

Neural Constraints on the Complexity of Avian Song

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Key Words

Sexual selection · Song system · Evolution · Motor learning · Auditory perception · Individual recognition · HVC · NCM

Abstract

Why do birds sing? In many species, because the song attracts or retains a mate. Why do females pay attention? This paper reviews evidence that females may do so because male song can be an honest indicator of attributes of a male's brain that could contribute to his fitness or that of his young. Male songbirds learn and produce their songs using a set of brain regions collectively known as the song system. The learning has distinct auditory and motor components, and current data suggest that the neural changes that encode these forms of learning primarily occur in different subdivisions of the song system. There are positive correlations between song complexity and the volume of motor song system nucleus HVC, both between and within species. The correlations appear to arise because individual differences in volume lead to differences in capacity for learning. The differences in HVC volume are correlated with differences in the volumes of other song system components and with the volume of the forebrain. They are heritable. Thus, a complex song can be a signal to a female of

immediate fitness (the male has a larger brain) and ultimate fitness (he has attractive characteristics that will be passed on to progeny).

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Introduction

Oscine bird species are able to learn features of the song that they produce. The species differ dramatically in the complexity of the song that is learned and produced by males. This has been known and commented on as long as there have been naturalists. Finding reasons for the differences has often seemed intractable. However, relatively recent observations on contrasting uses of song between species and individual differences in song content within species have led to novel insights into the evolution of song. Many of these insights relate to the interplay between behavior and ecology, the focus of the International Society for Behavioral Ecology and its conferences. In this brief overview, I argue that studying the brain systems responsible for song acquisition and production can provide complementary insights into the possibilities and constraints on song selection and evolution (more extensive information on the neurobiology of avian song and on these ideas can be found in DeVoogd and Szekely [1998], and DeVoogd and Lauay [2001]).

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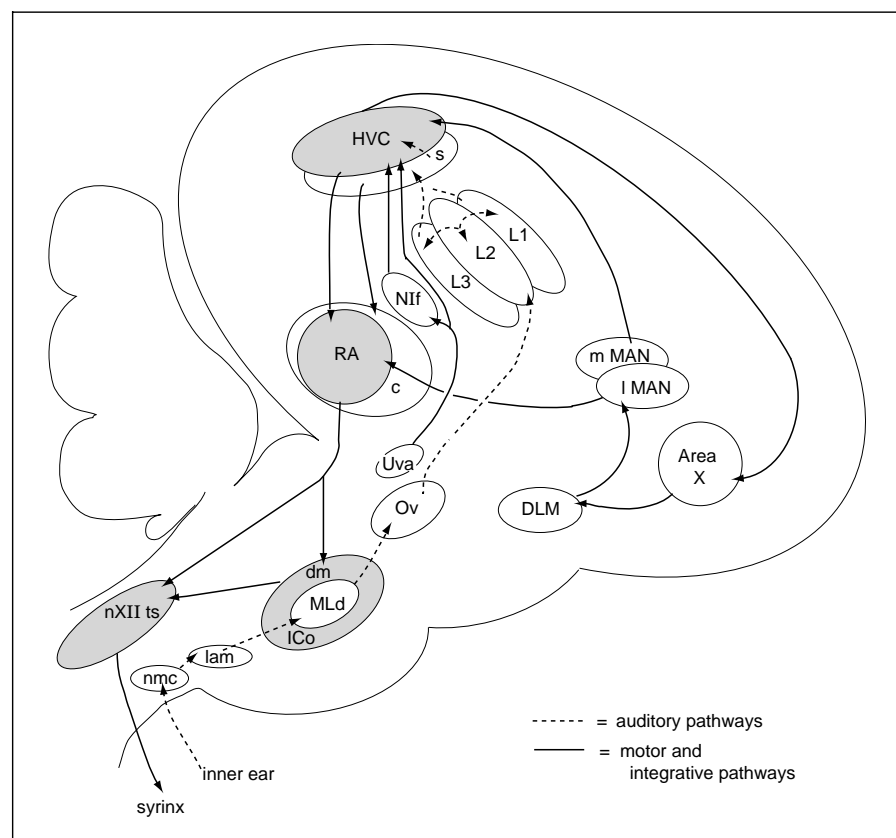
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Fig. 1. Major nuclei of the avian song system and their connections. Areas known to be essential for song production are shaded [from DeVogd and Lauay, 2000].



The Brain System Responsible for Song

The brain areas directly responsible for producing song were first described by Nottebohm and colleagues (fig. 1) [Nottebohm et al., 1976; reviewed by DeVogd and Lauay, 2001]. These brain nuclei include HVC in the dorsal telencephalon, which projects to RA more ventrally in the telencephalon. RA projects directly and indirectly to the hypoglossal nucleus in the brainstem where neurons that innervate the syrinx, the avian vocal organ, are found. HVC also projects to Area X, a basal ganglia-like structure in the rostral telencephalon [Luo et al., 2001], which projects via the thalamus to l-MAN, which in turn projects directly and indirectly back to HVC and RA. This latter series of connections appears homologous to projections from motor cortex in mammals that loop through the basal ganglia and are needed for acquisition of finely coordinated sequential motor tasks. Together, these areas comprise the motor song system: HVC, RA and the hypoglossal nucleus are necessary to produce song, and Area X and MAN are essential for correctly acquiring the vocalizations of song.

Brain-Behavior Relations in Areas Used to Learn and Produce Song

The brain areas of the motor song system vary in size between males of different songbird species. Although some of this variation is associated with species differences in body and brain size, much of it is not. In a survey of 41 oscine species, DeVogd et al. [1993] found a significant relation between HVC size (relative to overall telencephalon size) and published estimates of the song complexity typically found within the species (fig. 2). The behavioral and brain measures were compared using the method of independent contrasts, a procedure that deals with the non-independence of different species by forming a series of pairwise comparisons at equivalent degrees of relatedness. Thus, differences in brain and in behavior measures for the two most closely related species within each genus are tabulated. Further pairs are generated by comparing a composite measure of the scores of this first pair to their closest relative(s). Ultimately scores reflecting an entire genus are compared to the scores of the nearest other genus, and finally families are compared to fami-

