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Singing-driven gene expression in the developing songbird brain

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Abstract

Neural and behavioral development arises from an integration of genetic and environmental influences, yet specifying the nature of this interaction remains a primary problem in neuroscience. Here, we review molecular and behavioral studies that focus on the role of singing-driven gene expression during neural and vocal development in the male zebra finch (*Taeniopygia guttata*), a songbird that learns a species-typical vocal pattern during juvenile development by imitating an adult male tutor. A primary aim of our lab has been to identify naturally-occurring environmental influences that shape the propensity to sing. This ethological approach underlies our theoretical perspective, which is to integrate the significance of singing-driven gene expression into a broader ecological context.

Keywords

Zebra finch; Song production; Telencephalon; Food availability; ZENK; Post-transcriptional regulation

As juveniles, male zebra finches learn a vocal pattern that they will use to communicate with conspecifics [1,2]. Young males typically learn their father's song and produce the paternal vocal pattern throughout adult life in a stereotyped, unmodified fashion. Vocal learning involves at least two partially overlapping phases termed 'auditory' and 'sensory-motor'. During the auditory phase, young males focus their attention on the song of an adult male, memorizing it. The sensory-motor phase then begins with the production of highly variable song-like babbling; at this time young males begin to shift their auditory attention to their own vocalizations. Gradually, vocal patterns become less variable and more stereotyped as the bird modifies its vocal output to match the adult song memorized during the auditory phase. In adulthood, males produce their vocal pattern in the form of 'directed song', elicited by the presence of females, but they also produce 'undirected song' in solo contexts where they are unable to see and/or hear conspecifics.

While songbird vocal learning shares many behavioral similarities with human language learning, similarities in CNS organization between songbirds and humans have only recently

begun to receive serious attention [3,4]. Partly this has stemmed from mistaken homologies in the nomenclature for the avian telencephalon that have only recently been corrected [5]. Assessing the mammalian/avian homology of specific telencephalic subregions is now a vigorous area of current research, particularly with respect to the basal ganglia [6]. Nonetheless, at a more general level we know that both the learning of zebra finch song and the learning of human language include cognitive and behavioral processes that originate largely within the telencephalon, and that both species are characterized by an unusually generous allotment of total brain space to telencephalon. Fig. 1 shows a comparison of the proportional brain organization of four species, two that do not show vocal learning (rat, pigeon) and two that do (zebra finch, human). The human and the zebra finch are striking in that well over 50% of total brain space is occupied by the telencephalon. While a proportionally large telencephalon need not imply the presence of vocal learning in a particular species, it does appear that vocal learning is associated with an increase in the amount of brain dedicated to the telencephalon. In general terms then, both zebra finches and humans are “telencephalic specialists”, where the emergence of a large telencephalon seems linked to selection pressure for higher cognitive function and complex behavior, including but not limited to vocal learning.

Within the zebra finch telencephalon, brain regions and circuitry for song behavior can be divided into two functionally distinct pathways (see sagittal CNS schematic in Fig. 1). Both pathways emanate from HVC (proper name) and terminate in RA (robust nucleus of the arcopallium). The main pathway for vocal production is a projection from HVC to RA (shown in black); patterned neural activity in HVC drives RA neurons that in turn activate the brainstem motor neurons (nXIIts, tracheo-syringeal portion of the hypoglossal nucleus) that innervate the vocal organ (syrinx). In contrast, an anterior forebrain pathway (AFP, shown in white) is necessary for vocal plasticity during song learning. The AFP originates in HVC and includes Area X (proper name), DLM (medial portion of the dorsolateral nucleus of the thalamus), and LMAN (lateral magnocellular nucleus of the anterior nidopallium) before terminating in RA. Lesions of the AFP in juvenile males prevent the development of normal song behavior, whereas identical lesions in adults do not affect the recitation of already-learned song.

1. The promise and significance of singing-driven gene expression

In male zebra finches song control brain regions undergo substantial changes in volume, neuron number, and axonal connectivity during song learning and it has been suggested that these developmental changes are timed so as to coincide with the period of vocal learning [7–9]. Female zebra finches do not learn to sing and their lack of well-defined song control brain regions reinforces the idea that morphological development of the song system is timed so as to influence the ability of juvenile males to learn song. That is, some female song regions are monomorphic to those of males at the beginning of song learning (most notably LMAN and RA), but these regions then atrophy as the phase of song learning is played out. Thus, by adulthood song regions in vocalizing males are much larger and contain many more neurons, whereas female song regions have lost both volume and neurons and the ability to learn vocal behavior.

