



Visual and song nuclei correlate with courtship skills in brown-headed cowbirds

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(Received 3 November 1997; initial acceptance 5 January 1998;
final acceptance 5 January 1998; MS. number: A7920R)

ABSTRACT

We assessed courtship success in 14 adult male brown-headed cowbirds, *Molothrus ater*. Volumes of song control nuclei and visual nuclei were measured in Nissl stained tissue. Variation in courtship success was found to be related to variation in two areas of the avian brain: the song control nucleus, area X, and the thalamic visual area, nucleus rotundus. Volume of area X was negatively correlated with song potency, as assessed by female playback, and with rate of vocalizing. Volume of nucleus rotundus was positively correlated with song potency, vocalizing to females and courtship persistence. These data are the first to implicate a visual nucleus in the use of song. The data also complement previous findings with cowbirds suggesting that song learning involves visual attention to females. Taken as a whole, these results suggest that use of song depends on integration of auditory, vocal and visual information. In that female songbirds in many species assess multimodal performance of song, these findings with cowbirds suggest that future studies of brain and behaviour include a broader view of possible behavioural and neural correlates.

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The aim of the research reported here was to explore interrelationships between song use and brain organization in brown-headed cowbirds, *Molothrus ater*. Bird song has been a particularly rich source of information about the integration of species-typical behaviour and neural structures (reviewed in Nottebohm 1991; DeVoogd 1994; Brenowitz & Kroodsma 1996). For the most part, however, studies have focused on mechanisms underlying acquisition of song structure as opposed to acquisition of song skills, that is, how birds learn to use their songs when interacting with conspecifics (but see Kroodsma 1988; Spector et al. 1989; Freeberg et al. 1995).

Cowbirds represent ideal subjects for integrative study because much is already known about the development of vocal structure (King & West 1988, 1990; O'Loughlen & Rothstein 1993). As in all other songbirds studied to date, deprivation from species-typical stimulation leads to atypical song production. Self-produced vocal activity is also important as a male's rate of vocal activity affects the ontogenetic pace of song crystallization: the more a male sings, the faster he acquires stable song morphology

(King et al. 1996). Cowbirds also show considerable malleability while learning song and will acquire heterospecific sounds under certain conditions (Freeberg et al. 1995). Learning also occurs in a male's second year, with most males producing more and different song types (males have one to seven stable song patterns and one or two flight whistles). Changes beyond the second year have not been reported. Geographical variation in song, and in some populations, flight whistles, also occurs, with learning playing an important role for both vocalizations (O'Loughlen & Rothstein 1993). Thus, although the species is a brood parasite, leading many to expect highly constrained developmental programmes (Mayr 1974; Todd & Miller 1993), cowbirds appear as malleable as other nonparasitic songbird species.

In addition to sensitivity to auditory influences, male cowbirds are also influenced by visual stimulation, including social feedback from females (King & West 1983, 1988; West & King 1988). Young male cowbirds modify developing song content in response to visual responses from conspecific females, for example, wing strokes (West & King 1988). Observations of males with females reveal conspicuous visual attention: males approach and closely examine the female that has produced the wing stroke (West & King 1988). Adult males

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are also influenced by female stimulation (West & King 1980). Visual attentiveness to females is also crucial to courtship. Males attend to a female vigilantly as they approach her to vocalize and as they guard her from other males.

Studies of female cowbirds indicate the importance of song use to courtship. Songs elicit copulatory postures in male-deprived females and females show strong preferences for local song, as well as for the songs of dominant males (West et al. 1998). The playback measure has been validated by playing back songs of males with known courtship histories: based on hearing their song, females respond most often to the songs of more successful males, even though they have no information beyond song structure (West et al. 1981). In aviary settings and in the field, males and females develop consortships prior to mating: the consortships are defined by repeated singing directed from the male to the female (West et al. 1981; Yokel & Rothstein 1991). Males typically direct many vocalizations for several days before the pair copulates. A neural examination of the song system in the female cowbird's brain affirms the importance of song stimulation. We found that volume of 1MAN, a nucleus in the song control pathway, was monomorphic in cowbirds, even though females do not sing. Moreover, the volume and neuronal number of female 1MAN was positively correlated with the selectivity of copulatory response to playback of song (Hamilton et al. 1997).