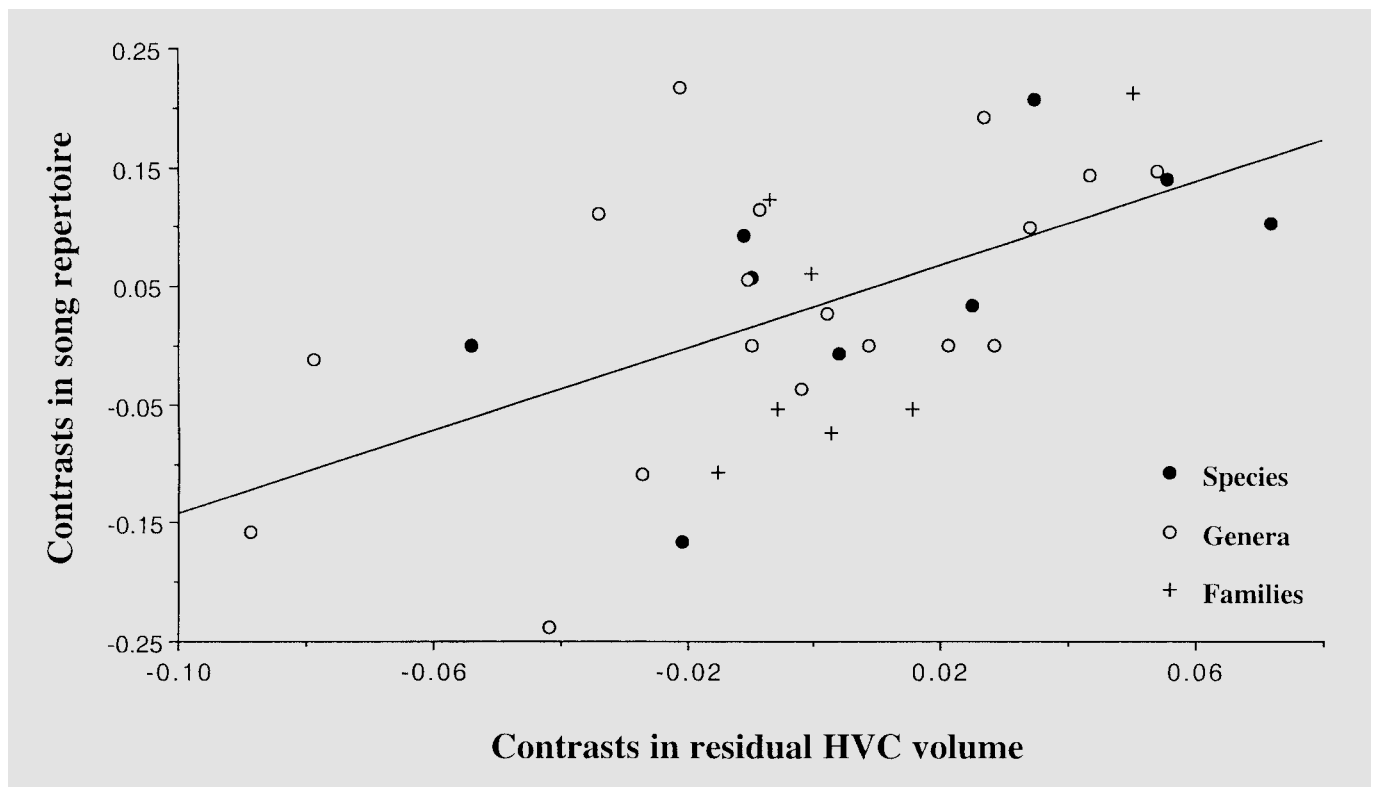


Fig. 2. Differences in the relative volume of HVC tend to be associated with differences in the song repertoire typically attained by males of a species. The method of independent contrasts was used in order to control statistically for varying degrees of relatedness in this sample of 41 oscine species. This relation is present in contrasts between contemporary species as well as in contrasts between contemporary genera and families, suggesting that the qualities that cause this relation now have been present since the divergence of the genera and the families [from DeVoogd et al., 1993].

lies. These broader comparisons are parsimonious estimates of ancestral state, respectively for the founding species of a genus or for the founding species of a family. The positive relation between relative HVC size and song complexity occurs at each of these levels of comparison (fig. 2), suggesting that the larger relative size of HVC permits more elaborate song among species now alive, and that this association has existed for a very long time.

The strength of these relations depends on comparability of the behavior and of the brain measures across the phylogeny. Clearly, there are comparability problems across such a wide phylogeny arising from differences in song form, in song quantification and in brain function. It is difficult to compare quantitatively, for example, the very simple repetitive song of a chaffinch (*Phylloscopus collybita*) with the complex song of a starling (*Sturnus vulgaris*). Furthermore, there are differences between labs in which aspects of song are measured in a species and what

these features are called. Species within the genus *Acrocephalus* typically produce songs in which there are units consisting of 1–3 notes that are produced in different parts of a song bout or in different renditions of song with the same phonology [Catchpole, 1980]. These are called syllables and are often the song feature used for quantifying song complexity. Zebra finch (*Taeniopygia guttata*) song also contains sound units, but these are very tightly linked and are produced in a fixed sequence. The units are sometimes called syllables but more often elements or motifs, within a single fixed song. Other species produce a brief series of notes repeatedly for a time and then shift to another series, which is usually described as a repertoire of multiple syllables within multiple songs. Finally, comparability problems can arise if the function of nuclei in the motor song system has diverged over the very long interval since the oscine species within a comparative study have had common ancestors. These uncertainties

can be improved by studying brain-behavior relations in narrower phylogenies.

Relatively little research has assessed relations between anatomy of the motor song system and song complexity within more closely related groups. Székely et al. [1996] used the method of independent contrasts on eight species of European warblers [Sylviidae] from two genera (forming 8 independent pair-wise comparisons) to show a positive correlation between the number of syllables in the typical repertoire and the relative volume of HVC. As in the study on the broader phylogeny, this method derives parsimonious estimates of ancestral state for brain and behavior. These estimates suggest that HVC has become larger in *Acrocephalus* lineages and has decreased in *Locustella* since the divergence of two genera, whereas IMAN appears to have increased in *Locustella* [DeVoogd and Székely, 1998]. If sexual selection on the basis of song has been occurring in both lineages, these results suggest that females have used different aspects of song in choosing males: perhaps heterogeneity or complexity for *Acrocephalus* and duration or stereotypy for *Locustella*. Such selection could lead to differences in the anatomy of the song system in the two lineages that would support these song characteristics.

There are also strong positive associations between aspects of song and of the motor song system within species. This was first observed in canaries (*Serinus canaria*), in which the number of syllables in an individual's repertoire is correlated with the volume of HVC and RA [Nottebohm et al., 1981]. Canady et al. [1984] found in marsh wrens (*Cistothorus palustris*) that HVC and RA are 30–50% larger in birds from western North America than in birds from eastern North America – a difference paralleled by a three fold difference in song repertoire between the two groups. Furthermore, there was a significant correlation within both the eastern and western samples between song repertoire and HVC volume. Logically, the difference in song repertoire could be due to differences in the quality or content of tutoring. However, the differences between the eastern and the western birds in song repertoire and in volumes of motor song nuclei are present even when the two are hand-reared in the lab with the same (very large) song repertoires available for copying [Kroodsma and Canady, 1985]. This suggests that the correlated differences in brain and behavior do not depend on post-hatching factors, and are likely to be genetic.

More recently, we have found that the number of song syllables in the repertoire of European sedge warblers (*A. schoenobaenus*) recorded on their territories is posi-

tively correlated with the volume of HVC [Airey et al., 2000a]. Several aspects of song were quantified for these individuals. We found that HVC volume was also correlated with the number of different syllables used in a song bout and with the total number of syllables in a song bout. These findings underscore the links that exist between the morphology of the motor song system and aspects of singing behavior. However, they also emphasize a caveat: both the brain and the song features that are quantified are selected by the experimenter. Thus, the aspect of song measured may be a byproduct of the aspect that the bird is striving to achieve, and the morphological feature may be a covariate of neural control that is expressed at a finer level of analysis, or even in another brain region. We will need to dissociate the behavioral features, either by assessing the reaction of the intended audience to songs artificially altered to vary these attributes independently, or by measuring brain and behavior in species in which this has occurred naturally. We will need to measure brain morphology in multiple areas and at multiple levels of analysis. Doing so will permit strong hypotheses both about present function of brain features, and about how selection has acted to permit these functions.

