

BRIEF REPORT

A Brain of Her Own: A Neural Correlate of Song Assessment in a Female Songbird

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The song control region in the avian forebrain is a series of discrete, interconnected nuclei mediating song learning and production. It has been studied in males or in species where both sexes sing. Little is known about the neural correlates of song perception in nonsinging females, often the intended recipients of song. We studied cowbirds (*Molothrus ater*), a species in which only males sing but in which females discriminate between males on the basis of song. We focused on nucleus IMAN because it has been implicated in early song acquisition, a stage relevant to both sexes to choose among competing acoustic models. We found that volume of IMAN was monomorphic in cowbirds. Moreover, the volume and neuronal number of female IMAN were positively correlated with selectivity of copulatory responding. The results provide strong evidence of nonsinging female's use of "song" control nuclei for song perception without the possibility of song production. © 1997 Academic Press

Brown-headed female cowbirds are ideal candidates in which to study neural correlates of song assessment. Females do not sing, but they discriminate songs at species, subspecies, and individual levels of analysis, preferring the songs of local, dominant males (King & West, 1990). In the field and in the laboratory, their response to song is rapid and unambiguous: females adopt copulatory postures to preferred songs while songs are still in progress (Fig. 1A). In addition to discriminating among songs before mating, female cowbirds react with social displays to songs for many months before courtship when young males are still developing repertoires (Fig. 1B) (West & King, 1988). Male cowbirds modify their vocal output in response to such stimulation from females, even during subsong and plastic song, points at which species-specific features are barely recognizable (King & West, 1988). Thus, females act as song tutors and song recipients, roles clearly necessitating perceptual comparisons of songs.

To look for correlates of females' perceptual capacities, we focused on the

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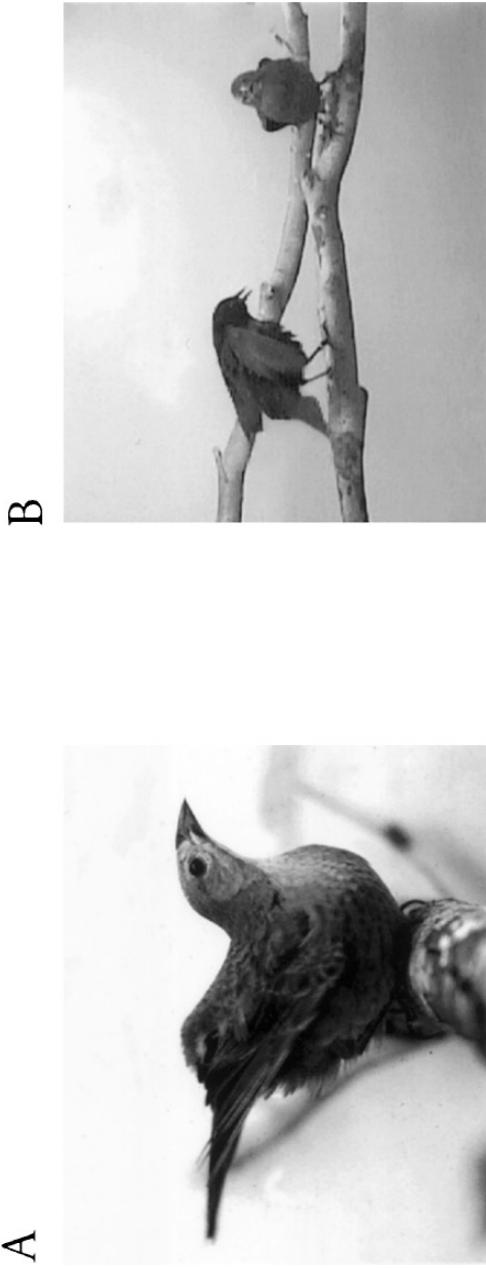


FIG. 1. (A) The female's copulatory posture to playback in a sound-attenuating chamber. A positive response is scored if the female arches her back, crouches, and separates the feathers around the cloacal area within one second of song onset (King & West, 1977). (B) The image is from a videotape in early March, 2 months prior to the onset of breeding. The female's rapid raising of her wings ("wing strokes") is occurring while the male's song is ongoing. Wing strokes affect which songs males retain in their repertoire and appear to be precursors of copulatory behavior (West & King, 1988). Contributed by A. P. King.

anterior forebrain pathway within the song control system (HVC–Area X–DLM–IMAN–RA). In male zebra finches (*Poephila guttata*), neurons responsive to song have been recorded along this pathway, with IMAN showing the most sensitivity and possessing the most neurons responsive to the male's own song (Doupe & Konishi, 1991; Margoliash, 1994; Vicario & Yohay, 1993). Lesions of IMAN or Area X lead to severe detriments in acquiring song, but do not affect adult production (Bottjer, Miesner, & Arnold, 1984; Scharff & Nottebohm, 1991). Nucleus IMAN also undergoes neuronal changes during the perceptual phase of song acquisition (Bottjer & Sengelaub, 1989).