The development of RA is perhaps most illustrative of sex-specific neurodegeneration in the song system (see Fig. 2). In males, RA volume doubles during the phase of vocal learning, an increase attributable to greater spacing between a fixed number of neurons and the addition of glial cells [7,10–15]. In contrast, female RA is roughly monomorphic to male RA at the time song learning begins, but then nearly half of RA neurons die, the surviving neurons increase their density (i.e., move closer together), and the overall volume of female RA diminishes significantly [7,11,13,16]. The result of this pattern of development is that the volume and number of RA neurons are substantially greater in adult males than in adult females. Given the magnitude of the sex difference in the morphology of the song control system, a surprising yet consistent result over the past 25 years is that gonadal hormones alone are not sufficient (although they appear to be necessary) to direct the sexual differentiation of the zebra finch song control system (reviewed by Arnold [17] but cf. [18,19]). These findings present an interesting problem of development – there must be other factors besides sex hormones that influence song learning and the sex-specific development of these neural populations.

Our approach to this problem is based on Jarvis and Nottebohm's [20], Kimpo and Doupe's [21], and Jin and Clayton's [22] discovery that singing by male birds drives expression of transcription factors (i.e. ZENK; *c-fos*) in several song control brain regions in the motor pathway (HVC, RA) and the AFP (Area X, LMAN). These transcription factors have a potentially broad influence on the expression of other genes in the developing song system. That is, the act of singing by juvenile males could play a specific role in vocal learning (e.g., in storing the effects of vocal practice during sensory-motor learning) and, because only males sing, singing-driven gene expression will produce a sex difference in gene expression within developing song regions that could contribute to the sex-specific neural fates of these regions.

Therefore, our working hypothesis is that the quantity of vocal practice by juvenile males may influence the quality of song learning as well as the morphological development of song regions. We emphasize that a focus on the influence of sensory and/or motor experience on specific aspects of neural and behavioral development has an extensive history that includes classic work by Lehrman [23] and more recently Gottlieb [24] (although see a recent review by Marler [25] for a different perspective on the influence of Lehrman). Also, the idea that song behavior might influence the development of song regions in a use-dependent way was conceived previously by both G. Ball and M. Konishi (see p.14 in Hauser [26]). However, the discovery of singing-driven gene expression in the song control system [20–22] provided a mechanism that allowed us to conceptualize how behavior, via the regulation of gene expression, might influence neural and behavioral development in songbirds. Focusing on a similar gene regulatory mechanism, readers should also see recent papers by Ball et al. [27] and Nottebohm [28] that evaluate the possible contribution of singing to seasonal changes in the morphology of song control regions in adult songbirds.

While the remainder of this paper will highlight developmental and behavioral studies from our own laboratory, readers should refer to several recent anthologies of songbird neuroethology for additional information on the enormous breadth and integrative

complexity of songbird research (Journal of Neurobiology, November 1997; Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology, December 2002; Annals of the New York Academy of Sciences, June 2004).

2. Singing-driven ZENK expression: implications for sensory-motor learning

With the discoveries of Jarvis and Nottebohm [20], Kimpo and Doupe [21], and Jin and Clayton [22] as impetus, we conducted our own descriptive study of singing-driven ZENK, where we measured ZENK expression in juvenile and adult birds at distinct stages of vocal development [29]. The initial developmental studies of ZENK measured expression only at the level of mRNA, and not protein [20,22,30], so we re-evaluated the role of ZENK in RA and HVC by measuring its expression at both mRNA and protein levels during undirected singing in juvenile and adult birds.

Our primary finding was a developmental increase in singing-driven ZENK immunoreactivity in RA during the juvenile emergence and adult maintenance of stereotyped singing [29]. That is, ZENK protein labeling was low in RA of birds producing subsong, while ZENK protein labeling in RA was substantially increased in birds producing plastic song or adult song. Moreover, this effect was specific to RA; ZENK protein labeling in HVC was present at similar levels regardless of stage of vocal development. Examination of ZENK mRNA labeling revealed a robust induction of ZENK mRNA in both HVC and RA at all developmental stages, suggesting a specific post-transcriptional inhibition of ZENK in RA that is apparently released as the vocal pattern matures. In other words, any form of singing (subsong, plastic song, or adult song) appears to drive transcription of ZENK in RA, but the subsequent translation of the ZENK transcript appears conditional, requiring other developmental or associative factors for complete induction of the ZENK protein.