The correlation between HVC volume and song attributes even extends to zebra finches, a species with a single song in which individuals vary in the number of motifs or elements (note clusters) that comprise the song. Ward et al. [1998] found that a larger number of neurons in HVC is associated with greater accuracy in copying from a model. Using somewhat different arrangements for rearing zebra finches, we found that the volume of HVC is correlated with the number of elements in the songs, regardless of their source [Airey and DeVoogd, 2000]. HVC volume is also correlated with song length, again underscoring the caution that finding a brain association with a particular behavioral measure does not guarantee that this is the feature that brain region was responsible for producing. In this study, we also measured the volumes of other nuclei of the motor song system. We found that number of sound elements in song is positively related to IMAN volume and negatively related to Area X volume. A multiple regression including these additional anatomical measures accounted for 76% of the behavioral variance. We do not presently understand the roles played by either of these nuclei in song acquisition or in modulating song production. However, this result emphasizes that song is produced by a network of brain nuclei and that interactions between the nuclei are critical to acquisition and expression of song. A configuration in an individual of a relatively large HVC and a relatively small Area X

may be especially effective in acquiring and producing a song with many elements. We are currently carrying out further comparative research to investigate whether other patterns of emphasis across the motor song system are associated with other patterns of song production.

Each of the experiments described above is correlational – they relate an aspect of song performance to an aspect of motor song system morphology. Clearly such correlations could arise either because the brain feature permits or causes the behavioral capacity, because features of the behavior such as learning or practicing song influence the brain feature, or because both are caused by some unmeasured brain attribute. Several experiments have assessed the extent to which song learning (or preventing it) affect the development and adult anatomy of the motor song system. Most radically, deafening zebra finches prior to their sensitive period for song acquisition profoundly impairs song acquisition. However, it does not result in a significant decrease in the volumes or number of neurons in any of the major nuclei of the motor song system compared to unmanipulated birds [Burek et al., 1991]. Less radically, Brenowitz et al. [1995] raised hand-reared male marsh wrens in the lab and exposed them to recordings of song repertoires that were either approximately normal (45 song types) or severely limited (5 song types). At one year of age, the birds were singing songs with repertoires similar to those to which they had been exposed. However, the volumes of HVC and RA and the number of neurons within these nuclei did not differ between the two treatment groups. Single hemispheres from these same individuals were Golgi-stained (a procedure that stains the dendrites, cell body and part of the axon of a subset of the neurons present within a nucleus). The peak number of synaptic spines on dendrites in HVC (but not RA) was greater in the birds that heard and acquired a large song repertoire than in those that heard and acquired the small song repertoire [Airey et al., 2000b]. More recently, we have replicated the observation that rearing male zebra finches without adult males present results in acquisition of a severely abnormal song, with fewer elements than normal and greater use of call notes in the song. This treatment also causes a decrease in the number of dendritic spines on neurons in HVC of the deprived animals as adults [Lauay et al., 2002]. Together, these studies suggest that associations between gross anatomy of HVC and the extent of song acquisition and expression are due to the brain affecting behavioral capacity rather than the other way around. In other words, the size of HVC constrains how elaborate a song can be acquired. The process of acquiring a song may alter con-

nections within motor song system nuclei, but does not noticeably affect this overall framework. Genetic or ontogenetic differences between individuals in the size of HVC and number of neurons then result in differences in the complexity of the song produced by individuals, in environments in which sufficient song models are available. This model is further supported by close examination of the Brenowitz et al. [1995] data on the differentially reared marsh wrens: within the group that was tutored with elaborate songs, there are positive correlations between the volumes of HVC and RA and song repertoire size, as would be predicted if some individuals had nuclei small enough that they were unable to retain and express all the song models presented to them.

None of the studies on the influence of learning on neuronal structure reviewed above can discriminate between the brain effects of auditory vs. motor learning. In other words, are we seeing effects of what the bird heard or of what he then produced? Leitner et al. [2002] have recently published data suggesting that it is the latter. Sedge warblers were hand-reared either in isolation or with exposure to tapes containing songs with normal syllable repertoires. In adulthood, both groups produced complex songs (in fact with larger syllable repertoires in the isolated than in the tutored birds). No group differences were found in the number of dendritic spines on neurons in HVC. Thus, in spite of huge differences in acoustic experience, isolated birds were able to improvise complex songs and to build or maintain HVC connectivity while doing so. Obviously, this raises the question of why bother learning a song if one can improvise a song without auditory learning. As mentioned above, this can only be answered by assessing the reaction of the intended receiver to learned and to improvised song.

Recently, Bolhuis and Macphail [2001] have criticized studies such as those reviewed above that compare aspects of brain anatomy and of behavior across species and relate these to selective processes. They argue that relating morphology of song system brain regions to song learning is inappropriate because much of auditory learning and memory goes on elsewhere in the avian brain. Although it is true that all bird species can learn to discriminate sounds, including species that do not have the nuclei of the song system, this overlooks what is special about the songbirds: that they can use auditory learning to mimic sounds that they hear. This linkage between auditory learning and motor production characterizes the behavior of oscine birds and physically takes place in the nuclei of the song system. Comparative analysis across orders of birds has shown that these nuclei or homologous ones are

found in the three lineages of birds that show vocal imitation, and not in the lineages that do not imitate [Jarvis et al., 2000]. Thus, by studying diverse species, we have found a behavioral specialization for learning and its underlying neural substrate, which would not have occurred in a typical single species study.

Bolhuis and Macphail [2001] also suggest that comparative analysis cannot lead to advances in understanding mechanisms of learning or memory, in contrast to explicitly experimental approaches. However, as reviewed above, comparative studies within songbirds can reveal strong associations between aspects of singing behavior and aspects of song system morphology – associations that are consistent whether widely divergent species are being studied, or a closely related clade, or variation within a species. Such associations can lead to testable hypotheses on the functions of nuclei and neural network characteristics, which can then be tested either with experimental interventions or through further selective comparative study. Thus, looking at relations between brain and behavior in a broader evolutionary context reveals patterns of brain function that would not otherwise be seen, and comparative approaches are a powerful adjunct to conventional experiments in assessing these patterns.

Brain Correlates of Song Perception

Behavioral Correlates of Song Perception

In many songbird species, males sing to females as part of the courtship process. In some species, song is used both as an agonistic signal to other males and as a means of attracting females [function of song reviewed by Andersson, 1994]. In species that use different songs in these two contexts, female-directed song is typically more complex than song that is male-directed or undirected [for example, Catchpole and Leisler, 1996]. Thus, for many species, females listen and respond to a class of complex songs that the male produces.