These studies suggest that IMAN's function includes perception of song and thus make it a likely target for use by female songbirds, particularly in species where females must discriminate among many variations of songs. In European warblers (*Sylviidae*), a positive relationship exists between female IMAN volume and male song complexity (DeVoogd, Cardin, Szekely, Buki, & Newman, 1996). In canaries (*Serinus canaria*), where females sing occasionally, lesioning of female HVC eliminates species specificity of copulatory responsiveness to song (Brenowitz, 1991). In that lesions of HVC reduce input to IMAN, these data are also consistent with the view that IMAN is used by listeners to recognize songs.

We used copulatory responses to song playback in captive females as a perceptual assay (Fig. 1A) (King & West, 1977). Playback responses correlate with mate preferences in captive colonies, indicating the reproductive significance of the measure (West, King, & Freeberg, 1994). We chose a set of songs known to differ in their ability to elicit copulatory responses (King & West, 1989; West & King, 1986). Six of the songs selected had elicited copulatory responses on 50% or more of all playback trials [labeled high potency (HP) songs], whereas the other six had elicited responding on fewer than 25% of all trials [labeled as low potency (LP) songs]. Male cowbirds tutored with the HP–LP songs copied HP songs significantly more often than LP songs, providing independent evidence of the differential properties of the songs. In tests with four cohorts of females over several years, all reliably preferred the set of HP songs, although some females displayed a greater categorical division between HP and LP songs than did others (King & West, 1989, 1990; West & King, 1986). These differences in HP–LP song discrimination suggest that some females are more selective or “choosy” than others, a trait many assume affects reproductive outcome (Searcy & Yasukawa, 1996).

In the present study, we tested 7 new females (four yearlings and three adults) using the HP–LP song set. Three additional females were used for neural analyses. All 10 females were collected in Orange Co., North Carolina (NC) in August, 1989, and had resided with NC male cowbirds until 1 or 2 weeks before playback tests in May. During testing, the playback females resided in sound-attenuating chambers. The remaining 3 females came from resident colonies living in aviaries. The set of six HP songs contained three common and three rare *M. a. ater* song types, as did the LP set. We played six songs/day with one song/trial every 90 min. Three HP and LP were played each day in varying orders. All songs were played four to six times to each female. We scored a positive response if a female adopted a copulatory posture within 1 s from onset of the song (Fig. 1A).

All seven females responded more to the HP songs, with some females ignor-

TABLE 1
Summary of Behavioral and Neural Measures
for the Seven Females Tested by Playback

	Female						
	CH-W	X-BL	CH-2Y	G-CH-G	LB-CH	2DB	2CH
Difference score ^a	0.70	0.50	0.47	0.30	0.24	0.23	0.13
HP response ^b	0.70	0.71	0.678	0.60	0.35	0.93	0.96
LP response ^b	0.00	0.21	0.21	0.30	0.11	0.70	0.83
Overall responsivity ^b	0.35	0.46	0.45	0.45	0.23	0.82	0.90
IMAN volume (mm ³)	0.424	0.378	0.184	0.373	0.177	0.171	0.096
IMAN neuron number ($\times 10^{-3}$)	21.10	14.44	10.76	15.97	8.70	5.34	3.39
IMAN neuronal density ($\times 10^{-4}$ neurons/mm ³)	4.97	3.81	5.84	4.28	4.92	3.12	3.53
IMAN somal area (mm ²)	125.4	99.1	112.4	117.0	120.1	103.2	136.0
Area \times volume (mm ³)	0.18	0.19	0.4	0.22	0.18	0.15	NA
Rt volume (mm ³)	0.97	1.70	0.67	1.66	1.00	1.26	1.04
Neostriatum volume (-IMAN volume; mm ³)	41.03	52.87	51.19	37.98	34.06	37.25	41.13
Brain weight (g)	1.20	1.37	1.20	1.31	0.99	1.31	1.27
Body weight (g)	42.28	38.22	42.42	39.81	38.70	39.33	42.25

^a Difference between HP and LP responses.

