

Birdsong Memory: A Neural Dissociation between Song Recognition and Production

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Summary

Songbirds learn their song from an adult conspecific tutor when they are young, much like the acquisition of speech in human infants [1, 2]. When an adult zebra finch is re-exposed to its tutor's song, there is increased neuronal activation in the caudomedial nidopallium (NCM), the songbird equivalent of the auditory association cortex. This neuronal activation is related to the fidelity of song imitation [3–6], suggesting that the NCM may contain the neural representation of song memory [7]. We found that bilateral neurotoxic lesions to the NCM of adult male zebra finches impaired tutor-song recognition but did not affect the males' song production or their ability to discriminate calls. These findings demonstrate that the NCM performs an essential role in the representation of tutor-song memory. In addition, our results show that tutor-song memory and a motor program for the bird's own song have separate neural representations in the songbird brain. Thus, in both humans and songbirds, the cognitive systems of vocal production and auditory recognition memory are subserved by distinct brain regions.

Results and Discussion

There are strong behavioral, cognitive, and neural parallels between speech acquisition in humans and song learning in songbirds [1, 2, 7]. In both cases, there is a sensitive period for auditory learning, and vocal learning proceeds through a practice stage that is called “babbling” in human infants and “subsong” in songbirds. In addition, it has become apparent that there are analogies and homologies between the brains of birds and mammals [8], prompting a complete overhaul of the nomenclature of the avian brain [9]. Despite these similarities, we know relatively little about the neural substrate of song memory. In the present study, we investigated whether, as in humans, the neural representation of auditory memory and that of vocal production and motor memory are separate in songbirds.

In recent analyses of song-related neuronal activation [3–6], the caudomedial nidopallium (NCM) has emerged as a candidate brain region that could contain the neural substrate for tutor-song memory. The NCM, which is

located outside the conventional song system (Figure 1A), is the likely avian equivalent of the auditory association cortex in the mammalian temporal lobe [1, 7]. If the NCM contains the neural substrate for the representation of tutor-song memory, lesions to this structure should impair recognition of the tutor song. In addition, if there are distinct neural representations of tutor-song memory and a vocal-motor program, then lesions to the NCM should not affect production of the bird's own song (BOS). We investigated this hypothesis by placing bilateral neurotoxic lesions in the NCM of adult zebra finch males (see Supplemental Experimental Procedures and Figure S1 in the Supplemental Data available online). We examined the effects of the lesions on tutor-song recognition by means of song preference tests and used spectrogram analysis before and after surgery to evaluate any changes in the bird's own song.

Lesions to the Caudomedial Nidopallium Impair Song Recognition

Zebra finches prefer the song of their tutor to a novel song [10–12], which suggests that they have learned the characteristics of tutor song and have formed an auditory memory of it. We measured the birds' song preferences by calculating the amount of time spent near a speaker that broadcast the song of their tutor compared to a speaker that broadcast a novel song (see Supplemental Experimental Procedures). Before surgery, the birds showed a significant mean preference for the tutor song (control group, $t(6) = 15.32$, $p < 0.0001$; experimental group, $t(9) = 17.01$, $p < 0.0001$). There was a significant effect of NCM lesions on preference for the tutor song [$F(1,5) = 22.36$; $p < 0.0001$; see Figure 2A]. These results suggest that tutor-song recognition was impaired after lesions to the NCM.

After surgery, the mean preference for the tutor song in the lesioned group was significantly different from the chance level ($t(9) = 2.84$, $p < 0.05$). It is possible that the remaining preference is subserved by parts of the brain that are outside the lesioned region.

An alternative interpretation of these findings is that birds with lesions to the NCM were less motivated to express a preference. Such a lack of motivation would be reflected in reduced activity directed at the stimulus songs in the preference tests. However, there were no significant differences in the number of transitions made between zones in the preference test, before and after surgery in either group (see Supplemental Data). Thus, an explanation of the effects of NCM lesions in terms of reduced motivation is unlikely.