There is substantial evidence that females pay attention to male song, and some evidence that they can be highly precise in recognizing the songs of individuals and in discriminating songs that differ in subtle ways. Adult female zebra finches choose to spend time near the song they had heard from their father or their tutor rather than near the song of an unfamiliar male [Miller, 1979a; Clayton, 1988; Clayton and Pröve, 1989; Collins et al., 1994; Riebel, 2000; Lauay et al., 2004], even if cross-fostered (and tutored) by birds from another related species [Clayton, 1990]. Female zebra finches also prefer the song of

their mate to that of other males [Miller, 1979b], as do song sparrows (*Melospiza melodia*), which prefer the songs of neighbors over the songs of strangers as well [O’Loghlen and Beecher, 1999]. Female white-crowned sparrows (*Zonotrichia leucophrys*) prefer songs from their natal dialect to songs from foreign dialects [MacDougall-Shackleton et al., 2001]. Female canaries respond more to the songs of their mates than to songs of other males, even when recent experience with the songs is equal [Beguín et al., 1998].

Female preferences in each of these studies may be due to a familiar vs. unfamiliar decision, rather than providing evidence for acute discrimination. However, several studies have found such evidence. For example, female song sparrows and swamp sparrows (*M. georgiana*) show greater responses to conspecific songs with large than with small repertoires [Searcy, 1984; Searcy et al., 1982]. Female sedge warblers prefer songs with greater numbers of unique syllables, both in the wild [Catchpole, 1980] and in the lab [Catchpole et al., 1984]. Female song sparrows captured in the wild as adults respond more to songs that lab-reared males had copied accurately from tutors than to songs that had been copied less accurately, and also more to songs incorporating more tutor notes than songs with more improvised notes [Nowicki et al., 2002a]. Indeed, there is some evidence that females can discriminate song features that males do not. For example, male red-winged blackbirds (*Agelaius phoeniceus*) react equally aggressively to recordings of normal blackbird song and recordings of mockingbird (*Mimus polyglottos*) imitation of blackbird song, but females are four times as likely to respond to normal song as to the imitation [Searcy and Brenowitz, 1988]. Perhaps the most extensive experiments on female choice have been done in great reed warblers (*A. arundinaceus*). In the lab, females respond more to elaborate than to simple repertoires when other aspects of song composition are held constant [Catchpole et al., 1986]. In the field, males with larger syllable repertoires attract more females and produce more young [Catchpole, 1986]. Females appear to sample the songs of multiple males when they return from migration, as part of the process of selecting a mate [Bensch and Hasselquist, 1992]. They continue to pay attention to males’ songs even after mating-young fathered by a male other than the social mate were found in 10 of 162 broods and the biological father of every one of these nestlings had a song repertoire greater than that of the social father [Hasselquist et al., 1996]. As indicated above, although researchers quantify song or syllable repertoire in such studies, it is not clear whether this is the specific song feature

that females are discriminating. In canaries, for example, females respond preferentially to syllables comprised of two brief notes that are rapidly repeated than to other sorts of syllables [Vallet and Kreutzer, 1995; Nagle et al., 2002]. This suggests that females may actually be attending to the incidence of such notes in a male's song rather than to the total number of unique syllables he produces, and these 'sexy' syllables may be more easily produced by males who are also able to acquire large syllable repertoires.

These experiments indicate that females use song not just to recognize conspecifics, but to distinguish among individual males and, in some species, to select males producing the most complex songs, or the songs most characterized by particular features. How females gain and use this ability is relatively unexplored. The experiments in which females discriminate between songs that are equally complex or are equally novel suggest that this capacity is learned. In a recent experiment, we have found direct evidence for learning. Adult female zebra finches that had been reared normally prefer normal male song to the song produced by males reared in isolation. However, females reared in a complex social and auditory environment (with sibs and mothers) but without hearing normal male song are equally likely to select either sort of song [Lauay et al., 2002, 2004]. It is not yet known whether females have an early sensitive phase during which song learning is enhanced as it is in males of many species.

Neural Correlates of Song Perception

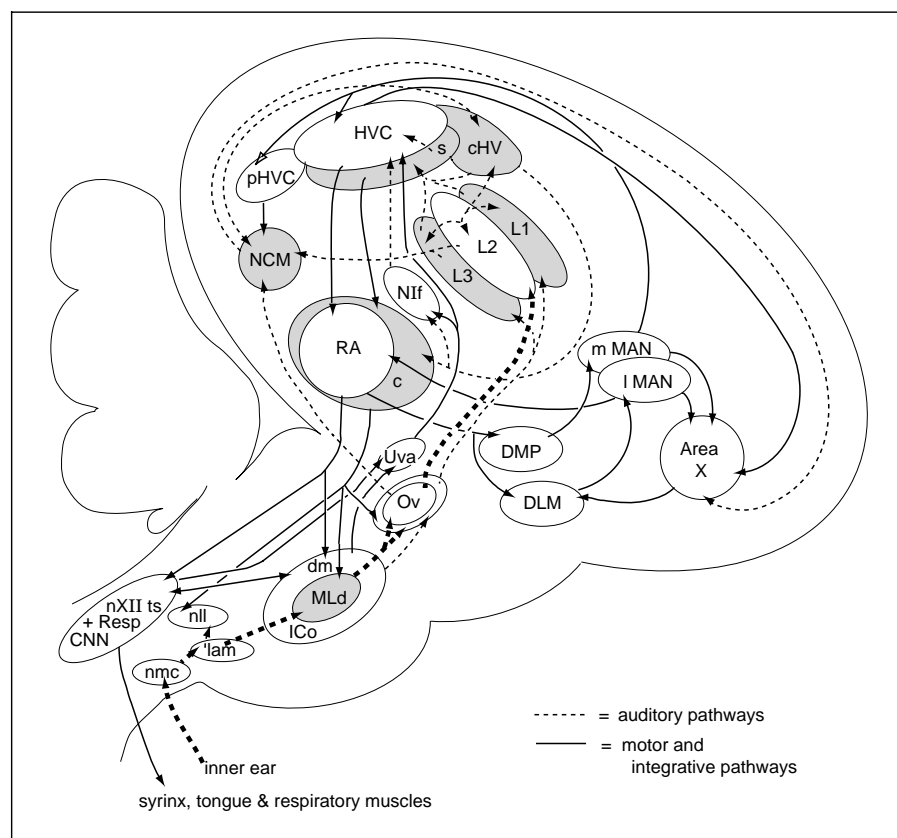
Avian song is a medium of communication. Logically, at least, the neural processing associated with decoding and assessing song should be as sophisticated as that associated with accurate motor acquisition and production. Recent experiments have begun to explore the neurobiology underlying auditory song acquisition and discrimination [reviewed by DeVoogd and Lauay, 2001; Ball and Balthazart, 2001]. Many of these studies have measured neuronal expression of immediate early genes (IEGs) in response to hearing or producing song. These are genes that are expressed when activity in individual neurons passes some (poorly understood) threshold, and whose protein products induce the expression of structural and regulatory genes [reviewed by Dubnau and Tully, 1998]. Gene expression can be quantified using *in situ* hybridization; the proteins can be visualized with immunocytochemistry. Not surprisingly, all major nuclei of the motor song system show IEG expression in males who are singing [Jarvis and Nottebohm, 1997; Jin and Clayton, 1997; Kimpo and Doupe, 1997]. This was first observed in a

context in which birds were isolated from their social group and tape recorded so that the amount of singing could be related to the amount of IEG expression. Under these circumstances, zebra finches produce 'undirected' song. In contrast, in a social group, finches show both undirected song and song focused on another individual. Interestingly, there is no IEG expression in Area X and MAN following this directed song [Jarvis et al., 1998]. This difference in IEG expression suggests different functions for the rostral nuclei in the two contexts for song – perhaps the nuclei are more closely engaged in monitoring and potentially modifying the song when the bird is less directly involved in social interactions.