^b Proportion of total playbacks eliciting a copulatory response.

ing the LP songs more often than other females, i.e., showing more choosiness. For each female, we computed a difference score, subtracting the mean proportion of responses to LP songs from the mean proportion of responses to HP songs, yielding a measure of preference for HP songs (Table 1).

We studied the brains of these seven females and three additional females. Measurements of the brains were carried out by persons with no knowledge of the outcome of the perceptual measures. At the time the brains were taken, the females were reproductively responsive, as indicated by egg laying and copulations. Seven males from the same NC population, who had been courting the females, were also studied. The birds were overdosed with chloroform and perfused with saline followed by buffered formalin. Brains were frozen-sectioned in the coronal plane at 40 μ m, and every third section was collected and stained with thionin.

To estimate the volumes of IMAN and Area X, sections were viewed with a projection microscope at a magnification of 32 \times . We measured several other brain areas used by others to assess relationships between the behavior of interest, selectivity, and nonsong related parts of the brain (Brenowitz, Lent, & Kroodsma, 1995). We measured a thalamic area, nucleus rotundus (Rt), which is involved in visual processing, and the neostriatum, measured from the first section in which both lamina hyperstriatica and lamina medullaris were visible to the first section in which lamina striatica no longer extended to the medial edge, subtracting IMAN. Boundaries of the nuclei were traced in each section in which they appeared, and the area of each was calculated. The summed areas were divided by the sampling ratio and multiplied by the thickness of

the section corrected for the percentage of the structure sampled. For three males and two females, nuclei HVC and RA were also measured.

An unbiased stereological counting technique (e.g., Wellman, Logue, & Sengelaub, 1995) was used to estimate neuronal number in IMAN. Sections were viewed at a magnification of 750 \times . Numerical densities of neurons were obtained with an optical disector procedure. Neuronal cell bodies falling within an ocular grid covering an area of 120 \times 120 μm were counted using an unbiased counting frame (cell bodies touching the left and lower edges of the grid as well as those in the top plane of focus were not counted). Neuronal density was multiplied by volume to estimate total neuron number within IMAN. Three evenly spaced sections per animal were sampled such that the location along the dorsal-ventral and mediolateral axes were randomly selected. Neurons were identified by the presence of a large pale nucleus with a single spherical nucleolus, and scant darkly stained cytoplasm. Soma size was assessed by projecting sections onto a computer screen at a final magnification of 2000 \times . Neuronal cell bodies were traced and areas calculated using a digitizing pad and a computer-based morphometry system (Java; Jandel, San Raphael, CA). Approximately 25 cells were measured in each animal. Brain weight and body weight were also measured.

The data revealed that IMAN volumes were monomorphic, $U(17) = 44$, ns (Fig. 2). Area X, HVC, and RA were present in the females but were consistently smaller than those in the males. We used the difference scores for the seven females from the HP-LP playback in a Spearman correlation with the volume of IMAN, yielding a value of $r_s(7) = .964$, $p < .005$. Size variation in IMAN was at least partially accounted for by differences in neuronal number, yielding a correlation with the difference scores of $r_s(7) = .893$, $p < .02$ (Table 1). We also correlated the difference scores with the measures for Rt, Area X, and the neostriatum (subtracting IMAN volume). No significant correlations occurred: $r_s = -.143$ for Rt; $r_s = .377$ for Area X; $r_s = .429$ for neostriatum). Neither body weight nor brain weight correlated with selectivity (body weight, $r_s = .152$; brain weight, $r_s = .143$).

Finally, we chose a second behavioral measure, overall responsivity to all playback songs (the proportion of responding to all 12 songs divided by number of playback presentations, see Table 1). Responsivity was not correlated with IMAN ($r_s(7) = .50$, $p > .20$) or with any other neural measure.

In summary, these data add three important points to our understanding of avian communication and its neural basis. First, these are the first data demonstrating that a nonsinging female songbird possesses a song nucleus thought to be implicated in song acquisition. Second, the size monomorphism in this nucleus suggests the potential significance of the female's receptive "song system" for understanding contributions to song learning. Third, the strong correlation within females between IMAN volume and song selectivity, but not overall responsivity, indicates the need to differentiate among perceptual capacities as the females themselves differ on these dimensions.