Taken as a whole, these findings led us to focus on five measures of male performance, three converging on song use and two on song content. First, we measured the rate at which vocalizing occurred in the 2 months prior to the breeding season, as well as during the breeding season, because previous work suggested that a greater rate of vocalizing is associated with more rapid song development (King et al. 1996). Second, we looked at female-directed vocalizing during the breeding season because such singing is a necessary part of consortship formation. Males that sing frequently but direct most of their songs to males form few, if any, consortships and rarely copulate (Freeberg et al. 1995; West et al. 1996). Third, we observed consortship formation, that is, directed singing to the same females over at least 3 days. Then, by removing females once consortships were formed, we looked at how quickly males focused attention on another specific female and formed a new consortship. In this way, we measured consortship persistence, that is, how successful males could be with new females, a measure that correlates highly with subsequent copulations (Eastzer et al. 1985; Freeberg et al. 1995; West et al. 1996).

Fourth, to examine song content, we measured the potency of the males' songs by playbacks to females. Song potency is also predictive of copulatory success in captive settings if the males also direct songs to females (West et al. 1981; Freeberg et al. 1995). Finally, we measured repertoire size, because it is the measure used most often in correlation studies of brain and behaviour in songbirds. Although we have looked for evidence that repertoire size is related to courtship success or song potency in cowbirds, we have not found any consistent relationship

across studies and thus we included it here only to provide comparative data on HVC and repertoire size (DeVoogd et al. 1993).

Our past studies of vocal development also influenced our choice of neural correlates, especially the focus on area X. The song control system in the forebrain of songbirds consists of two major pathways, with nucleus HVC participating in both (Margoliash et al. 1994). The first pathway is assumed to affect the motor production of song (HVC-RA). The second pathway (HVC-Area X-DLM-1MAN-RA) is thought to affect song acquisition, perception, and possibly song improvisation. Lesions of nuclei in the second pathway disrupt initial learning but do not affect adult production (Bottjer et al. 1985). Our attention was drawn to area X for three reasons. First, it shows the most sexual dimorphism between males and females in other songbirds (Sohrabji et al. 1990; Arnold 1992). In cowbirds, there appears to be a five-fold difference in size favouring males (Cleal et al. 1996). The nucleus also appears to be a site of synaptic plasticity (Wallhauser-Franke et al. 1995a). One function of such plasticity may be to modulate the period of song learning after song material has been acquired but before songs have crystallized (Sohrabji et al. 1990; Scharff & Nottebohm 1991). This is the developmental period in which we have found that cowbirds are especially sensitive to social stimulation and show evidence of improvisation affecting song potency (King & West 1989). Of the five behavioural variables described earlier, we expected song potency to be the most likely candidate for correlations with the volume of area X, as this measure of song content is clearly affected by social and vocal experiences prior to song crystallization. Given that we had found correlations between song stereotypy and rate of singing in two previous studies, we also studied possible relationships between area X and rates of vocalizing (King & West 1988; King et al. 1996).

We also measured three other major nuclei in the song system (HVC, RA and 1MAN). HVC stimulates both pathways, receives input from other areas of the brain including visual stimulation and has been shown to show volumetric relationships with repertoire size in some songbirds (Bischof & Engelage 1985; DeVoogd 1994). The other nuclei measured (RA and 1MAN) allowed us to determine whether any correlational effects found with area X were specific to that nucleus or a general feature of the song system.

