitment in fall (Kim et al., 1994). (2) The period of recruitment of neurons born during a similar time window is extended in fall compared to spring (Nottebohm et al., 1994), which might be due to a reduced survival probability for neurons born in spring. (3) Neurons born both in fall and in spring may survive for long periods but the probability of prolonged survival is higher in fall (Nottebohm et al., 1994). The survival probabilities do not depend on the new neurons but on their postmigratory environment. (4) Newly recruited neurons differentiate into either interneurons or RA-projecting HVC neurons (Nottebohm et al., 1994; Scharff et al., 2000). (5) Estrogens appear to increase the recruitment and/or survival of new neurons (Hidalgo et al., 1995). (6) Testosterone appears not to affect neuronal recruitment but to increase the survival probability of new HVC neurons that develop the RA-projecting phenotype (Goldman and Nottebohm, 1983; Rasika et al., 1994). The observation that estrogens are the active hormones in regulating survival of migrating neurons and/or their recruitment and survival in the HVC might explain the lack of correlation between seasonal testosterone production and seasonal changes in neuron recruitment in the HVC (Fig. 4A).

Estrogens are unlikely to act directly as a neurotrophic factor in this system since new neurons neither express estrogen receptors during migration (Gahr, 1990b; Hidalgo et al., 1995) nor after recruitment into HVC (Gahr, 1990a). This led to the idea that the estrogen receptor expressing neurons constitute a layer of mitotically quiescent neurons, which might modulate the survival of the new neurons during their first days of migration (Hidalgo et al., 1995). In the medial part of the caudal neostriatum, the new neurons need to travel through a layer of estrogen receptor-containing, mainly X-projecting HVC neurons. The estrogen receptor-containing neurons are sandwiched between the ventricular zone and a neostriatal area of high aromatase activity (Metzdorf et al., 1999; Fusani et al., 2000) (Fig. 3), i.e. the new neurons also need to migrate through an area with high local estrogen production but without estrogen receptors. Thus estrogens could affect new neurons indirectly due to factors released from estrogen receptor-expressing neurons, which they encounter during migration or in the HVC. Alternatively, estrogens could act directly on the new neurons via non-genomic mechanisms, not requiring estrogen receptor expression (Fig. 3).

Estrogenic action on migratory neurons appears to involve the estrogen-dependent regulation of cell adhesion molecules such as NgCAM (Goldman et al., 1996a; Williams et al., 1999). NgCAM is of particular interest in this respect since the addition of antibodies directed against NgCAM disrupts neuronal migration in cultures of the zebra finch ventricular zone. NgCAM affects calcium-mediated signaling in new neurons (Goldman et al., 1996a; Williams et al., 1999). Since new neurons of estrogen-treated cultures enter apoptosis significantly later compared to untreated cultures (Goldman et al., 1996a), estrogen might increase the survival probability of new neurons during migration via the above mentioned mechanisms (Fig. 3). This scenario requires that new HVC neurons originate from the caudomedial lateral ventricle, but it would not account for neurons departing from the proliferating zone anterior to the HVC and the rostroventral part of the ventricle, due to the lack of estrogen receptor and/or aromatase expression there (Gahr et al., 1993; Metzdorf et al., 1999).

Alternatively or in addition to an estrogen-dependent alteration of survival during migration, estrogens might affect the recruitment and/or survival of new neurons that travel through the HVC. Testosterone-dependent BDNF expression is thought
Fig. 4. Plotted are the monthly amounts (means) of new HVC neurons, pycnotic HVC cells, new song syllables, and testosterone plasma levels of adult male canaries. There is no correlation between the testosterone levels and new neurons collected in the same month (A) or the following months (data not shown). There is no correlation between the number of pycnotic cells and that of new neurons of the same month (data not shown) or of the following month (B) or of that of 2 months later (data not shown). There is further no correlation between the number of new neurons and of new syllables of the same month (C) or the following month (data not shown). Finally, there is no relation between testosterone levels and new neurons of the same (D) or following month (not shown) or second following month (not shown) (\( P > 0.1 \) in all cases, Pearson correlation test). (Values of new syllables are from Nottebohm et al., 1986 (Fig. 4), of new HVC neurons from Kirn et al., 1994 (Fig. 3A), of pycnotic cells from Kirn et al., 1994 (Fig. 3B), and of testosterone from Nottebohm et al., 1997 (Fig. 5).) Number of new neurons and pycnotic cells are per 1000 HVC neurons. In C and D there are only 11 data points due to the lack of behavioral data for 1 month (see Nottebohm et al., 1986).