The presence of nucleus IMAN in females and its correlated status with song selectivity suggest that it serves a perceptual function for females as they listen to song. The absence of correlations with other brain areas suggests that selectivity is specific to IMAN, not a property of the brain as a whole, nor of the entire song system. Although we have stressed a possible perceptual role

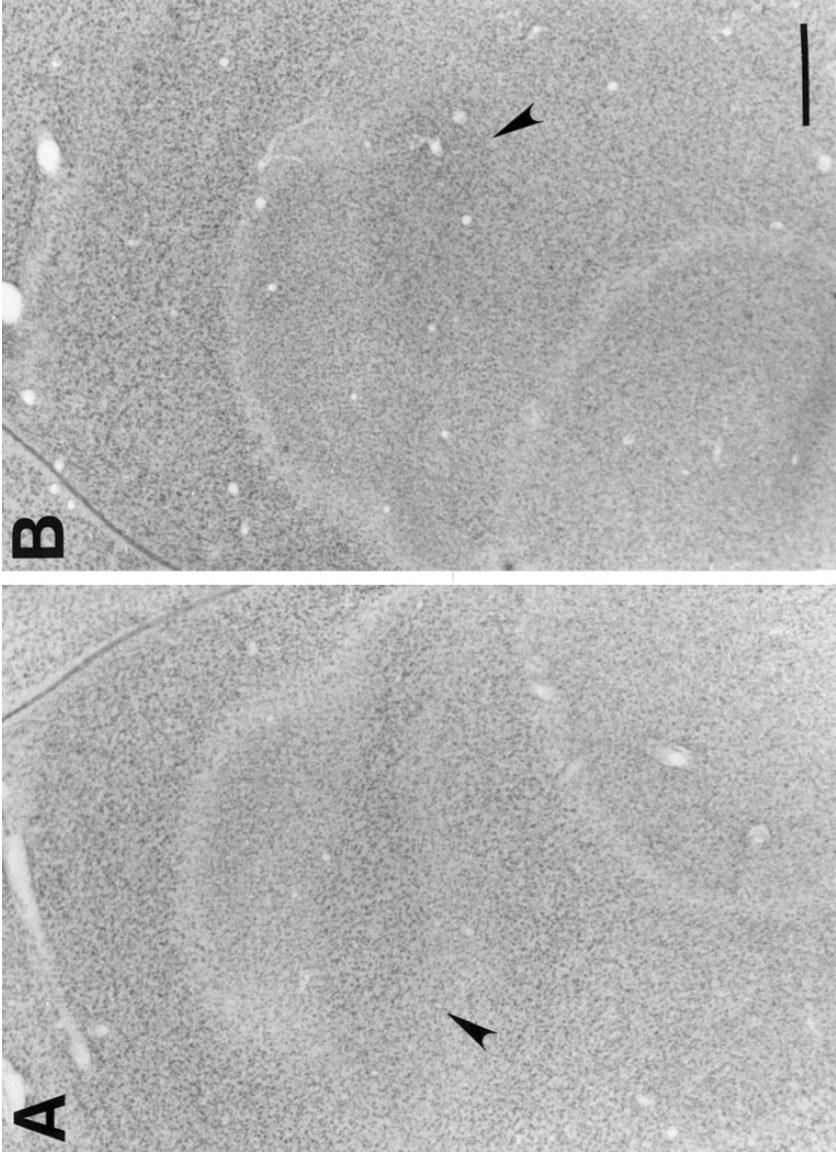


FIG. 2. Frontal sections were matched for anterior-posterior position through the lateral magnocellular nucleus of the anterior neostriatum (IMAN) in an adult male (A) and an adult female (B) cowbird. Arrowheads indicate the ventral border of the nucleus in both cases. Thionin stain; scale bar, 500 μm .

for IMAN, its role cannot be purely perceptual as it modulates copulatory responsiveness. Whether it also plays a role in social responsivity during male song learning now needs to be explored to determine if such reactivity seen in the early spring is a related precursor of copulatory functioning. From as early as we can record male song-like vocalizations, we find differences in song ontogeny if males are housed with adult female conspecifics displaying different song preferences (King & West, 1988). Thus, we must also entertain the possibility that female assessment and male song growth are ontogenetically coupled. We now need to examine IMAN in naive and experienced females to see if acoustic experience affects IMAN growth and structural differentiation. Neural differences in the interior IMAN_{core} and exterior IMAN_{shell} may explain IMAN's ability to integrate perception and action (Johnson, Sablan, & Bottjer, 1995).

Changes in IMAN size as a result of cell death have been discussed in relation to the role of experience in shaping male neural structures (Bottjer & Sengelaub, 1989). Whether song selectivity in females is influenced by experience remains to be explored. Little evidence of perceptual modifiability exists in female cowbirds at macrogeographic levels of song discrimination (King & West, 1990). But the present data on IMAN reveal microgeographic differences in song selectivity, i.e., at the individual level where reproductive decisions are actually made. As such, the data call for greater attention to female songbirds' responses to local males to provide relevant behavioral correlates.