Initially we asked whether this change in ZENK protein labeling in RA was simply age-related; for example, did the change reflect an endogenous genetic program of development or was it instead related to vocal development? To answer this question we used bilateral LMAN lesions to disrupt vocal development in a group of birds producing subsong (such lesions in juvenile birds induce an abnormal, premature vocal stereotypy [31]). Relative to age-matched subsong controls, juveniles with bilateral LMAN lesions sang prematurely stereotyped songs and showed dramatically increased ZENK protein labeling in RA, suggesting a specific relationship to vocal development instead of age [29]. Because LMAN is one of two major sources of afferent input to RA (along with HVC), LMAN synapses in RA will be lost following these lesions. Therefore, our lesion findings also suggest that the processing of ZENK protein in RA is normally inhibited by LMAN afferent input during subsong and that the premature induction of ZENK protein in RA neurons may be related to the abnormal vocal patterns produced by juvenile birds with LMAN lesions.

As a convergence point for two major streams of neural activity within the song system, RA is clearly positioned to serve an associative function during singing (see Fig. 1). Based on the findings described above, we hypothesize that induction of ZENK protein in RA could

be a mechanism to establish associations between motor information (from HVC) and learning/plasticity information (from LMAN). What is novel and interesting about such an arrangement is that transcriptional and translational stages of gene expression become two distinct steps in a hypothetical associative mechanism, with HVC input acting at the level of ZENK mRNA and LMAN input acting at the level of ZENK protein.

We do not know whether LMAN input (1) regulates expression of a translation factor required by the ZENK transcript (2) increases ZENK protein degradation or (3) regulates a modulator of ZENK activity that interferes with detection by antibody. Nevertheless, all of these mechanisms would require that neurons in HVC and LMAN form synapses on the same individual RA neurons and evidence of this specific neural connectivity has been established in zebra finches [32–35]. Moreover, different ionotropic glutamate receptors mediate HVC vs. LMAN synaptic inputs to RA; HVC terminals activate NMDA and non-NMDA receptors on RA neurons, while LMAN terminals activate predominantly NMDA receptors [32–35]. One possibility is that HVC depolarization of RA neurons could mediate ZENK transcription, whereas LMAN input to RA neurons could activate second-messenger systems that regulate ZENK translation.

Important questions remain as to the nature of the signals conveyed by HVC and LMAN to RA, and therefore what information could be represented by the induction of ZENK protein in RA. While the role of HVC in driving vocal production seems clear [36,37], the role of LMAN in vocal behavior does not. Original observations by Bottjer et al. [31] that LMAN lesions in juvenile birds specifically impaired sensory-motor learning (the same lesions in adult birds had no effect on already-learned song) suggested a role for this pathway in learning. However, Brainard and Doupe's [38] demonstration that LMAN lesions in adult birds blocked deafening-induced deterioration of song indicated a more general role in vocal plasticity. These findings led to the hypothesis that LMAN activity encodes an error signal generated by comparing auditory feedback with the memory of the tutor song [38,39]. According to this hypothesis, LMAN input to RA is instructive and a large signal should be generated when auditory feedback indicates a mismatch to the memory of the tutor song.

However, a recent test of the error-signal hypothesis has failed to find support; Leonardo [40] found that LMAN activity was unaffected by bursts of white noise that were presented while birds were engaged in directed singing, and instead LMAN activity remained time-locked to production of the song. Given these and other recent findings [41], it is difficult at present to define LMAN's role beyond its necessity for vocal plasticity. However, in defense of the error-signal hypothesis it should be noted that (1) the pattern of LMAN activity is different during undirected song [42] and (2) the pattern of singing-driven ZENK expression is different when birds are producing directed song, suggesting that the vocal-control system is in a different functional state [30]. Attention is clearly focused on the female during directed singing (perhaps at the expense of auditory feedback) so measuring the effect of altered auditory feedback on LMAN activity during undirected singing may eventually reveal a different result.

Future experiments where singing-driven ZENK expression is measured in different auditory contexts may also shed light on the role of LMAN, not to mention the correlation

between vocal development and ZENK protein labeling in RA. For example, Clayton [43] has theorized that ZENK expression may serve as a 'genomic action potential' with the capacity to integrate information across a time scale of seconds to minutes instead of the millisecond synaptic integration times that lead to an electrophysiological action potential. Therefore, perhaps ZENK expression in RA reflects a longer time-scale integration of synaptic input from HVC and LMAN that is functionally distinct from the rapid (millisecond) changes in electrophysiological activity predicted by the error-signal hypothesis. Indeed, Clayton [43] has suggested that the ZENK response may be preparatory for learning or storage of memory, rather than participating directly in these processes. Given the integrative synaptic connectivity of RA, such a role is consistent with the emergence of robust ZENK protein labeling during plastic song, when levels of vocal practice peak [44] and characteristics of the mature vocal pattern first appear.