to increase neuronal survival in the HVC (Rasika et al., 1999). We propose that this happens via an estrogen-dependent regulation of BDNF in the HVC that does not involve estrogen receptors (Dittrich et al., 1999; Fusani et al., 2002). In the HVC, BDNF is expressed (among others) in RA-projecting neurons that do not express the BDNF-receptor trkB (Dittrich et al., 1999). A further source of steroid-driven BDNF production appears to be of endothelial origin (Louissaint et al., 2002). Estrogens induce the expression of the receptor of vascular endothelial growth factor, which appears to promote mitotic angiogenesis in the HVC. Adenoviral inhibition of the tyrosine kinase activity of this receptor reduces the number of new HVC-neurons in adult canaries, which indicates a causal interaction between testosterone-induced angiogenesis and neurogenesis in adult songbirds (Louissaint et al., 2002). Thus, estrogen-dependent BDNF expression might either increase survival of new HVC neurons via unknown paracrine mechanisms or due to postsynaptic activity at the HVC to RA synapse and subsequent retrograde signaling that supports survival of RA-projecting HVC neurons. Unfortunately, it is not known if survival of new HVC neurons depends on the presence of their RA target neurons in adulthood.

Another hormone that might regulate seasonal recruitment and survival of HVC neurons is melatonin, which is produced in strict relation to the photoperiod. The HVC of songbirds expresses high levels
of melatonin binding sites (Gahr and Kosar, 1996; Bentley and Ball, 2000). Adult canaries maintained on long-day photoperiods have fewer dying cells in the HVC (indicated by the number of pycnotic cells) compared to animals maintained on short-day photoperiods (Kirn and Schwabl, 1997). This effect of photoperiod appears to be independent of the circulating levels of testosterone (Kirn and Schwabl, 1997), although the photoperiod is likely to affect testosterone production in canaries (Nottebohm et al., 1987; Leitner et al., 2001a). The suggestion of melatonin as a seasonal factor in neural replacement merits further scrutiny in light of melatonin’s role as a neuroprotective agent (e.g. Persengiev, 2001), which is in contrast to the increased number of dying cells in canaries maintained on short-day photoperiods.

Other trophic factors involved in recruitment and postmigratory survival include retinoic acid, and the insulin-like growth factors IGF-1 and IGF-2. IGF-1 supports the survival and differentiation of neural progenitor cells in rodents (DiCicco-Bloom and Black, 1988; Drago et al., 1991; Sockanathan and Jessel, 1998). IGF-1 is heavily expressed in the radial-guide cells of the new neurons and IGF-2 is expressed in parenchymal astrocytes and thus might support migratory neurons (Jiang et al., 1998). However, in vitro, neither factor appears to extend the lifespan of the new migratory neurons (Jiang et al., 1998). In the HVC, IGF-2 protein and retinoic acid-producing enzyme are found in the X-projecting neurons of adult canaries (Holzenberger et al., 1997; Denisenko-Nehrbass et al., 2000). However, since X-projecting neurons are neither born nor replaced in adulthood (Gahr, 1990; Hidalgo et al., 1994) nor does the experimental reduction of their abundance affect recruitment of other HVC neurons (Scharff et al., 2000), a role for retinoic acid and IGF-2 in the recruitment and survival of new HVC neurons is unlikely. Stress hormones, such as glucocorticoids, are involved in neural death and recruitment in the rodent hippocampus (Cameron and McKay, 1999; Gass et al., 2000) but have not been studied in songbirds in the context of neurogenesis.

Genomic or non-genomic effects of estrogens as well as those of other trophic factors on neuronal recruitment and survival are likely to be permissive. Since the number of new neurons in the zebra finch HVC is low compared to adult canaries, despite rather similar distributions of neostriatal aromatase activity, estrogen receptors (except the lateral HVC) (Gahr et al., 1993; Metzdorf et al., 1999), androgen receptors (Gahr and Metzdorf, 1997), melatonin receptors (Gahr and Kosar, 1996; unpublished) or BDNF expression (Dittrich et al., 1999; Rasika et al., 1999; Fusani et al., 2002) other events determining neuronal life-span (see below) need to be considered as potential agents active during neuronal recruitment.

Is neurogenesis related to neuronal death?