The more a bird has copied from its tutor, the more its own song will resemble the tutor song. The question thus arises whether the males expressed a preference for the tutor song or for their own song. In operant-choice tests, zebra finch males prefer the tutor song to their own song [13], which renders it likely that the preference we measured in the males was indeed a reflection of tutor-song recognition. In addition, there was no

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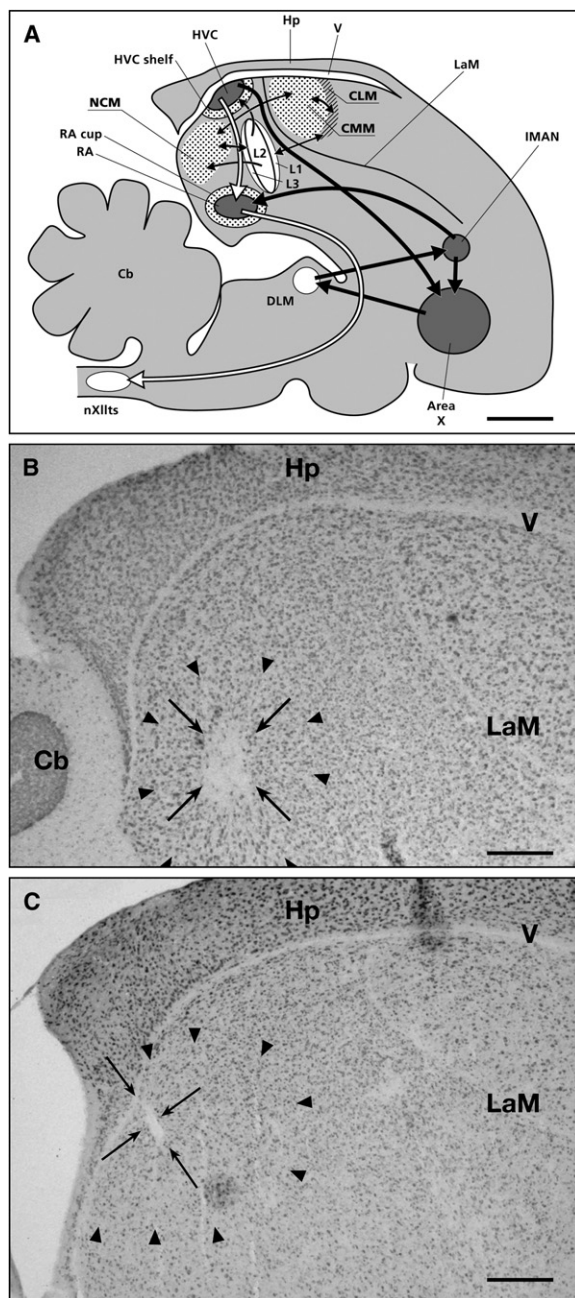


Figure 1. The Songbird Brain and Photomicrographs Showing Two Representative NCM Lesions

(A) Schematic diagram of a composite view of parasagittal sections of a songbird brain gives approximate positions of nuclei and brain regions involved in birdsong. The song system is a network of interconnected brain nuclei, consisting of a caudal pathway (white arrows), considered to be involved in the production of song, and a rostral pathway (thick black arrows), thought to have a role in song acquisition [36, 37]. Thin black arrows indicate known connections between the field L complex, a primary auditory-processing region, and some other forebrain regions. Dark gray nuclei show significantly enhanced expression of immediate early genes (IEGs) when the bird is singing [24]. Stippled areas represent brain regions that show increased IEG expression when the bird hears song [24, 26], including tutor song [3–5]. The scale bar represents 1 mm. Adapted by permission from Macmillan Publishers Ltd: Nature Reviews Neuroscience [7], copyright 2006.

(B and C) Parasagittal sections of the brains of two experimental birds, with immunohistochemical staining against neuron-specific

significant correlation between song similarity (i.e., the number of elements the bird had copied from its tutor song) and the preference for the tutor song, before or after surgery in either group ($r < 0.14$ in all cases). Thus, tutor song was preferred to novel song regardless of how much of the tutor song the birds had incorporated into their own song. These results suggest that lesions to the NCM impaired recognition of the tutor song.

