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Commentary

Songbirds and adult neurogenesis: A new role for hormones

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It is an intriguing property of birds that they remain able to generate large numbers of new neurons in adulthood (1). In all avian species studied thus far (including many non-songbird species), the ventricular zone continues to generate neurons throughout life, and these cells migrate and insert into many areas of the forebrain. This phenomenon has been studied most extensively, however, in the song system of passerine songbirds (2). The song system is a specialized set of brain structures that mediates vocal learning and is found only in birds that learn their song with reference to auditory information (3, 4). Furthermore, in most songbirds, both the song system and the singing behavior are sexually dimorphic and are under the control of sex steroids (5, 6). Nottebohm and colleagues (7–9), in a set of three articles in this issue of the *Proceedings*, shed new light on the interplay among hormones, seasons, and adult neurogenesis in canaries. This work raises numerous interesting questions about the regulation of the birth and differentiation of adult neurons in songbirds and about the role of these neurons in song learning.

Part of the interest in studying canaries is that they are "open" learners (10). That is, canaries modify their songs in adulthood, unlike many other songbird species ("closed" learners), in which song learning is restricted to a period in early life (11). A description of the essential neural differences between open and closed song learners could provide insight into general mechanisms of plasticity. The seasonal time course of the adult song modification in canaries is intriguing: most of the changes occur during summer and early fall, after the end of the breeding season, and in late winter, immediately before the onset of the next breeding season (12). These times correspond roughly to periods when the birds' testosterone (T) levels are low or beginning to rise again after having been low for several months (13). In contrast, in spring, when the birds have high T levels, they sing a great deal, but this song is very stable or "crystallized." This correlation suggests that changing levels of androgen may play a role in adult song modification, as they do in song crystallization (14, 15).

One likely location for the neural differences between closed and open learners is within the song system itself. In canaries as in other songbirds, the song system contains two distinct circuits. The first of these forms the motor pathway for song and consists of a chain of nuclei (Fig. 1) including the hyperstriatum ventrale, pars caudale (HVC; also known as high vocal center), the robust nucleus of the archistriatum (RA), and the tracheosyringeal portion of the hypoglossal nucleus (nXIIts). Lesions of these motor nuclei in birds of any age lead to abnormal song production (3). A second circuit, called the anterior forebrain pathway, consists of area X (X), the medial portion of the dorsolateral thalamus (DLM), and the lateral portion of the magnocellular nucleus of the anterior neostriatum (LMAN); this circuit indirectly connects HVC to RA (Fig. 1 and refs. 16 and 17). The HVC neurons that project to area X are different from those projecting directly to RA, although the two populations are intermixed in HVC (18–20). In contrast to the motor pathway, the song nuclei X, DLM, and LMAN are not essential for normal song production in adult birds with stable "crystallized" song. Lesions of this anterior forebrain pathway in young songbirds of any species or in adult canaries in the process of adding new syllables, however, result in markedly disrupted song (21–23). Thus this circuit plays a critical role during phases of song modification, while the motor pathway must be intact throughout life.

Where and when do new neurons enter the song system? In the HVC, the neurons born before hatching consist predominantly of the neurons that will project to X (19). LMAN, X output neurons, and RA neurons, like the HVC-to-X projecting neurons, also all appear to be born prior to hatching (24–26). Most HVC-to-RA projecting neurons, in contrast, are born after hatching (postnatal days 10–240 in the canary), when song learning is already well underway (18, 19). Thus these two circuits, the motor pathway and the anterior forebrain pathway, not only have different roles in learning but also are set up quite differently during development. In adult birds, newly generated neurons are inserted throughout much of the avian forebrain, including those regions not involved in song. In the adult song system, however, new neurons are found only in the HVC, in both males and females (1, 27). Moreover, most of the new HVC neurons in adult canaries become long projection neurons in the direct pathway from HVC to RA (28). How does this restriction of neuronal fates occur? The ventricular zone of adult songbirds may have become committed to generating only HVC (and nonsong system) precursors, just as in mammals the germinal zone generates neurons of particular fates at specific times in development (29, 54). Alternatively, the selection of neuronal types may not be at the level of the ventricular zone (VZ), but later, through selective recruitment (i.e., migration into HVC or differentiation into HVC neurons) or survival. Certainly, not all postmitotic neural precursors survive. Alvarez-Buylla and Nottebohm (27) estimated that only one-third of labeled precursor cells eventually become differentiated neurons. Factors favoring survival of particular neuronal precursors could determine which types of new neurons are observed in adults.