In the following, we discuss evidence relating each of the different steps of neurogenesis (birth of neuror progenitors, neuron migration, neuron recruitment, and final neuron differentiation) to neuronal death in a particular brain area. Again, we focus on the HVC of songbirds due to a lack of data for other brain regions. Direct evidence that neuron replacement occurs comes from the population of RA-projecting HVC neurons of adult domesticated canaries. The HVC of these canaries undergo seasonal changes in morphology on the background of an overall constant HVC volume (Gahr, 1990a, 1997; Kirn et al., 1991; Kirn and Schwabl, 1997; Leitner et al., 2001a; but Nottebohm, 1981; Nottebohm et al., 1994). Kirn and Nottebohm (1993) labeled the RA-projecting HVC neurons present in April with a vital retrograde marker, which enabled them to identify the same neurons many months later. Thirty to 50% of the projection neurons labeled in April are lost and replaced by October. It is unlikely that all such HVC neurons are replaced within the course of a year since a subpopulation of RA projecting neurons appears to be permanent (Paton et al., 1985, 1986). Furthermore, there is no correlation between monthly cell death and neuronal recruitment, even if we assume that cell death might precede neuronal recruitment by 1 or 2 months (Fig. 4B). The data on seasonal cell death in the HVC are difficult to interpret due to their variability but suggest increased cell death after the breeding period (Kirn et al., 1994).

A second study that addresses directly the relationship between neuron death and neuron recruitment was performed in the HVC of adult male zebra finches by means of a cell-specific ablation
technique (Scharff et al., 2000). In this study, RA-or X-projecting HVC neurons were retrogradely labeled with a laser-sensitive dye, which induces cell death after photostimulation. Only the induced death of the RA-projecting neurons leads to an increased incorporation of new HVC neurons, which are of the RA-projecting neuron and interneuron type. This suggests that the death of only certain neuron types increases the recruitment of new neurons (Scharff et al., 2000). Alternatively, the differences in the effect of the lesion of RA- or X-projecting neurons might result from a higher number of photolesioned neurons per unit area due to the approximately 3-times greater cell density of RA- versus X-projecting HVC neurons (see Fig. 2C). This scenario would indicate why similar lesions in juveniles lead to an increased recruitment of neurons in either case, since the density of X-projecting neurons per unit HVC volume is higher in juvenile zebra finches compared to adults (Gahr and Metzdorf, 1999). Nevertheless, the work of Scharff et al. (2000) suggests some relation between neuron death and recruitment, even if the underlying mechanisms remain unknown.

The suggestion that vacancies within a brain area due to neuron death are related to the cessation of migration of new neurons needs some mechanistic hypothesis, but appears not to guarantee network homeostasis since dead Area X projecting HVC neurons are not replaced (Scharff et al., 2000) and since the death of RA-projecting neurons also leads to a drastically increased recruitment of new HVC interneurons (Scharff et al., 2000). Apparently, the local environment in HVC appears to restrict differentiation of neurons recruited in adulthood but does not maintain the network. Thus neurogenesis would not be a repair mechanism in case that the photolytic death of RA-projecting HVC-neurons does not induce collateral neuronal death of interneurons. The recruitment of neurons into the adult HVC requires vital neighboring cells, since focal excitotoxic lesions of about 100 µm in diameter are not re-populated (Rybak and Gahr, unpublished). These findings in songbirds are of general interest since they indicate that: (1) neuronal death does not necessarily induce neuronal recruitment even if young neurons are ‘available’; and (2) enhanced neuronal recruitment following neuronal death does not lead to cell-type specific replacement. Although injury can induce neurogenesis in neocortical regions of adult mice that normally do not recruit new neurons (Magavi et al., 2000), the songbird data suggest that this does not guarantee replacement of all neuron types in local networks.

In summary, a whole battery of factors, which might be regulated differentially by internal and external environments, appear to influence neuron survival and recruitment in the HVC. For the HVC, the most parsimonious scenario of neuronal recruitment and survival is that there are permanent HVC-neurons and those with life-spans that depend on environmental signals. Further, production of new neurons is a continuous process in adult songbirds. Neurons that travel through HVC by chance might be recruited, i.e. some local signals arrest cell migration. Neurons recruited into the HVC start to differentiate into interneurons or RA-projecting neurons. Either neuron type needs to compete with other neurons or other recently recruited neurons for synaptic partners and trophic factors, reminiscent of neuron overproduction during early brain development (Cowan et al., 1984). This competition is facilitated in times of network instability, which might occur due to seasonal production of various hormones. Recruited neurons that compete successfully become long-lived neurons, those that do not die (Fig. 3). All new long-lived neurons compete with newly recruited neurons in the future and may eventually die. Whether neuron recruitment is then a by-product of hormone-induced plasticity, or whether hormones make certain neuron populations inherently less stable over time, altering the probability of cell death under stressful physiological conditions, and requiring recruitment of reserve neurons, remains to be seen.