In the avian visual system, we focused on the main visual pathway, the tectofugal pathway, especially the largest thalamic structure, nucleus rotundus (Rt). Our research indicates that males use visual cues from females to develop effective songs (West & King 1988), and more importantly that successful song use during courtship requires simultaneous visual attentiveness to females' movements and the behaviour of rival males. Nucleus Rt has often been measured in studies of songbirds' brains to correct for overall volumetric differences in brain size as there is no evidence to suggest specific forms of experience affects its size or that it is involved in song learning (Brenowitz et al. 1995). Recent studies, however, suggest that visual processing of texture,

motion, shape and colour is affected by lesions of Rt in pigeons, *Columbia livia* (Shimizu & Karten 1993; Wang et al. 1993; Shimizu et al. 1997). These qualities are clearly key components of social attention to conspecifics and thus could affect attentive singing behaviour. We also measured ectostriatum to explore the possibility of other visual relationships and neostriatum to look for effects of overall brain size differences.

METHODS

Aviary Subjects

Sixteen male cowbirds of two age cohorts served as subjects. All males were collected as juveniles (age, 50–75 days) in Monroe County, Indiana at the laboratory in a funnel trap attached to an aviary. Cohort 1 consisted of nine males collected in July 1992. Cohort 2 consisted of seven males collected in July 1993. At the onset of this study, cohort 1 males were coming into their third year, and cohort 2 males were coming into their second year. These males had been subjects in previous studies (King et al. 1996; West et al. 1996). Males in cohort 1 had been housed until May of their hatching year with heterospecifics and then housed with other Indiana male and female cowbirds in indoor–outdoor aviaries for a year. The males in cohort 2 had been housed with female conspecifics until May of their first year and then housed in indoor–outdoor aviaries with other Indiana male and female cowbirds until the onset of the present experiment.

All birds used in the study were banded with coloured leg rings to permit individual identification. All were maintained on a modified version of the Bronx Zoo diet for omnivorous birds and were given millet, canary seed and vitamin-treated water daily.

Playback Subjects

Eight adult females, also collected in Monroe County, Indiana, served as subjects for the playback of males' vocalizations in May and June 1995: all had served as playback subjects in the previous year as part of another experiment. Prior to the breeding season, they were housed in aviaries with male and female cowbirds.

Housing Conditions

Aviary subjects

In July 1994 at the onset of the present study, we housed the 16 males in two identical indoor–outdoor resident aviaries ($3.1 \times 10.9 \times 3.4$ m) until May. The two cohorts of males were each housed with seven female cowbirds, also collected from the same site. One starling was housed in each of the resident aviaries as part of another experiment. Aviary A consisted of four males from cohort 1 and four males from cohort 2. Aviary B consisted of five males from cohort 1 and three males from cohort 2. Two birds died early in the winter, leaving four males from cohort 1 and three males from cohort 2

in each resident aviary. During the courtship phase, in May and June, aviary subjects were housed for varying lengths of time in a larger, unfamiliar aviary. The aviary's outdoor section measured $9.1 \times 18.3 \times 3.4$ m and the indoor section measured $9.1 \times 3.0 \times 2.7$ m. Ten unfamiliar adult female cowbirds, also collected in Monroe County, Indiana, served as potential recipients of social behaviour during the courtship phase.

Playback subjects

Beginning in May, the playback females were housed singly or in pairs in sound-attenuating chambers ($1.2 \times 1.2 \times 1.2$ m). No effects of social housing on playback responsiveness have been found in previous studies (Freeberg et al. 1995).

Measures

We used the following measures of social behaviour throughout the study.

Vocalize

The male's song consists of a series of low-frequency tone bursts followed by a whistle; the entire song lasts between 800 and 1200 ms. Song was considered directed if the singer was within 0.3 m of another bird and if the song spread display typically accompanying the song was oriented towards that individual (Friedmann 1929). Male cowbirds also use flight whistles, modulated tones between 4 and 12 kHz, between 500 and 2000 ms. If flight whistles were directed to another individual as described above, they were counted as directed vocalizations, but they occurred infrequently in the context of directed vocalizations (but see West et al. 1998 for details of whistle use in relation to mating).