Song Production Is Not Affected by Lesions to the NCM

Song analysis based on multiple song characteristics (see Supplemental Experimental Procedures) revealed that song output was not affected by NCM lesions. That is, within subjects there were no significant differences in songs recorded before surgery and those recorded immediately after surgery [$F(1,12) = 0.38$; group \times time interaction: $F(1,12) = 2.84$], or at 11 days [$F(1,15) = 0.29$; interaction: $F(1,15) = 0.24$], or at 56 days [$F(1,6) = 0.26$; interaction: $F(1,6) = 1.00$] after surgery (Figure 3; Table S1). In addition, songs did not change significantly within subjects in the period of 8 weeks after surgery [$F(2,5) = 0.36$; group \times time interaction: $F(2,5) = 0.47$]. Thus, within a period of 8 weeks, NCM lesions did not significantly affect the males' vocal production.

In zebra finches, modifications in song production may be protracted, as seen in birds deafened during adulthood [14]. In birds that were older than those used in the present study (664–2090 days old), effects of deafening were not apparent until 100 weeks after surgery, in contrast to younger birds (<140 days), of which the songs were affected within 4 weeks after deafening. The authors suggested that every time a bird sings, “a little bit of learning—motor engrainment—occurs” ([14], p. 5054). It may be that we would have found significant changes in song production if we had measured at a later time after surgery. Thus, we cannot exclude the possibility that adequate song production in adults—if it involves learning sensu Lombardino and Nottebohm [14]—would eventually require an intact NCM. However, our results show that in the short term, song production can proceed unaltered when tutor-song recognition is impaired after lesions to the NCM.

NCM Lesions Do Not Affect the Ability to Discriminate between Different Calls

In order to recognize the tutor song, birds must be able to discriminate between different complex vocalizations

nuclear protein (NeuN). Lesions to the NCM 2 weeks after surgery (B) and the NCM 8 weeks after surgery (C) are indicated with arrows. Arrowheads indicate the region that showed structurally changed morphological organization, surrounding the region with dead tissue. All lesions were located within the NCM, overlapping with the area sampled in gene-expression studies [3–5]. The scale bar represents 0.25 mm. Abbreviations are as follows: Cb, cerebellum; CLM, caudal lateral mesopallium; CMM, caudal medial mesopallium; DLM, nucleus dorsolateralis anterior, pars medialis; Hp, hippocampus; HVC, acronym used as a proper name; L1, L2, and L3, subdivisions of field L; LaM, lamina mesopallialis; IMAN, lateral magnocellular nucleus of the anterior nidopallium; NCM, caudal medial nidopallium; nXIIIts, tracheosyringeal portion of the nucleus hypoglossus; RA, robust nucleus of the arcopallium; and V, ventricle.

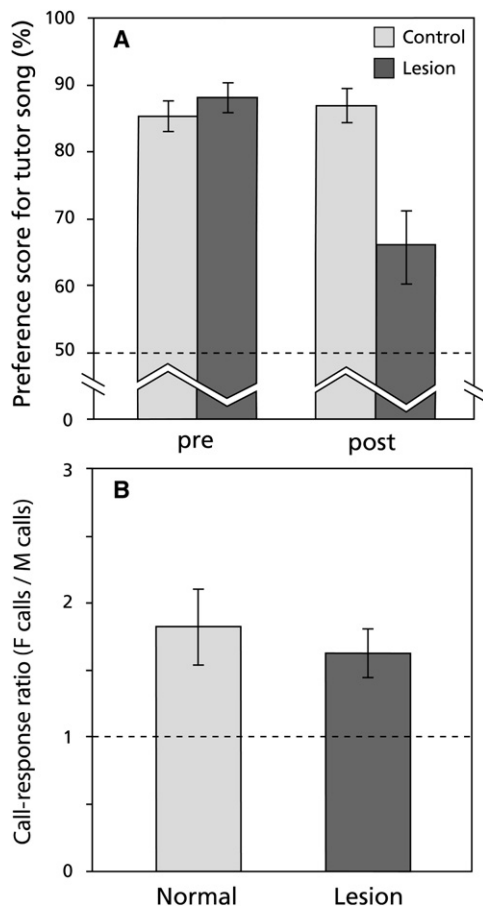


Figure 2. Lesions to the NCM Impair Song Recognition without Affecting Call Discrimination

(A) Mean preference scores (\pm SEM) for tutor song in birds in the two groups.