Survival mechanisms involving hormonal action in the bird neostriatum and striatum must be different for neurons originating from different parts of the ventricular zone. In songbirds, only neurons originating from progenitor cells of the caudomedial and the HVC ventricular zones would experience an estrogen-sensitive environment during early migration. Thus, only neuronal recruitment and death in the HVC, but not in Area X or most other forebrain areas, would be sensitive to hormone receptor-dependent mechanisms. These conclusions are based on the lack of androgen receptors, estrogen recep-
tors, and aromatase in Area X (and the entire lobus paraolfactorius) and most forebrain areas and on the lack of these proteins in the parenchyme adjacent to the dorsolateral and rostroventral ventricular zone of songbirds (see Fig. 3) (Gahr and Metzdorf, 1997; Bernard et al., 1999; Metzdorf et al., 1999). Estrogen and androgen receptor expression as well as aromatase activity is rare or completely absent in the forebrain of non-songbirds with the exception of the hippocampus (Gahr et al., 1993; Gahr and Metzdorf, 1997; Saldanha et al., 1998, 2000; Metzdorf et al., 1999; Gahr, 2000), suggesting that gonadal hormone-dependent survival and recruitment of new neurons or other cell types in the forebrain of non-songbirds is unlikely, except in the hippocampus.

The role of neurogenesis in brain function

The most problematic question concerns the ultimate mechanism of neurogenesis, i.e. its adaptive role in brain function and behavior. One must evaluate the need for neurogenesis against the background of a general plasticity of permanent neural components in brain areas involved in behavioral control, such as the HVC and the hippocampus. Neural plasticity is demonstrated in numerous studies of the songbird forebrain. Such plasticity, seasonal or hormone-dependent or injury-induced, occurs in areas that do not show neurogenesis (e.g. adult RA: DeV oogd and Nottebohm, 1981; DeV oogd et al., 1985), in permanent neuron populations of the HVC (Gahr, 1990a; Johnson and Bottjer, 1993; Halle et al., 2002), and in neuronal populations with adult neuronal recruitment (Johnson and Bottjer, 1993; Dittrich et al., 1999; Rasika et al., 1999).

Besides a role in behavioral plasticity, seasonal neuronal loss and recruitment is proposed as a mechanism to adjust the energy budget to the environment. Here we do not discuss this idea further for the following reasons: songbirds use between 1.5 and 2.1% of their forebrain for song production (Gahr, unpublished). The only areas that recruit new neurons in adulthood are the Area X and the HVC, which constitute about 1.1% and 0.2% of the telencephalon, respectively, but are not related to forebrain size and show high individual variability (Airey and DeV oogd, 2000; Gahr, unpublished). Neuronal investments in Area X are not special for singing since it develops as a subpart of the lobus paraolfactorius, a structure that is similar in size in singing males and non-singing females, such as the female zebra finch. The special investment hypothesis remains viable for HVC neurons. However, neurogenesis is an ongoing process in adult birds and is not related to whether the birds differentiate forebrain vocal areas or not, i.e. it is not related to the differentiation of vocal control neurons (Ling et al., 1997). The cost argument would only warrant further discussion if vocal learning and/or changes in vocal pattern were related to increased stem cell mitogenesis.

The concept that neurogenesis in adulthood is a process for updating and renewing memories (Nottebohm, 1991) has received support from inter- and intraspecies comparisons of food-storing behavior and vocal behavior. In young food-storing animals, initial experience in food-storing increases the amount of neurogenesis in the hippocampus, which correlates with increases in hippocampus size (Patel et al., 1997). This raises the question of whether the motor activity of performing this behavior increases the capacity of the hippocampus (via neurogenesis) to acquire spatial memories during later life. When the amount of adult neurogenesis was not shown to correlate with the intensity of food-storing behavior, the latter was suggested to correlate with hippocampus size. However, among closely related species such as woodpeckers, there are food-storing and non-storing species with no obvious differences in hippocampus volume (Volman et al., 1997). Similarly, the lack of seasonal changes in volume or neuron numbers of the hippocampus of food-caching gray squirrels, a long-lived mammal, does not support the hypothesis that seasonal variations in food-storing behavior correlates with morphological changes (Lavenex et al., 2000). Such species discrepancies suggest that neurogenesis may be permissive for spatial learning but is not determining it.