3. The behavioral ecology of singing-driven gene expression

The discovery of singing-driven gene expression [20–22] also generated several new purely behavioral questions about zebra finch vocal development. First, in order to quantify the effects of singing-driven gene expression, we conducted a quantitative behavioral study of zebra finch vocal development. Second, we undertook a series of studies to identify environmental conditions that influence the propensity to sing; these factors would presumably determine the 'dose' of singing-driven gene expression received by developing birds, allowing us to begin to understand singing-driven gene expression in an ecological context.

The classic studies of Immelmann [1] and Arnold [2] represent the traditional sources of our understanding of zebra finch sensory-motor learning. These authors provided key qualitative observations on the stage-like progression of song development from subsong (initial highly variable vocalizations) to plastic song (note elements present but produced in variable sequences) to song crystallization (note and sequence stability achieved). More recently Tchernichovski et al. [45] applied quantitative techniques to describe the development of individual song notes, discovering that the variety of adult note types emerges from only a few prototypical notes produced at the beginning of sensory-motor learning. However, because our working hypothesis stipulates that gene expression related to neural development and song learning is in part under behavioral regulation, it became important to know the quantity of singing produced by juvenile birds as they learned their vocal pattern. Therefore, using a computerized sound–event triggered recording system, we measured 24 h song bout totals at weekly intervals in a group of developing male zebra finches housed on a 14:10 light:dark cycle [44].

We found that song production showed an inverted U-shaped function across vocal development; birds sang the least while in subsong, dramatically increased vocal production during plastic song and song crystallization, then decreased vocal production as they matured into adulthood [44]. Analysis of the diurnal pattern of song production (birds never sang in the dark phase) revealed that the high levels of vocal production generated during sensory-motor learning were accomplished in part by distinct morning and afternoon peaks in singing, whereas in adulthood 60%–70% of all singing was produced in the morning. The

significance of the developmental shift in the diurnal distribution of singing is unknown. However, with Clayton's 'genomic action potential' in mind [42], we have speculated [44] that the high level of morning singing by juveniles may produce a saturation of ZENK transcriptional activity, such that a brief refractory period is required before afternoon singing can drive further ZENK expression. That is, juvenile birds may distribute daily singing in such a way to optimize the molecular benefits of vocal practice.

Together, our quantitative data suggest that sensory-motor learning is indeed a form of trials-based learning, with more trials required to learn than to maintain the behavior. A corollary would be that more trials should produce better learning, and we found a strong positive correlation between the level of vocal production during plastic song and the sequence stability of the vocal pattern in adulthood ($R^2=0.77$, [44]). Our data further suggest a positive correlation between daily song bout production and the morphological development of song regions in males. For example, in earlier studies of the neural development of the song system (e.g., [9,11,46]) a pattern of morphological growth and regression was reported that is strikingly similar to the inverted U-shaped pattern of daily song bout production that we observed during vocal development [44]. However, it is important to note that we cannot say whether larger song regions cause high levels of singing, or whether higher levels of singing lead to larger song regions.

Testing the role of singing-driven gene expression in neural and behavioral development would be facilitated by a means to manipulate the amount of singing by individual birds. Previously, Slater and Jones [47] used a natural stimulus (playback of aviary sounds) to increase singing while Li et al. [48] used the presence of a human observer in the aviary to decrease singing. However, playback of aviary sounds produced only a small increase in singing, while the continuous presence of a human observer is not practical for long-term studies. Deactivation of the muscles of vocalization (the syrinx) has also been used to prevent birds from singing (e.g., [49,50]), but birds continue to attempt vocalizations following such peripheral manipulations. Moreover, the vocal distortion that accompanies such manipulations, although temporary, alters auditory feedback and produces a combined motor/auditory insult that complicates interpretation of results.

Like Slater and Jones [47], we sought to identify natural environmental stimuli that influence the propensity to sing. Recall that presentation of a female is a stimulus that will elicit directed song by a male zebra finch, but this and other social manipulations (e.g., playback of aviary sounds) are compromised by the fact that males continue to produce hundreds of bouts of undirected song each day when housed in complete social isolation. Instead, we took inspiration from ethological field and laboratory studies of songbirds suggesting that food availability may serve as a natural sign stimulus for song production [51–54].