Perhaps the most intriguing finding from the studies of IEG expression has been the discovery that brain areas distinct from the motor song system are activated by hearing song (fig. 3) [Mello et al., 1992; Mello and Clayton, 1994; Jarvis and Nottebohm, 1997]. Song activates substantial IEG expression in the caudomedial portion of the neopallium (NCM), as well as in zones adjacent to HVC and RA and portions of Field L (the primary telencephalic target of thalamic auditory projections), that can be thought of as an auditory song system. In contrast to the sexual dimorphisms usually seen in the nuclei of the motor song system, these auditory areas appear similar in the two sexes and show similar IEG expression in response to song [Chew et al., 1996; Mello and Ribeiro, 1998; Duffy et al., 1999]. In NCM of both sexes, the IEG response habituates with repeated presentation of the same song, an effect that lasts at least 24 hours [Chew et al., 1996]. Thus, NCM may participate in discriminating songs and may be a site at which auditory memories for songs are stored.

The discovery of the structures of the auditory song system has led to several studies assessing where and how song discrimination takes place. Female European starlings prefer longer to shorter songs [Gentner and Hulse, 2000], and longer songs induce greater IEG expression in ventral NCM than do shorter songs [Gentner et al., 2000]. Canaries form songs from syllables that can be sorted into different classes based on the frequencies used, the presence of multiple notes and the amount of frequency modulation within the syllable [Güttinger, 1985]. When females hear syllables restricted to a particular class, IEG expression in NCM is restricted to a portion of the structure that is consistent among birds within a syllable class and is different across syllable classes [Ribeiro et al., 1998]. Far less expression occurs to synthesized components of the syllables, suggesting that in adult females, NCM is wired to respond to the full entity that comprises

Fig. 3. Song system nuclei and pathways as currently understood. Dashed lines indicate projections that appear to be primarily auditory; solid lines indicate projections that are primarily motor or integrative. Bold lines indicate the primary auditory reception pathways. Shaded areas show immediate early gene activation in birds that are hearing song [from DeVoogd and Lauay, 2000].



conspecific song. An activation program such as this would be well-suited to register and initiate behavioral responses on the basis of song syllable variety or incidence of especially salient syllables – as female canaries in fact do [Kroodsma, 1976; Vallet and Kreutzer, 1995].

The neurobiology of female discrimination has also been studied using other approaches. Lesions to HVC in female canaries result in an increased likelihood of showing a sexual response to sparrow song as well as to canary song [Brenowitz, 1991], and also increases the responses to canary songs that would normally be ineffective stimuli [Del Negro et al., 1998]. In contrast, lesioning HVC in female zebra finches does not increase responses to nightingale song. However, lesioning caudal hyperstriatum ventrale (cHV – an area near NCM in which song evokes IEG expression) does increase the responses [MacDougall-Shackleton et al., 1998]. These studies do not resolve whether increased responding to inappropriate stimuli is due to impaired discrimination. They also suffer from the limitations of all lesion studies – the most complete (and most effective) lesions are also the ones that invade areas adjacent to the target, and are also the ones most likely to

cause substantial degeneration in regions connected to the target. Because the auditory and motor song system structures are physically close together and extensively interconnected, it is difficult to determine the site at which changes in neural processing have led to the changes in behavior. In an alternate approach, Leitner and Catchpole [2002] have found that female canaries that are most discriminating in their responses to ‘sexy’ versus non-sexy syllables are those with the largest HVCs. Although this, too would seem to indicate that HVC in females is involved in song discrimination, two other explanations are also possible. First, in male zebra finches at least, variation in the volume of HVC is positively correlated with variation in the volumes of Area X and RA [Airey et al., 2000c], so discriminatory functions may be carried out by these or brain regions of the auditory song system that are closely linked to HVC, and the variation measured in the volume of HVC reflects variation in the acuity of these other regions. Also, the volume of HVC varies seasonally, in phase with variation in levels of gonadal steroids [Nottebohm, 1981; reviewed by Tramontin and Brenowitz, 2000], as does the incidence of sexual displays in the

birds, suggesting that individual differences in both HVC volume and sexual responses to song could be covariates of the levels of steroids or receptors. We have reasoned that if female song discrimination is tuned through early learning, it might be possible to find anatomical consequences of developmental song deprivation in brain sites important for the learning. To date, we have found that such females have fewer dendritic spines in NCM than do normally-reared females [Lauay et al., 2002]. Similarly, we reasoned that within a genus, females of a species in which males produce more elaborate songs should show greater IEG expression in response to conspecific song than females from a species with simpler songs (as the females should differ in how fine their discriminations need to be). In preliminary data from African marsh (*A. baeticatus*) and Cape reed (*A. gracilirostris*) warblers we find more ZENK-immunopositive cells in NCM in the African marsh females, supporting this hypothesis [Sundberg et al., 2001]. These too are correlative studies that will need to be extended and supplemented by other techniques. Clearly, the available data indicate that female perception of conspecific song and discrimination among songs can be highly precise, even among extensive, complex male songs. However, much remains to be done to understand how these functions are carried out.

Implications of Brain Heritability and Development

Why do male songbirds learn song and sing, and why do females pay attention to the songs? This question is rarely raised by neurobiologists, but has received extensive thought by ecologists and behavioral biologists. For song to be a signal that is selected by females, it must convey reliable information about the male's proximate or ultimate fitness, in other words, a male's song should either indicate that he has attributes that are immediately useful to the female like a good territory or good foraging skills, or that he has genes that would increase the chances of survival of his offspring [Zahavi, 1975; Dawkins and Krebs, 1978; Andersson, 1994; reviewed by Nowicki et al., 2002b]. Several different song-encoded indicators of fitness have been suggested. For example, sustained complex song might carry a handicap, by exposing a male to predation and preventing him from foraging [Zahavi, 1975]. Then singing such a song would indicate to a female that the male has evaded predation or is especially efficient or aggressive about foraging, and so is superior to other males. Alternatively, the ability to sing a complex

song might indicate favorable nutrition during rearing, thereby optimizing learning during the sensitive phase [Nowicki et al., 2002b] or favorable current body condition, perhaps associated with enhanced immunocompetence or reduced parasite load [Möller, 1991]. Such factors could easily lead to enhanced ability to maintain a favorable territory and to forage, and potentially could result from traits that are heritable, such that song would be an honest indicator of male qualities related to both proximate and ultimate fitness.