We suggest, therefore, that observations of neurogenesis in homologous brain areas like the hippocampus, of species with clear differences in the behavior thought to be sensitive to neurogenesis does not really aid in the evaluation of the adaptive role of neurogenesis. Consequently, it is doubtful if species differences in the rate of neuronal recruitment in the HVC of songbirds with differences in adult vocal learning, such as the canary, the Bengalese finch
and the zebra finch (Scott et al., 2000) will provide much insight into a functional role for neurogenesis. Similarly, the lack of neurogenesis in most parts of the vocal control system such as the RA of songbirds (Goldman and Nottebohm, 1983) suggests that anatomical and functional plasticity (e.g. seasonal changes in RA synapse densities (DeVoogd and Nottebohm, 1981) and production of song syllables) are possible without neurogenesis. Clearly, different parts of a neural circuit could have evolved different mechanisms underlying behavioral plasticity, but this will not value or devalue the role of new neurons in other areas.

In the following, we discuss the pros and cons of an adaptive role for neurogenesis in the function of the vocal system of the canary, but first give some background data on vocal plasticity and learning of songbirds. Despite indications that Area X plays an important role in vocal learning and maintenance of learned vocal patterns (Scharff and Nottebohm, 1991; Brainard and Doupe, 2000), the functional role of new neurons in Area X has not been studied. In the HVC, protracted neurogenesis and neuronal death are thought to be involved in auditory–vocal learning, in loss of vocal motor memories, and in seasonal alterations of song temporal patterns (Nottebohm et al., 1986; Rasika et al., 1994). We will not discuss the role of new neurons in seasonal changes in the size of the HVC in detail (Nottebohm, 1981; Nottebohm et al., 1994; but see Gahr, 1990a, 1997; Kirn et al., 1991; Kirn and Schwabl, 1997; Leitner et al., 2001a,b), as this problem has been the subject of previous reviews (Gahr, 1997; Tramontin and Brenowitz, 2000; Bolhuis and Macphail, 2001). Currently, there is no theory to indicate that the size or total number of neurons of any forebrain vocal control area (or the hippocampus) matters for behavioral control, beyond a minimum area size and neuron number (Gahr et al., 1998).

Oscine songs are species-specific vocalizations composed of more or less frequency-modulated sounds that are produced following a particular temporal pattern. In domesticated and free-living canaries, the song of reproductively active males is characterized by long repetitions of individual song units (syllables). A syllable is composed of one or several frequency modulated units (elements) (Güttinger, 1985; Leitner et al., 2001b). Since songbird song is not innate, birds need to form an auditory memory and then learn the muscle movements for its production, which requires auditory–motor feedback (Konishi, 1965). The learning period is species-specific. In some species, it appears restricted to a defined ontogenetic window (e.g. the zebra finch) while other species seem to learn new vocalizations life-long or to periodically enter new learning windows (e.g. the canary) (for a review see Marler, 1991). However, even in species with a restricted learning period such as the zebra finch, continued auditory-motor feedback is necessary to maintain the learned song (Nordeen and Nordeen, 1992; Leonardo and Konishi, 1999). An individual might memorize many more auditory patterns (depending on adequate access to such patterns) than it finally produces and might select parts of different memories to recombine into its own song. This selection process is thought to be influenced by sociosexual experience (West and King, 1988; Marler, 1997) and might change during an animal’s life-time, leading to the activation of different vocal memories (Hough et al., 2000). Further, the extent to which birds copy temporal and/or spectral patterns from external models appears to vary between species (Marler, 1991). In the canary, the temporal pattern develops according to innate rules, while most of the spectral features are learned (Güttinger, 1979, 1981). Their learning of spectral features (syllables) appears to occur seasonally with a focus of new learning during the autumnal non-breeding period, although new syllables appear also in the breeding period (Nottebohm et al., 1986).