Vocalizing rate

We calculated vocalization rate for 3 months, March, April and May. These rates were defined as the number of vocalizations per minute averaged across all samples during each month. In addition, we calculated female-directed vocalizing rate during May in the courtship aviary. To obtain the males' proportion of female-directed vocalizing (FDV), we divided the number of songs directed to females during each focal sample by the total number of songs, directed or undirected, during that sample. For each male, these ratios were averaged across all samples.

Consort day/consortship

A consort day was scored if a male directed at least 10 vocalizations to females during focal sampling, of which at least one-third were to a particular female. For a consortship to be established, three consecutive consort days with the same female had to occur. If a copulation occurred on the second day of a developing consortship, we judged that the pair of birds formed a consortship. A copulation was scored when a male mounted a female that was in a copulatory posture, that is, her back was arched and her wing feathers were spread apart.

Consort persistence level (CPL)

This measure was defined as the number of consort days divided by the number of days in the aviary (Eastzer et al. 1985).

Observation Procedures

During the spring, we observed each male in each cohort in the resident aviaries during 5-min focal samples on six occasions in March and seven occasions in April. In May, two observers recorded vocal behaviour and copulations in the courtship aviary between 0600 and 0945 hours, 7 days a week, totalling one to three units of focal sampling of either 10, 15, or 20 min per male (average 26.7 min observed daily; range 10–60 min). On a given day, the length of focal sampling units were the same for each male but differed over days to accommodate limiting weather conditions (e.g. strong winds or rain). The observers noted each vocalization and the identity of the recipient if it was directed to an individual.

Courtship Testing Procedures

To assess the males' courtship skills, we observed the birds in the courtship aviary beginning on 9 May 1995 and ending on 27 May 1995. The females to be courted ($N=10$) were drawn from other aviaries and were unfamiliar to the males. From 9 May to 16 May, we observed four males from each resident aviary, balancing the number representing each cohort as much as possible. On 17 May, we reduced the number to three males from each resident aviary, and six females. Once a consortship was established, we removed either the male or the female from the aviary and replaced it with another bird of the same sex. This procedure allowed us to determine whether males could be successful at forming consortships with multiple females, although it reduced the opportunity to observe copulations as these typically do not occur until after the consortship is clearly established (West et al. 1981; Freeberg 1996). Males averaged 9 days in the aviary (range 5–13 days). When males were rotated out of the courtship aviary, they were returned to their resident aviaries with other birds with which they had overwintered.

Recording Procedures and Analysis of Song Types

We recorded the males in the resident aviaries in early May when their vocalizations had crystallized. We recorded vocalizations on a Sony TCD-D10 PRO II digital audio tape recorder with Sennheiser RF condenser microphones situated less than 0.3 m on axis from the singer.

Zero-crossings-analyser (ZCA) displays were used to determine repertoire size and frequency of song use. These displays provide an instantaneous frequency \times time display (West et al. 1979). Song types (different frequency by time patterns sung repeatedly by a male) were classified by two observers. For use in playbacks, we estimated the two most frequently sung song types of each male. One example of each was chosen on the basis of recording quality from a total of 983 recorded songs

(average/male=70, range 17–153). We then dubbed the recordings chosen for playback to an Otari MX5050 MK III half-track recorder at 38.1 cps.

Playback Testing

In May and June 1995, we played back songs using an Otari MX5050 recorder, a Urei 537 1/3-octave equalizer, and a Crown D75 power amplifier through JBL 2105 speakers situated in each chamber. The Urei equalizer was adjusted by playing white noise through the playback system and recording speaker output with a Brüel & Kjaer 2033 spectrum analyser. The SPL was 85 ± 2 dB adjusted with a Brüel & Kjaer 2209 sound pressure meter, set to A weighting, impulse reading.

We played six songs/day to the females, with one vocalization/trial, separated in time by 90 min. Vocalizations were played in varying orders each day, but each vocalization was presented an equal number of times at different times of day. We played back each vocalization eight times over the course of the experiment. We scored a positive response if a female adopted a copulatory posture within 1 second from the onset of the sound, that is, if she arched her neck and back and separated the feathers around the cloacal area. Song potency (SP) for each male was determined by the average proportion of positive responses to playbacks of each of his songs.