Our data suggest that song complexity can be an honest indicator of male quality through what it conveys about brain structure. As reviewed above, male songbirds have a network of nuclei involved in song acquisition and production. Both males and females have an auditory song system, structures for which increasing evidence indicates that they play a role in song perception and discrimination. There are close relations between the sizes, or the balance of sizes, of the motor song system nuclei and how complex a song an individual male can learn and produce. Thus, females that select males on the basis of song complexity are selecting for particular configurations of the neuroanatomy of the song system. One way then to evaluate how song can be an honest indicator for females is to assess consequences and correlates of particular patterns of song system anatomy. David Airey, working in my lab, has done this by measuring brain features including the sizes of nuclei of the motor song system in 190 zebra finches from 38 pairs and their progeny [Airey et al., 2000c]. We find that variation among individuals in the volumes of these nuclei are positively correlated with the volumes of nuclei that are one synapse removed as well as with the overall volume of the telencephalon (fig. 4). Thus, should a female finch select a male on the basis of song complexity, on average, she will be choosing a male with a larger HVC, larger Area X and RA, and larger overall forebrain (the brain subdivision in which vertebrate complex learning occurs). It is plausible that male songbirds with larger forebrains are better at many behaviors besides song, perhaps including learning and remembering generally, and that these abilities would contribute to the immediate fitness of their offspring.

We also find that the variation in the volumes of HVC, RA and nXII is heritable (fig. 5). The heritabilities of these structures directly involved in song production are higher than for Area X and IMAN, suggesting that variation in the latter structures is less tied to genetics and may be more tied to the environment during development. Evolvability, a relative measure of the predicted response to selection, is higher for HVC and RA volumes than for

Fig. 4. Across male zebra finches, variation in the volume of a nucleus of the motor song system is significantly correlated with variation in the volumes of song system nuclei with which it is monosynaptically connected. In addition, variation in the volumes of HVC and Area X is correlated with variation in telencephalon volume [Airey et al., 2000c].

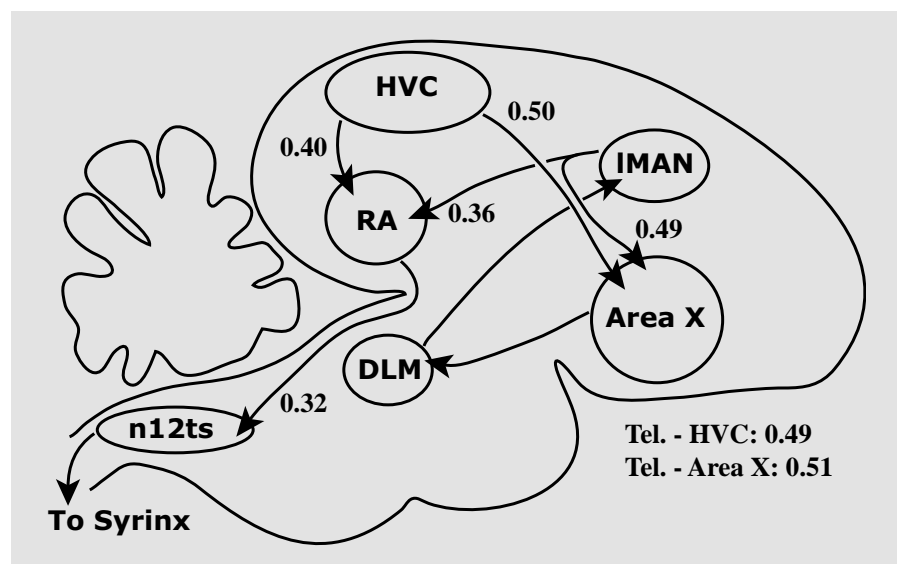
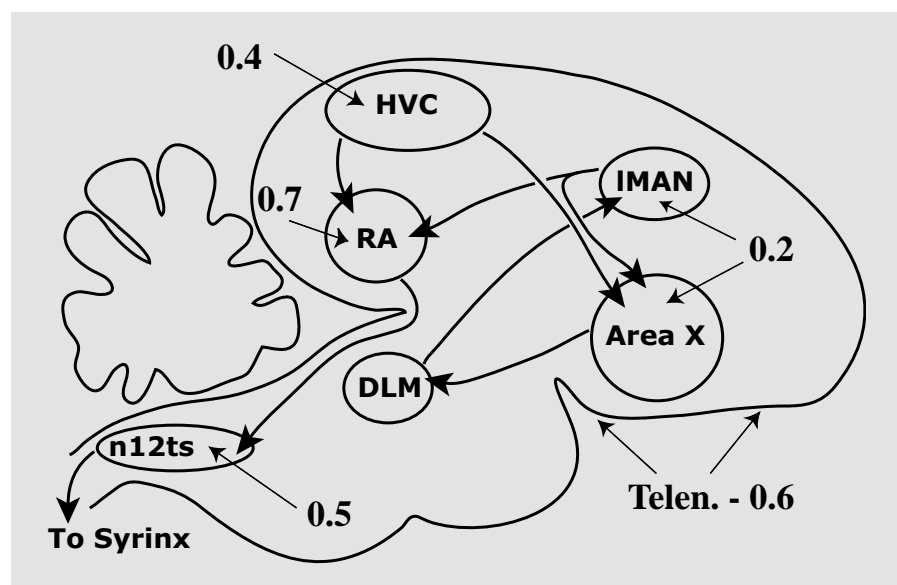


Fig. 5. Variation in the volumes of nuclei of the motor song system that are directly involved in song production is heritable in male zebra finches. Variation in rostral nuclei that are part of a basal ganglia-like loop that monitors and modulates these caudal nuclei is not significantly heritable [Airey et al., 2000c].



all the other traits measured. Thus, should a female select a male on the basis of song complexity, she will be choosing a trait that, to the extent that it depends on the volumes of these nuclei of the motor song system, is likely to be passed on to their sons. In other words, she is choosing a trait that, because it is associated with increased fitness in the male, will contribute to the ultimate fitness of their offspring.

These data also raise questions about constraints on song acquisition. Why should evolvability or the expected capacity for change in response to selection be high, if females are persistently selecting on the basis of song com-

plexity? It is possible that this is a consequence of the relaxation from selection associated with many generations of domestication. If so, evolvability should be lower in wild-caught individuals. The correlations of HVC volume with the volumes of related nuclei of the song system as well as with the volume of the entire remaining telencephalon suggest that it might not be possible to build and sustain a larger HVC without increasing the size of afferent and efferent structures and of more general integrative brain regions. This would mean that overall brain weight and energetic demands could be constraints on expression of a larger HVC.