Besides vocal learning, a second source of plasticity in vocalizations of adult songbirds comes from a dependency on gonadal steroid hormones, testosterone and its estrogenic metabolites. Testosterone is required for the development and production of songs of reproductively active male birds (Pröve, 1974; Heid et al., 1985; Nottebohm et al., 1987; Rost, 1990; Schwabl and Kreiner, 1991; Smith et al., 1995, 1997; Leitner et al., 2001a). Testosterone treatment induces male-typical song pattern in females in many species, in which females normally sing only rudimentary songs or female-typical songs (Leonard, 1939; Nottebohm, 1980; Vallet et al., 1996). Testosterone-dependent induction of songs typical of a reproductively active male, takes several
weeks (Pröve, 1974; Nottebohm, 1980; Heid et al., 1985; Vallet et al., 1996). Thus, the seasonal change in vocal activity and the structure of vocalizations of seasonal breeding species is thought to depend mainly on seasonal changes in testosterone production. Other seasonal factors such as the nightly period of melatonin production have been examined but appear to be of minor relevance for singing (Dlonek and Deviche, 2001), although morphogenetic effects of melatonin on vocal control areas have been reported (Bentley et al., 1999). A role for gonadal hormones in vocal learning has been proposed for both juvenile (Marler et al., 1988; Bottjer and Hewer, 1992) and adult (Nottebohm et al., 1987) songbirds.

Androgens and estrogens might have differential roles for the control of various temporal features, the type of syllables produced, and new syllable learning. In the domesticated canary, estrogens are necessary for one parameter of the song motor pattern, the syllable repetition rate, while other motor features such as the duration of syllable repetitions are androgen-dependent (Fusani et al., 2002). This suggests that seasonal changes (Nottebohm et al., 1986, 1987; Leitner et al., 2001a) and the effect of castration (Heid et al., 1985) on the temporal song pattern of male canaries depends both on estrogens (repetition rate) and androgens (tour duration). In free-living canaries the seasonal change in the syllable repertoire composition correlates with seasonal changes in the temporal organization of the song (Leitner et al., 2001a) (Fig. 5). In particular, fast-frequency modulated syllables (which are sexually attractive for female canaries (Kreutzer and Vallet, 1991; Leitner et al., 2001b)) correlate with high repetition rates and are more frequent during the breeding period (Leitner et al., 2001a). The increase in the repertoire of sexually attractive syllables and

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**Fig. 5.** Seasonal and annual changes in the total number of song syllables (A), the number of permanent song syllables (B), testosterone plasma levels (C), and the size of the forebrain vocal areas HVC and RA (D) of individual free-living wild male canaries. The size of the syllable repertoire and the size of the vocal control areas remained unchanged, whereas a remarkable seasonal change occurred in the plasma testosterone (T) level and in the repertoire composition. The individuals recovered, however, many syllables that were seasonally lost on an annual basis. Volumes are medians and quartiles. T levels are medians and quartiles of the entire breeding or non-breeding seasons. Connected dots in (C) indicate seasonal changes in the T levels of the same individuals (From Leitner et al., 2001a.)
repetition rates correlates with seasonal increase in testosterone production of free-living canaries (Leitner et al., 2001a) (Fig. 5) The question of whether androgens and estrogens affect the vocal learning in adult wild canaries has not been studied experimentally. For adult domesticated canaries, testosterone was thought to be negatively correlated with the occurrence of new syllables (Nottebohm et al., 1987; Kirn et al., 1994). Our analysis, however, shows that there is no such relationship (Fig. 4D).

The endocrine approach of Fusani et al. (2002) indicates that the HVC controls high repetition rates, because it is the only part of the vocal system that expresses estrogen receptors in adult canaries. Electrophysiological studies show that the activity of HVC units in the zebra finch correlate with the generation of song syllables (Vu et al., 1994; Yu and Margoliash, 1996). The temporal organization within a syllable of adult male canaries can change seasonally (Leitner et al., 2001b). These data suggest that the HVC is involved both in the control of learned (spectral) and innate (temporal) features of the canary song. We discuss neuroanatomical changes in the HVC in relation to neurogenesis, vocal learning (syllables), and seasonal plasticity in non-learned (temporal) song pattern of adult canaries below.

Plasticity of permanent neurons, neurogenesis, and innate vocal pattern

The temporal song pattern of canaries develops according to innate rules as long as testosterone levels are high either in development or in adulthood (Leonard, 1939; Güttinger, 1979; Nottebohm, 1980; Nottebohm et al., 1986; Güttinger et al., 1990; Fusani et al., 2002). In testosterone-treated females that produce a male-typical temporal pattern and those that produce reduced high-repetition rates of syllables, the volume of HVC is identical; the latter were also deprived of estrogen production (Fusani et al., 2002). This indicates that: (1) the masculinization of HVC size depends on testosterone or its androgenic metabolites but not on estrogens; and (2) the androgen-dependent masculinization of the HVC is not sufficient for the production of a sexually attractive temporal pattern, such as high syllable repetition rates (Fusani et al., 2002). Testosterone treatment of female canaries has been shown to increase HVC volume, which correlates with an increase in the number of new RA-projecting HVC neurons, although the total number of this neuron type is not affected by testosterone (Rasika et al., 1994). Thus the reduced syllable repetition rates in the estrogen-deprived testosterone treated females (Fusani et al., 2002) might be due to a reduced number of RA-projection neurons. As discussed above, estrogens might stimulate the migration and incorporation of new neurons in the canary forebrain (Hidalgo et al., 1995; Williams et al., 1999). This question requires further work, since other temporal features such as song-length are masculinized in testosterone-treated, estrogen-deprived female canaries (Fusani et al., 2002). We currently have no hypotheses to explain how new HVC neurons could be relevant for syllable repetition rates but not for other vocal temporal patterns.