The first paper (7) in this series of three speaks to this issue of survival of neuronal precursors and the factors that might control it. The authors examined the effect of T treatment on new neurons in female canaries. Female canaries have smaller song nuclei than males and sing much less (30). When they do produce song, the song has fewer syllable types than the song of adult males and is less stable. It has been known for some time that administration of T to female canaries induces them to sing much more and to produce stable song. Furthermore, the hormone causes dramatic changes in morphology of the song nuclei, including a 50–90% increase in the volume of the HVC and RA, increased HVC and RA neuronal size, and a major increase in dendrites and dendritic length in both of these nuclei (31, 32). In this paper (7), the authors examined the effect of T on neurogenesis in female canaries in two ways, which allowed them to separate effects on proliferation from effects on neuronal survival and/or recruitment. (*i*) They implanted adult female canaries with T;

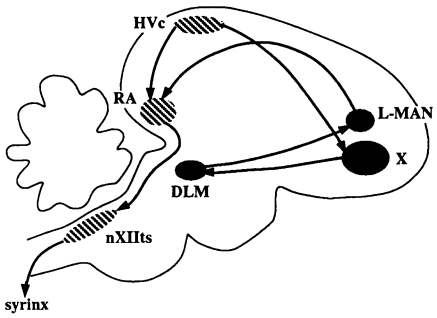

FIG. 1. Simplified schematic of the song system. The cross-hatched nuclei, HVC, RA, and nXIIts, form part of the descending motor pathway for song. The nuclei X, DLM, and LMAN, shown in solid areas, form a pathway indirectly connecting HVC to RA and play a special role during song learning. The HVC neurons that project to each of these pathways are separate populations of neurons intermixed throughout RA.

after 2 days, they gave a 1-day pulse of [^{3}H]thymidine and counted the number of labeled cells in the VZ above, rostral, and caudal to the HVC. They found that T does not increase the overall rate of proliferation of germinal zone precursors; this replicates the recent result of Brown *et al.* (33). Both groups of researchers point out that, since the exact location of the cells fated to give rise to HVC neurons is not known, this result does not rule out a selective increase in a subpopulation of HVC precursor VZ cells, which might not be detected. Furthermore, if precursors committed to particular neuronal phenotypes exist, there might be a hormone-induced change in the fate of VZ cells, for example, a shift toward HVC neuronal precursors from other precursor cells, without a change in the labeling index. (*ii*) The authors then examined the effects of androgen on new neurons at a slightly later stage after their birth in the VZ. Since 20 days is about the amount of time it takes for a newly postmitotic precursor to migrate out of the VZ and become a recognizable neuron (27), the experimenters began treating female canaries with T starting only 20 days after labeling with [^{3}H]thymidine. After 40 days of T treatment, they selectively labeled RA-projecting HVC neurons by retrogradely filling them from RA and then assessed the fraction of these neurons that were [^{3}H]thymidine-labeled. The result was that with T there is no increase in the overall number of back-labeled neurons, but there is an increase in the percentage that are newly generated. Thus T apparently increases the number of new neurons that survive or are recruited into the HVC-to-RA pathway. Other possibilities are that T increases the likelihood of a new neuron making the connection to RA or its rate of doing so, or even the extent of its connections in RA (thus perhaps influencing the frequency of backlabeling). All of these possibilities could be due to direct hormonal effects on the HVC neurons, since many of them have androgen receptors (20, 34), to indirect effects on targets (RA) or afferents to HVC, or to a combination. There is ample precedent for hormonal effects on neuronal survival (35–37, 55) and increasing evidence for interactions between steroids and neurotrophic factors (38). Regardless of the mechanism of the hormone-induced change, the result suggests that much of the action of T on new neurons occurs subsequent to their birth and migration out of the germinal zone.

In the other two papers (8, 9), the authors turned their attention to the adult male canary and examined new neurons through the seasons. They labeled new neurons with [^{3}H]thymidine each month of the year, and, 27 days later, counted the number of new neurons and pyknotic cells per 1000 total HVC neurons (8). In this manner, they showed that there is HVC neuronal birth and death throughout the year and added to the evidence from their previous work (39) that new neurons replace older ones, as the overall number of neurons in HVC does not change. Furthermore, they reported that there are 2 months, October and March, that show the highest ratios of newly born to total HVC neurons (i.e., the most replacement; the number of new neurons per thousand is 5–7 in these months vs. 1–3 in the others). In addition, a small peak of pyknotic cells immediately precedes each of the peaks in cell replacement. These findings are correlated with the known seasonal peaks and troughs of T in male canaries: serum T levels are low in July–August and January–February, and rise again in spring and late fall/winter (13). This raises the possibility that in males as well as female canaries, androgen increases the survival and incorporation of new neurons into the HVC. It further suggests that periods of low T might be associated with neuronal cell death. The latter possibility is reminiscent of neurogenesis in the postnatal hippocampus of rats, where a lack of adrenal steroids is associated with increased granule cell death, and increased steroids suppress cell birth (37, 38, 41). The linkage between neuronal death and neurogenesis is poorly understood in many systems, and there have been speculations about feedback to neuroblasts from dying cells (42). In the song system as well, the absence of an overall increase in HVC neuron number requires some coordinated regulation of cell birth and death. If both established and newly generated HVC neurons depend on hormone, the seasonal changes in androgen could well explain this phenomenon. It is further intriguing that the times of neuron loss and replacement are correlated with periods of song instability and restabilization, respectively, raising the possibility that these processes play a role in seasonal modification of song as well.