Neuroanatomical Methods

Birds were overdosed with chloropent and perfused with saline followed by buffered formalin in June 1996. Brains were frozen-sectioned in the coronal plane at 40 μ m, collecting every third section, then stained with thionin. To estimate the volume of neural structures, sections were viewed with a projection microscope at a magnification of $\times 32$. We traced the boundaries of each nucleus or the region in each section in which it appeared. We calculated the area of each nucleus using a computer-based morphometry system (Sigmascan; Jandel, San Raphael, California). 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. Volume was calculated in this way for area X, the lateral portion of the magnocellular nucleus of the anterior neostriatum (1MAN), the hyperstriatum ventrale, pars caudale (HVc), robustus archistriatalis (RA), nucleus rotundus (Rt), and the ectostriatum. Area X was not distinguishable in two males. HVc was not distinguishable in one male. 'Core' and 'belt' regions of the ectostriatum (described in Engelage & Bischof 1993) were not reliably distinguishable so we included both regions in ectostriatum volume. As a control for possible differences in overall brain or body size, we measured neostriatum in 10 males from the first section in which both lamina hyperstriatica and lamina medullaris dorsalis were visible to the last section in which the ectostriatum appeared.

We used an unbiased stereological counting technique (e.g. Wellman et al. 1995) to estimate neuronal number

Table 1. Behavioural measures for both cohorts

Males	FDV*	CPL [†]	SP [‡]	VR Mar [§]	VR Apr [§]	VR May [§]	Number of song types
Cohort 1							
MRN ^a	0.59	1.00	0.75	1.80	0.80	1.27	5
MWN ^a	0.58	0.80	0.86	1.47	1.69	1.54	3
MBW ^a	0.51	1.00	0.64	2.93	1.77	1.71	4
M2B ^a	0.38	0.75	0.62	1.10	1.40	1.35	3
MBY ^b	0.37	0.36	0.74	1.60	1.77	0.89	2
MGN ^b	0.20	0.38	0.73	2.27	1.91	1.71	4
MBP ^b	0.06	0.43	0.43	2.03	0.69	1.25	6
MBR ^b	0.03	0.00	0.40	0.07	0.71	0.35	1
Cohort 2							
MLR ^b	0.43	0.63	0.64	1.17	1.94	0.98	4
MDPD ^a	0.16	0.61	0.68	1.03	0.94	1.09	4
MLPL ^a	0.35	0.45	0.62	1.03	1.14	0.63	5
MGBG ^b	0.18	0.64	0.59	3.30	0.83	1.09	4
MLW ^a	0.08	0.09	0.57	1.00	1.03	0.22	4
MBLL ^b	0.00	0.00	0.53	2.83	0.74	0.92	3

*Female-directed vocalizations: average proportion of vocalizations directed to females in May.

[†]Consort persistence level: average proportion of days in aviary meeting consortship criteria.

[‡]Song potency: average proportion of song playbacks eliciting female copulatory postures.

[§]Vocalizing rate: average vocalizations per minute in March, April and May.

^aMales housed in aviary A.

^bMales housed in aviary B.

in area X and nucleus rotundus (Rt). We viewed sections at a final magnification of $\times 750$. We obtained numerical densities of neurones using an optical disector procedure (Srivastava et al. 1993). We counted neuronal cell bodies falling within an ocular grid covering an area of $120 \times 120 \mu\text{m}$ 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). We sampled six evenly spaced sections per animal along the dorsal-ventral and medio-lateral axes at random. We identified neurones by the presence of a large pale nucleus with a single spherical nucleolus, and scant darkly stained cytoplasm. We multiplied volume by neuronal density to obtain an estimate of total neurone number.

We assessed somal area by projecting sections onto a computer screen at a final magnification of $\times 2000$. We traced neuronal cell bodies and calculated areas using a digitizing pad and a computer-based morphometry system (Java; Jandel, San Raphael, California). We measured 30 cells in each nucleus in each animal.