By testing adult male zebra finches under conditions of a 2-day food deprivation we discovered a prompt (within 2 h) cessation of undirected singing when food was first removed [55]. In contrast, exposing birds to other non-social environmental manipulations (water deprivation, cold ambient temperature) did not have specific or acute effects on undirected song production [55,56]. The food removal effect was striking in that song

production was attenuated several hours prior to any change in metabolic rate, demonstrating that this was not a deprivation effect due to energy depletion [55].

In free-feeding adult zebra finches, measurement of the diurnal pattern of undirected song production shows that 60%–70% of daily song production is generated in the first half of a 14 h light phase [44,55]. If food availability is a stimulus for undirected singing, we reasoned that removing food for part of the light phase should reduce singing. In order to maximally reduce song production we developed a feeding regimen where food was absent during the first 8 h of the light phase and then made available during the last 6 h – a manipulation we now call Timed Access to Food (TAF). Birds remained on TAF for an 11-day period; body mass and total daily food intake were maintained at free-feeding levels throughout, indicating that access to food during the last 6 h of the light phase was sufficient for the birds to maintain normal energy balance [56]. However, in contrast to these null effects on body mass and food intake, TAF reduced daily undirected song production by more than two-thirds. Moreover, song production diminished rapidly on the first day of TAF and returned promptly to baseline when food was again made available for the entire light phase, suggesting that TAF influences singing via an acute neural mechanism.

Collectively, our findings support other field and laboratory reports that have identified food availability as a specific stimulus for undirected song production [51–55], but studies in additional songbird species should be conducted before more general conclusions are drawn. We have also found that directed song is not affected when birds are on TAF, indicating that the inhibitory effects of food availability on vocal production are specific to undirected song and can be rapidly overridden by the presentation of a female (Johnson, unpublished data).

4. A novel food/song relationship: how and why

Initially, clues to the mechanism of the food/singing relationship were obtained from metabolic measurements made during the course of the TAF manipulation [56]. Free-feeding zebra finches show a pronounced daily rhythm in usage of carbohydrate and lipid fuels, shifting from lipid to carbohydrate metabolism at the onset of the light phase and shifting back at the onset of the dark phase. However, birds on TAF do not shift from lipid to carbohydrate metabolism until food becomes available each day (8 h into the light phase) and then shift back shortly before the onset of the next light phase. These data suggest that the shift from lipid to carbohydrate metabolism at the onset of the light phase might normally play a role in gating the production of undirected song. We have tested this hypothesis by administering drugs that block carbohydrate metabolism (2-deoxyglucose or 2,5-anhydro-D-mannitol) at the onset of the light phase in a group of free-feeding birds. In so doing, we forced usage of lipid fuels and produced a decrease in undirected song production resembling that observed in birds on TAF [57].

In mammals, reliance on lipid metabolism is associated with increased endocannabinoid signaling in the CNS, which in part serves to convey peripheral metabolic information to relevant brain structures, including those for increased appetite [58]. Evidence suggests that a similar sequence of events may link TAF to a reduction in undirected song production. For example, Soderstrom et al. [59] have found that removal of food rapidly increases

telencephalic endocannabinoid levels, and that administration of a CB1 antagonist can rescue undirected singing following food removal. Zebra finch song regions express high levels of CB1 cannabinoid receptors [60] and we previously demonstrated inhibitory effects of a cannabinoid agonist on undirected singing [61] and song-evoked gene expression [62]. Therefore, by altering the daily rhythm of metabolic fuel usage, TAF may increase brain endocannabinoid levels, which could act directly on song regions to reduce undirected song production.

An understanding of the broader ecological significance of the food/singing relationship in songbirds has been facilitated by the work of Nowicki and colleagues [63–65], who advanced a nutritional-stress hypothesis that predicts a specific relationship between the quality of nutrition received by birds as nestlings and their resulting vocal development. Nowicki et al. [63] suggest that “learned features of song can provide an accurate indicator of male quality because they reflect variation in the development of brain areas mediating the learning process, which in turn reflects variation in the response of individuals to nutritional stresses faced early in life.” In this way, “song can be used by females to assess the quality of potential mates.”