(B) Mean call-response ratios (\pm SEM) in unoperated control males and males with NCM lesions.

The call-response ratio was calculated for each individual by division of the total amount of responses to female calls by the total amount of responses to male calls.

[15]. The effect of NCM lesions on tutor-song preference might be a result of an impaired ability to discriminate between different sounds. To control for this possibility, we subjected a subset of the experimental subjects and a group of unoperated control birds to a call-discrimination test (see Supplemental Experimental Procedures). Zebra finch long calls have the same frequency range as songs and consist of several harmonics similar to song syllables. Calls of males and females differ in fundamental frequency, frequency modulation, and duration [16] (see Figures S2A and S2B). The calls of males are characterized by a frequency modulation also seen in some types of song syllables. Zebra finch males are able to discriminate calls of the two sexes; they preferentially respond to female calls in playback tests [16].

Figure 2B shows the call-preference scores for birds with NCM lesions and an unoperated control group. Birds with lesions to the NCM exhibited a significant preference for female calls over male calls. In this group, the mean preference for female calls was significantly different from the chance level for both measures used

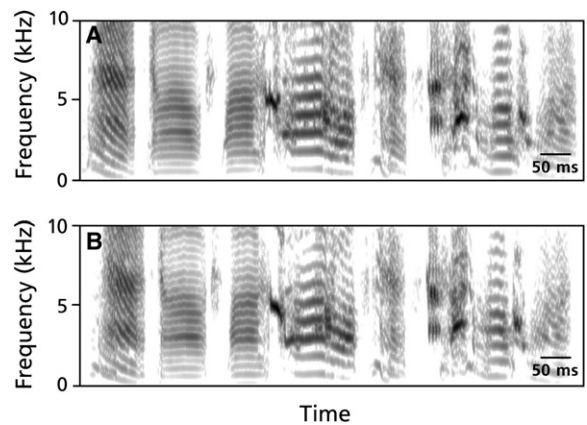


Figure 3. Song Production Is Not Affected by NCM Lesions

Spectrograms of one motif of an experimental bird before (A) and 8 weeks after (B) NCM lesioning. There were no changes in song production after NCM lesions.

(strength of response, shown in Figure 2B: $t(4) = 3.59$; $p < 0.05$. Probability of responding: mean, 1.48; SEM, 0.15; $t(4) = 3.26$; $p < 0.05$). A similar preference for female calls was found in the control group (strength of response, shown in Figure 2B: $t(9) = 2.98$; $p < 0.05$. Probability of responding: mean, 1.53; SEM, 0.21; $t(9) = 2.54$; $p < 0.05$). There was no significant difference in mean preference score between the two groups [$t(13) = 0.44$ for the strength of the response and $t(13) = 0.18$ for the probability of responding].

The preference for female calls shown by the two groups of birds, was smaller than reported by Vicario et al. [16]. It is important to note that the calls used in the present study (see Table S2) have different characteristics (duration, fundamental frequency) than the calls used by Vicario and coworkers. All these characteristics may influence the males' preference for female calls [16]. The fact that in both groups there was a significant preference for female calls demonstrates that discrimination of complex auditory stimuli was not affected by NCM lesions. Thus, it is unlikely that the effect of NCM lesions on tutor-song preference is due to an impaired ability to discriminate sounds.