These data also offer a challenge to discover how females perceive and categorize song in the absence of vocal feedback. Some evidence exists suggesting that songbirds use information from vocal-articulatory gestures to recognize conspecific songs, an idea with parallels to motor theories of speech perception (Williams & Nottebohm, 1985). The present study suggests other categorization mechanisms are also possible. As in studies of human language, however, information about vocal production far outweighs information about vocal comprehension. Until this imbalance is corrected, studies of vocal learning and function represent only half the story.

REFERENCES

- Bottjer, S. W., Miesner, E. A., & Arnold, A. P. (1984). Forebrain lesions disrupt development but not maintenance of song in Passerine birds. *Science*, **224**, 901–902.
- Bottjer, S. W., & Sengelaub, D. R. (1989). Cell death during development of a forebrain nucleus involved with vocal learning in zebra finches. *Journal of Neurobiology*, **20**(7), 609–618.
- Brenowitz, E. A. (1991). Altered perception of species-specific song by female birds after lesions of a forebrain nucleus. *Science*, **251**, 303–305.
- Brenowitz, E. A., Lent, K., & Kroodsma, D. E. (1995). Brain space for learned song develops independently of song learning. *Journal of Neuroscience*, **15**(9), 6281–6286.
- DeVoogd, T. J., Cardin, J. A., Szekely, T., Buki, J., & Newman, S. W. (1996). Relative volume of L-MAN in female warblers species varies with number of songs produced by conspecific males. *Society for Neuroscience Abstracts*, **22**, 1401.
- Doupe, A. J., & Konishi, M. (1991). Song-selective auditory circuits in the vocal control system of the zebra finch. *Proceedings of the National Academy of Science USA*, **88**, 11339–11343.
- Johnson, F., Sablan, M. M., & Bottjer, S. W. (1995). Topographic organization of a forebrain pathway involved with vocal learning in zebra finches. *Journal of Comparative Neurology*, **358**, 260–278.

- King, A. P., & West, M. J. (1977). Species identification in the North American cowbird: Appropriate responses to abnormal song. *Science*, **195**, 1002–1004.
- King, A. P., & West, M. J. (1988). Searching for the functional origins of cowbird song in eastern brown-headed cowbirds (*Molothrus ater ater*). *Animal Behaviour*, **36**, 1575–1588.
- King, A. P., & West, M. J. (1989). Presence of female cowbirds (*Molothrus ater ater*) affects vocal improvisation in males. *Journal of Comparative Psychology*, **103**, 39–44.
- King, A. P., & West, M. J. (1990). Variation in species-typical behavior: A contemporary theme for comparative psychology. In D. A. Dewsbury (Eds.), *Contemporary issues in comparative psychology* (pp. 331–339). Sunderland, MA: Sinauer.
- Margoliash, D. (1994). Distributed representation in the song system of Oscines: Evolutionary implications and functional consequences. *Brain Behavior and Evolution*, **44**, 247–264.
- 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. *Journal of Neuroscience*, **11**(9), 2896–2913.
- Searcy, W. A., & Yasukawa, K. (1996). Song and female choice. In D. E. Kroodsma & E. H. Miller (Eds.), *Ecology and Evolution of Acoustic Communication in Birds* (pp. 454–473). Ithaca, NY: Cornell Univ. Press.
- Vicario, D. S., & Yohay, K. H. (1993). Song-selective auditory input to a forebrain vocal control nucleus in the zebra finch. *Journal of Neurobiology*, **24**(4), 488–505.
- Wellman, C. L., Logue, S. F., & Sengelaub, D. R. (1995). Maze learning and morphology of frontal cortex in adult and aged basal forebrain-lesioned rats. *Behavioral Neuroscience*, **109**, 837–850.
- West, M. J., & King, A. P. (1986). Song repertoire development in male cowbirds (*Molothrus ater*): Its relation to female assessment of song. *Journal of Comparative Psychology*, **100**, 296–303.
- West, M. J., & King, A. P. (1988). Female visual displays affect the development of male song in the cowbird. *Nature*, **334**, 244–246.
- West, M. J., King, A. P., & Freeberg, T. M. (1994). The nature and nurture of neophenotypes. In L. A. Real (Eds.), *Behavioral mechanisms in Evolutionary Ecology* (pp. 238–257). Chicago: Univ. of Chicago Press.
- Williams, H., & Nottebohm, F. (1985). Auditory responses in avian vocal motor neurons: A motor theory for song perception in birds. *Science*, **229**, 279–282.