Finally, it is possible that simply selecting for a larger HVC does not necessarily lead to a more complex song. As indicated earlier, having a more complex song in zebra finches is associated with having a relatively small Area X in addition to a relatively large HVC. Results from lesion and electrophysiology studies indicate that the rostral nuclei in the motor song system play a role in modulating the caudal nuclei during song acquisition and performance [Bottjer et al., 1984; Scharff and Nottebohm, 1991; Rosen and Mooney, 2000]. The projection from Area X to the thalamus is inhibitory, whereas the projections from the thalamus through MAN and on to RA are excitatory [Luo and Perkel, 1999]. Thus, very simplistically, an Area X that is small with respect to the sizes of HVC and RA might permit more lMAN → RA excitation than a larger Area X, and this enhanced input might facilitate consolidation of the multiple configurations of the syringeal muscles needed for an elaborate syllable repertoire. If true, developmental processes leading to enlargement of HVC (such as prolonged neurogenesis and decreased apoptosis)

would also lead to an enhanced Area X, and so could prevent acquisition of a song as complex as HVC could encode. Again, the simplest biologically feasible means of increasing the volume of HVC while retaining or decreasing the relative size of Area X might be to increase overall brain size – thereby preserving song complexity as an honest signal to a female of male quality. Clearly, many of these suggestions although testable, are presently conjectural. Nonetheless, overall the data reviewed above indicate that females might select males on the basis of song complexity in some species because this trait conveys honest information about the males' brains, information that can be related both to proximate and to ultimate fitness.

Acknowledgements

Thanks to Sarah Newman for editorial suggestions and to NSF IBN 0090963 for support.

References

- Airey DC, DeVogd TJ (2000) Variation in song complexity and HVC volume are significantly related in zebra finches. *NeuroReport* 10: 2339–2344.
- Airey DC, Buchanan KL, Catchpole CK, DeVogd TJ (2000a) Song complexity, sexual selection and a song control nucleus (HVC) in the brains of European sedge warblers. *J Neurobiol* 44:1–6.
- Airey DC, Castillo-Juarez H, Casella G, Pollak EJ, DeVogd TJ (2000c) Variation in the volume of zebra finch song control nuclei is heritable: developmental and evolutionary implications. *Proc R Soc Lond B* 267:2099–2104.
- Airey DC, Kroodsma DE, DeVogd TJ (2000b) Learning a larger song repertoire increases spine density in a songbird telencephalic control nucleus. *Neurobiol Learn Mem* 73:274–281.
- Andersson M (1994) *Sexual Selection*. Princeton NJ: Princeton University Press.
- Ball GF, Balthazart J (2001) Ethological concepts revisited: Immediate early gene induction in response to sexual stimuli in birds. *Brain Behav Evol* 57:252–270.
- Beguín N, LeBoucher G, Kreutzer M (1998) Sexual preferences for mate song in female canaries (*Serinus canaria*). *Behav* 135:1185–1196.
- Bensch S, Hasselquist D (1992) Evidence for active female choice in a polygynous warbler. *Anim Behav* 44:301–311.
- Bolhuis JJ, Macphail EM, (2001) A critique of the neuroecology of learning and memory. *Trends Cog Sci* 4:426–433.
- Bottjer SW, Miesner E, Arnold AP (1984). Forebrain lesions disrupt development but not maintenance of song in passerine birds. *Science* 224:901–903.
- Brenowitz EA (1991) Altered perception of species-specific song by female birds after lesions of a forebrain nucleus. *Science* 251:303–305.
- Brenowitz EA, Lent K, Kroodsma DE (1995) Brain space for learned song in birds develops independently of song learning. *J Neurosci* 15: 6281–6286.
- Burek MJ, Nordeen KW, Nordeen EJ (1991). Neuron loss and addition in developing zebra finch song nuclei are independent of auditory experience during song learning. *J Neurobiol* 22:215–223.
- Canady RA, Kroodsma DE, Nottebohm F (1984). Population differences in complexity of a learned skill are correlated with the brain space involved. *Proc Nat Acad Sci USA* 81:6232–6234.
- Catchpole CK (1980) Sexual selection and the evolution of complex songs among European warblers of the genus *Acrocephalus*. *Behaviour* 74:149–166.
- Catchpole CK (1986) Song repertoires and reproductive success in the great reed warbler, *Acrocephalus arundinaceus*. *Behav Ecol Sociobiol* 19:439–445.
- Catchpole CK, Dittami J, Leisler B (1984) Differential responses to male song repertoires in female songbirds implanted with oestradiol. *Nature* 312:563–564.
- Catchpole CK, Dittami J, Leisler B (1986) Sexual differences in the responses of captive great reed warblers (*Acrocephalus arundinaceus*) to variation in song structure and size. *Ethology* 73:69–77.
- Catchpole CK, Leisler B (1996) Female aquatic warblers (*Acrocephalus paludicola*) are attracted by playback of longer and more complicated songs. *Behaviour* 133:1153–1164.
- Chew WJ, Vicario DS, Nottebohm F (1996). A large capacity memory system that recognizes the calls and songs of individual birds. *Proc Nat Acad Sci USA* 93:1950–1955.
- Clayton NS (1988) Song discrimination learning in zebra finches. *Anim Behav* 36:1016–1024.
- Clayton NS (1990) Subspecies recognition and song learning in zebra finches. *Anim Behav* 40: 1009–1017.
- Clayton N, Pröve E (1989) Song discrimination in female zebra finches and Bengalese finches. *Anim Behav* 38:352–354.
- Collins SA, Hubbard C, Houtman AM (1994). Female mate choice in the zebra finch: The effect of male beak colour and male song. *Behav Ecol Sociobiol* 35:21–25.
- Dawkins R, Krebs J (1978) Animal signals: information or manipulation? In: *Behavioural Ecology* (Krebs JR, Davies NB, eds), pp 282–309. Sunderland, MA: Sinauer.
- DelNegro C, Gahr M, LeBoucher G, Kreutzer M (1998) The selectivity of sexual responses to song displays: effects of partial chemical lesion of the HVC in female canaries. *Behav Brain Res* 96:151–159.