A Neural Dissociation—Parallels with Humans

These results suggest that the NCM is necessary for recognition of the tutor song, but that its integrity is not required for song production or sound discrimination. These findings are consistent with the hypothesis that the NCM is (part of) the neural substrate for the representation of tutor-song memory [3]. Furthermore, the results suggest a neural dissociation between brain regions (including the NCM) involved in auditory perception and memory and brain regions (including nuclei in the song system) involved in vocal production. This dissociation has a striking parallel in humans, where the neural substrate of speech perception and memory is primarily localized to the auditory association cortex in the superior temporal gyrus [17], whereas motor representations of speech involve frontal-cortex regions centered around Broca's area [18–20]. Presently, there is insufficient evidence for one-to-one homologies between the avian and the mammalian brain [8]. Nevertheless,

neuroanatomical evidence concerning auditory projections from the thalamus has led to the suggestion that the NCM (and possibly the caudomedial mesopallium) is analogous to the mammalian auditory association cortex [1, 7]. In human adults, fMRI studies have shown that, in particular, regions in the superior temporal gyrus are involved in speech perception and memory [17–19]. In addition, there is evidence from both lesion studies and gene-expression analyses that the auditory association cortex is involved in auditory recognition memory in monkeys and rats [21–23].

Analyses of expression of immediate early genes (IEGs) have revealed a dissociation between nuclei in the song system, which are activated when the bird is singing [24], and the NCM and the caudomedial mesopallium (CMM, Figure 1A), which are activated when the bird hears song [3–5, 25, 26]. There was increased IEG expression in the CMM of female zebra finches when they were exposed to their father's song, compared to novel song [25]. In zebra finch females, lesions to the CMM, but not to the song-system nucleus HVC, impaired a preference for conspecific over heterospecific song [27]. In contrast, lesions to song-system nuclei in songbirds disrupted song production but not recognition [15, 28]. Taken together with the present findings, showing that lesions to the NCM do not affect song production but impair song recognition, these results reveal a complete double dissociation of the effects of lesions to rostral and caudal brain regions on song in zebra finches.

Recent evidence shows that the functional distinction between the temporal and frontal cortex in human speech is not as strict as was thought previously [18, 19, 29–31]. Furthermore, in humans, temporal and frontal regions do not function in isolation but interact continually, already in juveniles. For instance, an fMRI study in 3-month-old infants revealed neural activation in response to speech not only in the temporal cortex, but also in Broca's area in the frontal cortex [30]. The latter was related to speech repetition, prompting the authors to suggest that in preverbal infants, Broca's area may be involved in memory for speech. This more dynamic view of the involvement of caudal and rostral brain regions in human auditory recognition and vocal production also has a parallel in songbirds. In adult songbirds, some nuclei in the song system are responsive to song, particularly to the bird's own song, with a minority of cells preferentially responding to tutor song [32]. In the early sensorimotor phase of song acquisition in juvenile zebra finches, neurons in the song-system nucleus HVC show transient preferential responding to the tutor song, whereas in adults there is preferential responsiveness to the bird's own song [33]. In the present study, the subjects were adults and had crystallized songs. It is possible that song acquisition in the sensorimotor phase requires an intact NCM because early song production involves access to the representation of the tutor song. In that case, lesions to the NCM of juvenile zebra finches are predicted to influence song development. In addition, plastic changes to adult song [34, 35] may also involve continued interactions between the song system and the NCM (and possibly the CMM [3, 25]).

In summary, we have demonstrated that lesions to the caudomedial nidopallium (NCM), a brain region outside

the conventional song system, impair song recognition without affecting song production or sound discrimination. These findings suggest that the NCM contains the neural representation of tutor-song memory, and that access to this representation is not necessary for song production. There is a parallel in human speech, where there is a similar dissociation between frontal brain regions mainly involved in speech production and caudal temporal regions mainly involved in speech perception and memory. In both systems, there appears to be continued dynamic interaction between these two brain regions throughout the life of an individual. The parallel between songbirds and humans suggests convergent evolution of the mechanisms underlying vocal learning.

Supplemental Data

Experimental Procedures, two figures, two tables, and four audio files are available at <http://www.current-biology.com/cgi/content/full/17/9/789/DC1/>.

Acknowledgments

We thank Thijs Zandbergen for technical assistance; Jeanette Pots, Kim Roosemalen, Gijs van de Sande, and Josine Verhaal for performing behavioral tests and histological analysis; Daniel Oberski and Simon Reader for comments on the manuscript; and Maarten Terlou for writing image-analysis software.

Received: December 18, 2006

Revised: March 22, 2007

Accepted: March 22, 2007

Published online: April 12, 2007

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