We completed neural measurements of song-related areas and Rt volume before looking at behavioural data so that the investigator was blind to the individual repertoire size, song potency and level of courtship success. For measurements taken after behavioural measures were complete (i.e. ectostriatum and neostriatum), we coded birds so that their identity was not known.

Statistical Analysis

We used Spearman's rank-order correlations for comparisons of behavioural and neural measures across individuals due to positive skew. All *P* values are for two-tailed tests.

RESULTS

Comparison of Age Cohorts

Inspection of the data revealed that males did not differ by age cohort or aviary on any behavioural measure (Table 1) or neural measure (Tables 2 and 3). The males were thus pooled for further analyses. As expected, the three courtship variables were positively correlated with one another across all males ($N=14$; SP and CPL, $r_s=0.562$, $P<0.05$; SP and FDV, $r_s=0.797$, $P<0.002$; CPL and FDV, $r_s=0.837$, $P<0.001$). Vocalizing rate and song potency were correlated only during courtship in May ($N=14$; VRMay and SP, $r_s=0.551$, $P<0.05$; VRMarch and SP, $r_s=-0.119$, NS; VRApril and SP, $r_s=0.398$, NS).

Behavioural Measures and Neural Volumes

Song potency was negatively correlated with area X volume such that area X volume decreased with higher song potency (Table 4; Fig. 1). Vocalizing rates were negatively correlated with the volume of area X. Area X was not significantly correlated with the remaining two measures of courtship success. No significant correlations with behavioural measures were found for song nuclei 1MAN, RA, or HVc. Repertoire size was not correlated with volume of HVc (Table 4; $r_s=-0.217$, NS) or with any other measure.

Nucleus rotundus was positively correlated with the three courtship measures (Table 4; Fig. 2a, b, c). Nucleus rotundus was not correlated with vocalizing rates (Table 4). Ectostriatum was not significantly correlated with Rt ($N=10$; $r_s=-0.079$, NS), or with any of the three courtship measures ($N=14$; SP ($r_s=-0.062$, NS), CPL ($r_s=-0.095$, NS) or FDV ($r_s=-0.077$, NS)). Neostriatum

Table 2. Neural measures of area X

Male	Area X (mm ³)	Neurones/ mm ³	Estimated neurone number	Somal area (mm ³)
Cohort 1				
MRN ^a	1.84	11 667	19 674	78.02
MWN ^a	1.66	11 852	21 467	85.72
MBW ^a	1.71	12 593	21 533	82.67
M2B ^a	2.38	8333	19 833	93.27
MBY ^b	2.25	11 667	26 250	82.56
MGN ^b	1.35	9444	12 750	79.59
MBR ^b	2.72	8704	23 674	101.27
Cohort 2				
MLR ^b	1.78	10 926	19 448	94.47
MDPD ^a	2.10	9815	20 611	83.34
MLPL ^a	2.22	11 481	25 489	81.15
MLW ^a	2.28	14 074	32 089	82.35
MBLL ^b	1.98	10 000	19 800	80.31

^aMales housed in aviary A.^bMales housed in aviary B.**Table 3.** Neural measures of nucleus rotundus

Male	Rt (mm ³)	Rt neurones/ mm ³	Rt estimated neurone number	Rt somal area (mm ³)
Cohort 1				
MRN ^a	1.78	9630	17 141	255.03
MWN ^a	1.50	6667	10 000	296.77
MBW ^a	1.28	10 370	13 274	228.51
M2B ^a	1.37	8519	11 670	255.10
MBY ^b	1.32	9259	12 222	233.35
MGN ^b	1.06	8519	9030	267.83
MBP ^b	1.06	9259	9815	276.49
MBR ^b	0.86	10 000	8600	194.86
Cohort 2				
MLR ^b	1.63	11 481	18 715	271.30
MDPD ^a	1.01	9630	9726	320.23
MLPL ^a	1.17	8148	9533	236.89
MGBG ^b	1.89	9630	18 200	264.71
MLW ^a	0.89	12 222	10 878	283.01
MBLL ^b	0.78	7407	5778	234.09