In a first test of their hypothesis, Nowicki et al. [64] found that the repertoire size of adult male great reed warblers shows a significant correlation with the development of a morphological feature (the length of the innermost primary feather) that reflects the quality of the nutritional environment that birds experienced as nestlings. More recently, Nowicki et al. [65] and Buchanan et al. [66] directly tested the nutritional stress hypothesis by rearing male swamp sparrow or zebra finch nestlings under conditions of poor nutrition or daily corticosterone injections. Adult birds exposed as nestlings to poor nutrition or stress (but not thereafter) displayed reduced brain size and song region size, and produced low quality songs. Together, the findings of Nowicki et al. [65] and Buchanan et al. [66] support the idea that the ability of a nestling to respond to nutritional stress will affect the subsequent growth of song control brain regions, which in turn will influence the quality of the adult song.

However, song production was not measured in the above studies so it is not clear whether the manipulations of nutrition and stress were also associated with changes in daily singing, and if so, whether reduced levels of singing could have contributed to impaired growth of song regions and/or poor quality songs. While this caveat does not lessen the support these data provide for the ‘nutritional stress hypothesis’, our observations on the food/singing relationship [55,56] suggest a potential addition to the theoretical framework of Nowicki et al. [63] that extends beyond parental feeding of nestlings to include the ability of juvenile birds to forage on their own. That is, the ability of juvenile males to locate and secure food during sensory-motor learning (when they cease to be fed by their parents) may have consequences for the amount of time they spend engaged in vocal practice. Given the positive relationship between the quantity of juvenile vocal practice and the quality of adult songs [44,49], we hypothesize that song quality may also indicate the ability of males to provision themselves with food during sensory-motor learning. In this scenario, the quality of adult male songs may also serve as a reliable indicator of cognitive/behavioral attributes

related to efficient foraging, in addition to indexing the adequacy of nutrition that males obtained as nestlings.

Songbirds are increasingly recognized for their large repertoire of cognitive abilities related to foraging and caching food, including tool use and episodic memory (reviewed in Ref. [4]), but little is known about how these traits evolved. Therefore, a link between foraging/caching efficiency, amount of vocal practice, and song learning suggests that female sexual selection for male song quality may have contributed in a general way to the evolution of the songbird telencephalon and the higher cognitive functions vested therein.

5. Summary

The hypothesis that singing-driven gene expression plays a specific role in neural and/or vocal development in the male zebra finch remains to be fully tested, but it has already begun to stimulate a deeper understanding of the dynamics of vocal production during juvenile development [44,45]. There are also new genetic details to consider, specifically a surprising differential transcriptional vs. translational regulation of singing-driven ZENK that potentially serves as a mechanism for associative learning [29]. However, as zebra finch microarrays [67,68] begin to allow rapid screening for additional singing-driven genes, it will be important to identify natural environmental influences on the propensity to sing so that the function of these genes can be experimentally tested and then understood in a broader ecological context. To that end, we were aided by ethological field and laboratory studies suggesting that food availability influences singing in natural environments [51–54], which led to experimental studies of the effects of food availability on undirected singing [55,56] and the potential metabolic [57] and neurochemical systems [59–62] that convey information about food availability to the brain. Finally, considered in the context of the ‘nutritional stress hypothesis’ [63], our data on the food/singing/gene expression relationship suggest that song quality may also allow female assessment of male cognitive abilities related to locating and securing food. In this way, female preference for higher quality songs may have contributed to the evolution of the disproportionately large telencephalon of songbirds.

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Fig. 1.

Vocal learning as a telencephalic specialization in songbirds and humans. The stacked column graph (above) shows the proportional organization of six major CNS subdivisions in four species, two that learn vocal patterns by imitation (zebra finch, human) and two that do not (rat, pigeon). Brain regions for vocal learning in zebra finches and humans are localized primarily to the telencephalon and both species show an atypically large allotment of total brain space to the telencephalon. Proportional data for the rat and human were replotted from Swanson [69] whereas CNS proportions for pigeon and zebra finch were generated by us (Johnson and Whitney, unpublished data). The sagittal CNS schematic (below) shows major brain regions and axonal connections involved in songbird vocal behavior. Within this network, there are distinct circuits for vocal production (black pathway [36,37]) and vocal plasticity (white pathway [31,38,41]). See text for additional details and abbreviations.

Fig. 2.

Sex-dependent neural fate during development of the song region RA. The volume, neuron density, and neuron number of RA are similar in juvenile males and females [7,10,11,16]. By adulthood, female RA has lost neurons and volume, whereas male RA increases in volume— neuron number in males remains stable due to a decrease in neuron density. Coronal plane of section, thionin-stained tissue, scale bar=200 μ m.