Alternatively, estrogens might be necessary to tune particular electrical properties of permanent HVC networks, neurochemical properties of HVC neurons, or synapse densities. The number of somatic electrical synapses increases after testosterone treatment in HVC (Gahr and García-Segura, 1996). Estrogens have been shown to mediate this testosterone-induced increase in the density of gap-junctions in a number of mammalian brain areas (for a review see García-Segura et al., 1994). In the HVC, estrogens could induce gap-junctions via estrogen-receptors expressed in the X-projecting HVC neurons because most of these cells are in close contact with the somata of RA-projecting neurons (Gahr, 1990a) (Fig. 2C). A group of factors that could mediate the effect of estrogens on synaptic and electric properties of HVC neurons are the members of the neurotrophin gene family, such as BDNF, due to their well known effects on differentiation and function of central neurons (Bonhoeffer, 1996; Marty et al., 1997; Altar and DiStefano, 1998). In the zebra finch, the estrogen-dependent expression of BDNF mRNA in the song system is limited to the HVC, and occurs particular in RA-projecting HVC neurons (Dittrich et al., 1999). In the canary HVC, testosterone up-regulates BDNF levels (Rasika et al., 1999). This effect of testosterone in the canary HVC can be blocked with aromatase inhibitors and is, therefore, estrogen mediated, similar to the zebra finch HVC (Fusani et al., 2002).
Plasticity of permanent neurons, neurogenesis, and vocal learning

In wild male canaries, there is a seasonal change in the composition of the syllable repertoire while the total number of syllables of individuals remains constant between seasons. The seasonal change concerns the more abundant production of sexually attractive syllables in the breeding season (Leitner et al., 2001a,b) (Fig. 5). These changes are accompanied with seasonal changes in testosterone-levels but not in changes of HVC volume and HVC neuron morphology (Leitner et al., 2001a,b). Thus, it appears unlikely that seasonal changes, even short-term changes, in HVC size (Nottebohm, 1981; Kirn et al., 1991) or morphology (Gahr, 1990a), as suggested for domesticated canaries, account for the increased production of sexually attractive syllables in the breeding season of wild canaries. Such a scenario would require that the forebrain vocal areas undergo two cycles of growth and degeneration between breeding seasons. The transition period between non-breeding and breeding singing of wild canaries is not accompanied by degenerative changes in HVC size (Leitner et al., 2001a,b). As a consequence, HVC size and neuron number do not appear to be functionally related to syllable repertoire compositions. We think it is prudent to be cautious about linking the number of learned motor patterns to the size of brain structures involved in this task (Nottebohm, 1981; Nottebohm et al., 1986; Ward et al., 1998), since the overt motor memories at any time point might differ from the total motor memory of individual birds (Leitner et al., 2001a).

Is the recruitment of new HVC neurons a possible explanation for the seasonal production of vocal repertoires in wild adult canaries? From a mechanistic standpoint, new neurons entering a brain area might either disturb existing neural networks, repair existing neural networks through neuron replacement, or lead to new networks if recruited.

Seasonal neuronal death and replacement in HVC under the control of seasonal changes in testosterone levels are suggested to underlie seasonal vocal learning in domesticated canaries (Rasika et al., 1994; Alvarez-Buylla and Kirn, 1997). Although we did not study neurogenesis in wild canaries, neurogenesis in the forebrain appears to be a general feature of birds, irrespective of whether they learn their songs by imitation or not (see above; Ling et al., 1997). It is, therefore, conceivable that particular neurons could be lost and replaced by others to modify HVC circuitry so that syllables are lost and added both in the fall and spring. However, with such a scenario it is difficult to understand how the wild canaries are able to exactly recover many of their seasonally lost syllables annually (Leitner et al., 2001a,b). If the autumnal new neurons are important for the syllables that appear to be new in fall, these neurons should die in spring in order to lose the syllables again. Yet the new HVC neurons added in fall have been shown to survive the following spring cell-death period (Nottebohm et al., 1994). Since 75% of the repertoire remains constant both seasonally and annually in wild canaries, cell death and replacement needs to spare a large part of the HVC in order to maintain behavioral continuity since many of the HVC neurons appear active during the production of the same syllable (Yu and Margoliash, 1996).