In the final paper (9), the authors report that the lifespan of HVC neurons depends in part on when they are generated. HVC neurons labeled with [^{3}H]thymidine to mark their birthdate in October are still present 4 months after labeling; in contrast, >50% of new HVC neurons labeled in May have disappeared 4 months later. A puzzling observation is that the number of neurons labeled in the fall continues to increase long after the thymidine is gone, raising the possibility of some complicated seasonal effects on labeling itself, or perhaps delayed migration and differentiation. Nonetheless, the results again show seasonal effects on HVC neuronal survival. What might cause these seasonal effects? One possibility, in light of the first two papers, is that androgens play a role here as well. Neurons generated in October experience the November–December peak of androgen shortly after birth, whereas neurons labeled in May undergo the summer drop in T not long after they are born. Perhaps HVC neurons are born at a constant rate but depend on T early in their life span to survive. If so, they must become less dependent on hormone thereafter, as it is clear from previous work that many September-born HVC-to-RA projecting neurons are present at least 8 months later (28). Thus, after the initial exposure to hormone, these neurons apparently survive the January–February drop in androgen. Alternatively, being born at a time of relatively higher T levels (May) might make a neuron differentially dependent on T or other factors, resulting in shorter survival of these cells than of neurons born in October. If T promotes survival of neurons by acting some time after their generation, how might it be acting? Is the establishment of connectivity to RA (or from afferents) crucial and hormone-dependent? If so, is the role of these connections due to activity or neurotrophic factors? Are these effects direct or indirect? The advantage of the song system is that these are all testable questions. For instance, what happens to new neurons when the normal seasonal changes in T are eliminated by exogenous replacement or by castration? These manipulations could be done while simultaneously eliminating inputs and outputs of HVC, to identify the site of action of the hormone. What happens in female zebra finches, which likely incorporate new neurons into HVC but have no effective innervation of RA (43)? The three papers in this issue of the *Proceedings* raise many such interesting questions and provide new insight into the development and regula-

tion of newly generated neurons in the adult brain.

The question remains as to how directly the new neurons in the song system and their turnover are related to song modification. One suggestion has been that the neurogenesis itself is responsible for the seasonal plasticity of learning displayed by canaries (2, 44, 45). It is important to remember, however, that androgen causes numerous other dramatic changes in the song system, including large increase in dendrites, and hence, synaptic sites, in a number of song nuclei (32). It seems likely that all of these other neuronal changes, as well as the increased turnover of neurons, are involved in seasonal plasticity. Furthermore, as has been seen with other correlations between song learning and development (e.g., ref. 46), it is possible that neurogenesis goes on in parallel with learning but is not directly responsible for it. This possibility is also raised by the fact that neurogenesis occurs (albeit at a somewhat lower level) in adult zebra finches as well, although zebra finches do not learn songs in adulthood (44). Crucial experiments to assess the role of new neurons in learning remain to be done: for instance, what are the effects on song of eliminating adult neurogenesis? These experiments are difficult, but perhaps new molecular tools such as cell-specific promoters coupled to genes involved in cell death or survival (e.g., *bcl-2* or c-*myc*; see ref. 42) may be helpful. A simpler approach to test the correlation would be to compare canaries not to zebra finches, which are not seasonal breeders, but rather to other highly seasonal birds that nonetheless do not modify their songs. Such an approach previously showed that seasonal changes in HVC volume are not necessarily correlated with changes in song repertoire (47). A particularly informative species might be one like the chaffinch, which shows transient instability in song structure each year at the end of the breeding season (ref. 48; presumably at a time of low T levels), but is nonetheless a closed learner. In some respects it is astonishing that songs can remain constant for many years, in the face of such dramatic neuronal turnover in the motor pathway for song. Perhaps the anterior forebrain circuit of the song system (Fig. 1), which is one of the few portions of the songbird telencephalon not to turn over, plays some special role in preserving song memory (although see refs. 49 and 50). The problem closed song learners face in retaining song is analogous to the issue of how memory persists in the mammalian olfactory system and hippocampus, where neurons also turn over throughout life (37, 38, 51–53). It may be particularly useful to tackle this problem of long-term memory in the song system, where much is known about the pathways involved and where the song itself provides an excellent behavioral assay for the results of learning.

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