- DeVoogd TJ, Lauay CHA (2001) Emerging psychobiology of the avian song system. In: Handbook of Behavioral Neurobiology, Vol 13 (Blass E, ed), pp356–392. New York: Kluwer Academic/Plenum.
- DeVoogd TJ, Szekely T (1998) Causes of avian song: Using neurobiology to integrate proximal and ultimate levels of analysis. In: Animal Cognition in Nature (Pepperberg I, Kamil A, Balda R, eds), pp 337–380. San Diego CA: Academic Press.
- DeVoogd TJ, Krebs JR, Healy SD, Purvis A (1993) Relations between song repertoire size and the volume of brain nuclei related to song: comparative evolutionary analyses amongst oscine birds. *Proc R Soc Lond B* 254:75–82.
- Dubnau J, Tully T (1998) Gene discovery in *Drosophila*: New insights for learning and memory. *Ann Rev Neurosci* 21:407–444.
- Duffy DL, Bentley GE, Ball GF (1999) Does sex or photoperiodic condition influence ZENK induction in response to song in European starlings? *Brain Res* 844:78–82.
- Gentner TQ, Hulse SH (2000) Female European starling preference and choice for variation in conspecific male song. *Anim Behav* 59:443–458.
- Gentner TQ, Hulse SH, Duffy D, Ball GF (2000) Response biases in auditory forebrain regions of female songbirds following exposure to sexually relevant variation in male song. *J Neurobiol* 46:48–58.
- Güttinger HR (1985) Consequences of domestication on the song structures in the canary. *Behaviour* 94:254–278.
- Hasselquist D, Bensch S, von Schantz T (1996) Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. *Nature* 381:229–332.
- Jarvis ED, Nottebohm F (1997) Motor-driven gene expression. *Proc Nat Acad Sci USA* 94:4097–4102.
- Jarvis ED, Ribeiro S, da Silva JL, Ventura D, Vielliard J, Mello CV (2000) Behaviourally driven gene expression reveals song nuclei in hummingbird brain. *Nature* 406:628–632.
- Jarvis ED, Scharff C, Grossman MR, Ramos JA, Nottebohm F (1998) For whom the bird sings: context-dependent gene expression. *Neuron* 21:775–788.
- Jin H, Clayton DF (1997) Localized changes in immediate-early gene regulation during sensory and motor learning in zebra finches. *Neuron* 19:1049–1059.
- Kimpo RR, Doupe AJ (1997) FOS is induced by singing in distinct neuronal populations in a motor network. *Neuron* 18:315–325.
- Kroodsmas DE (1976) Reproductive development in a female song bird: differential stimulation by quality of male song. *Science* 232:395–398.
- Kroodsmas DE, Canady RA (1985) Differences in repertoire size, singing behavior and associated neuroanatomy among Marsh Wren populations have a genetic basis. *Auk* 102:439–446.
- Lauay CHA, Gerlach NM, Regan EA, DeVoogd TJ (2004) Female zebra finches require early song exposure to prefer high quality song as adults. *Anim Behav*, in press.
- Lauay CHA, Pflaster A, Komorowski R, DeVoogd TJ (2002) In zebra finches, dendritic spine frequency in NCM varies with exposure to song during rearing. *Soc Neurosci Abstr* 382.4
- Leitner S, Catchpole CK (2002) Female canaries that respond and discriminate more between male songs of different quality have a larger song control nucleus (HVC) in the brain. *J Neurobiol* 52:294–301.
- Leitner S, Leisler B, DeVoogd TJ, Catchpole CK (2002) The size of a major song control nucleus (HVC) in the brains of European sedge warblers has a strong genetic component. *Proc R Soc Lond B* 269:2519–2524.
- Luo M, Perkel DJ (1999) Long-range GABAergic projection in a circuit essential for vocal learning. *J Comp Neurol* 403:68–84.
- Luo M, Ding L, Perkel DJ (2001) An avian basal ganglia pathway essential for vocal learning forms a closed topographic loop. *J Neurosci* 21:6836–6845.
- MacDougall-Shackleton SA, Hulse SH, Ball GF (1998) Neural bases of song preferences in female zebra finches (*Taeniopygia guttata*). *NeuroReport* 9:3047–3052.
- MacDougall-Shackleton SA, MacDougall-Shackleton EA, Hahn TP (2001) Physiological and behavioural responses of female mountain white-crowned sparrows to natal- and foreign-dialect song. *Can J Zool* 79:325–333.
- Mello CV, Clayton DF (1994). Song-induced ZENK gene expression in auditory pathways of songbird brain and its relation to the song control system. *J Neurosci* 14:6652–6666.
- Mello CV, Ribeiro S (1998) ZENK protein regulation by song in the brain of songbirds. *J Comp Neurol* 393:426–438.
- Mello CV, Vicario DS, Clayton DF (1992) Song presentation induces gene expression in the songbird forebrain. *Proc Nat Acad Sci USA* 89:6818–6822.
- Miller DB (1979a) The acoustic basis of mate recognition by female zebra finches (*Taeniopygia guttata*). *Anim Behav* 27:376–380.
- Miller DB (1979b) Long-term recognition of father's song by female zebra finches. *Nature* 280:389–391.
- Möller AP (1991) Parasite load reduces song output in a passerine bird. *Anim Behav* 41:723–730.
- Nagle L, Kreutzer M, Vallet E (2002) Adult female canaries respond to male song by calling. *Ethology* 108:463–472.
- Nottebohm F (1981) A brain for all seasons: Cyclical anatomical changes in song-control nuclei of the canary brain. *Science* 214:1368–1370.
- Nottebohm F, Kasparian S, Pandazis C (1981) Brain space for a learned task. *Brain Res* 213:99–109.
- Nottebohm F, Stokes TM, Leonard CM (1976) Central control of song in the canary. *J Comp Neurol* 165:457–68.
- Nowicki S, Searcy WA, Peters S (2002a) Quality of song learning affects female response to male bird song. *Proc R Soc Lond B* 269:1949–1954.
- Nowicki S, Searcy WA, Peters S (2002b) Brain development, song learning and mate choice in birds: a review and experimental test of the 'nutritional stress hypothesis'. *J Comp Physiol A* 188:1003–1014.
- O'Loughlin AL, Beecher MD (1999) Mate, neighbour and stranger songs: a female song sparrow perspective. *Anim Behav* 58:13–20.
- Ribeiro S, Cecchi GA, Magnasco MO, Mello CV (1998) Toward a song code: Evidence for a syllabic representation in the canary brain. *Neuron* 21:359–371.
- Riebel K (2000) Early exposure leads to repeatable preferences for male song in female zebra finches. *Proc R Soc Lond B* 267:2553–2558.
- Rosen MJ, Mooney R (2000) Intrinsic and extrinsic contributions to auditory selectivity in a song nucleus critical for vocal plasticity. *J Neurosci* 20:5437–5448.
- Scharff C, Nottebohm F (1991) A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: Implications for vocal learning. *J Neurosci* 11:2896–2913.
- Searcy WA (1984) Song repertoire size and female preferences in song sparrows. *Behav Ecol Sociobiol* 14:281–286.
- Searcy WA, Brenowitz EA (1988) Sexual differences in species recognition of avian song. *Nature* 332:152–154.
- Searcy WA, Searcy MH, Marler P (1982) The response of swamp sparrows to acoustically distinct song types. *Behaviour* 80:70–83.
- Sundberg KA, Newman SW, Buki J, DeVoogd TJ (2001) Female songbirds that differ in song experience or quality of song discrimination also differ in their IEG response to hearing song. *Soc Neurosci Abstr* 27.843.
- Szekely T, Catchpole CK, DeVoogd A, Marchl Z, DeVoogd TJ (1996) Evolutionary changes in a song control area of the brain (HVC) are associated with evolutionary changes in song repertoire among European warblers (Sylviidae). *Proc R Soc Lond B* 263:607–610.
- Tramontin AD, Brenowitz EA (2000) Seasonal plasticity in the adult brain. *Trends Neurosci* 23:251–258.
- Vallet E, Kreutzer M (1995) Female canaries are sexually responsive to special song phrases. *Anim Behav* 49:1603–1610.
- Ward BC, Nordeen EJ, Nordeen KW (1998) Individual variation in neuron number predicts differences in the propensity for avian vocal imitation. *Proc Nat Acad Sci USA* 95:1277–1282.
- Zahavi A (1975) Mate selection – a selection for a handicap. *J Theor Biol* 53:205–214.