Thus, it is unlikely that the seasonal increase in sexy syllables on the background of a stable repertoire size is due to seasonal neuronal death and replacement (Leitner et al., 2001a,b). Clearly, the study of neuron recruitment in HVC, particularly of the RA-projecting type, of wild canaries would be useful. However, statistical analyses comparing neural replacement in HVC (Kirn et al., 1994), cell death in HVC (Kirn et al., 1994), testosterone production (Nottebohm et al., 1987), and monthly production of new syllables (Nottebohm et al., 1986) do not result in any correlations between these physiological parameters and vocal learning (Fig. 4). Although testosterone masculinizes the female canary vocal system including an increased number of new RA-projecting HVC neurons (Rasika et al., 1994) and glia cells (Goldman and Nottebohm, 1983), such females can produce masculine temporal features (which are innate and hormone-sensitive, see above) but in most cases sing very few different syllables (Hartley et al., 1997; Fusani et al., 2002). This remains true even if they are tutored with male canary song or are exposed to such song during the testosterone treatment period. These examples suggest that recruitment of HVC neurons per se do not increase the probability to engage in vocal learning and that neuronal loss and replacement in HVC are not re-
Fig. 6. The effect of an excitotoxic partial bilateral lesion of the HVC on the song of an adult male zebra finch. The song is shown before the lesions (A), 2 days after the lesions (B), and 2 weeks after the lesions (C). Note that this male recovers its song pattern, although the lesion destroyed about 40% of the left and of the right HVC permanently. The only difference in the recovered song is that this male now sometimes sings a second strophe (C) in which it omits the syllables d* and e*, while the bird mostly sings the strophe shown in A (Rybak and Gahr, unpublished).

Experimentally induced neuron-type specific death so far provides the best evidence for a functional role of neurogenesis in behavioral homeostasis (Scharff et al., 2000). The induced death of a (unknown) number of RA-projecting HVC neurons caused dramatic distortions in the song of adult male zebra finches while induced death of X-projecting neurons has no effect. Furthermore, the behavioral recovery of the song of some males correlates with the increased recruitment of new neurons (Scharff et al., 2000). However, similar effects on the vocal pattern of adult male zebra finches are the result of excitotoxic, partial bilateral lesions of the HVCs (Fig. 6) (Rybak and Gahr, unpublished). Although we did not study neurogenesis in these animals, such lesioned animals recover their song without repair of the focal lesions over about the same time period as the photolesioned zebra finches in the study of Scharff et al. (2000). We demonstrated recently that lesions in the HVC, small or large, induce collateral modifications of the intact HVC areas; the expression of GABA-producing enzyme (GAD mRNA) is transiently down-regulated following such lesions in the HVC (Halle et al., 2002). Similar collateral dam-
age that would disturb electrophysiological patterns over the entire HVC is also conceivable in cell-type specific lesions. As a consequence, the behavioral recovery of zebra finches with photolytic lesions might be due to recovery of permanently existing neurons such as GABA-producing HVC neurons rather than to the recruitment of new neurons. The ultimate experiment with rigorous control over the recruitment or survival of HVC neurons in intact adult animals is required to shed light on the adaptive role of adult neurogenesis.

In summary, we suggest that there is little detailed evidence supporting any particular, definitive adaptive role for neurogenesis in adult birds, even in their vocal and food-storing behavior. Similarly, some adaptive role for neurogenesis also needs to be suggested for mammals. Neuronal recruitment in the mammalian hippocampus increases following an associated learning task in rats (Gould et al., 1999b) and following exposure to complex environments or to increased motor activity in adult mice (Van Praag et al., 1999; Bjorklund and Linduall, 2000). In these cases increased recruitment follows environmental changes, somewhat similar to the proposed increases in neuron recruitment in the canary HVC after increased vocal learning in the fall (if these are truly related, see above). Although a recent publication suggests reduced formation of trace memories subsequent to mitogenic arrest in mice (Shors et al., 2001), it remains to be seen whether this effect is due to reduced production of neurons only or due to effects of such treatment on body systems that require ongoing mitogenesis, such as the immune system.

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