^aMales housed in aviary A.^bMales housed in aviary B.**Table 4.** Spearman rank-order correlation coefficients between volume of the two target nuclei, area X and nucleus rotundus (Rt) and the behavioural measures

	Area X ^a	Rt ^b
Song potency (SP)	-0.618*	0.540*
Female-directed vocalizations (FDV)	-0.469	0.785***
Consort persistence level (CPL)	-0.484	0.781***
March vocalizing rate (VRMarch)	-0.704*	0.392
April vocalizing rate (VRApril)	-0.532	0.036
May vocalizing rate (VRMay)	-0.736**	0.522

^aN=12.^bN=14.

*P<0.05, **P<0.01, ***P<0.002.

was not correlated with any of the three courtship measures (N=14; SP ($r_s = -0.426$, NS), CPL ($r_s = -0.213$, NS) or FDV ($r_s = -0.261$, NS)).

Neuroanatomical Measurements

For the two targeted neural areas, area X and Rt, we compared neuronal density, estimated neurone number and neuronal somal areas with volume measurements in area X (Table 2) and in Rt (Table 3). In area X, volume was not reliably predicted by neuronal density (N=12; $r_s = -0.273$, NS) or by neuronal somal area ($r_s = 0.329$, NS). In Rt, volume was not correlated with neuronal density (N=14; $r_s = 0.024$, NS) or with neuronal somal area

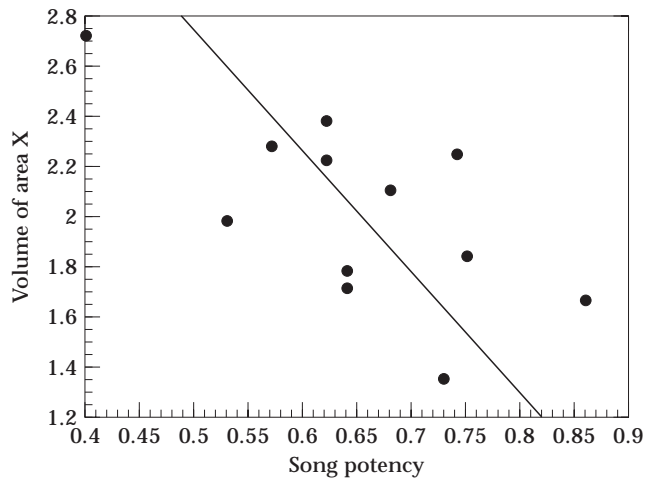


Figure 1. Scatterplot of the relationship between volume of area X and song potency.

($r_s=0.095$, NS). The lack of correlations with these measures suggests that neuronal number may be determining volume to a large degree.

DISCUSSION

The data implicate a visual nucleus in successful courtship behaviours in captive cowbirds and suggest that visual attention plays an integrative role in a male's development of effective song use. Bird song has often been described and studied as a strictly auditory/vocal process. Visual elements have typically not been conceptualized as part of the learning process. But other studies in our laboratory show that social experience (including visual stimulation) affects song learning itself in terms of acquisition of song content and pace of song maturation (King et al. 1996). Thus, these data on cowbirds call for the inclusion of other modalities at behavioural and neural levels of analysis. A successful male must not only attend to his own and his rivals' songs, but also to visual, auditory and other sensory cues displayed by conspecifics. Visual attention may also come into play in learning to direct songs to females as opposed to other males. Nucleus rotundus correlated with rate of female-directed singing, but not singing rate overall. Thus, the involvement of visual areas may serve to facilitate social discrimination of conspecifics.

The present data cannot address the question of whether volumetric differences found in nucleus rotundus reflect differences in visual experience. We now have data, however, from a new group of male cowbirds showing group differences in volume of Rt in males as a function of social context. We housed 12 males individually with female cowbirds: six were housed with females from their local population and six with females from a distant population. The females were known to prefer local versus distant song (West et al. 1998). The males housed with local females had larger volumes of Rt than did the males with distant females. The local females directed significantly more social behaviour towards their

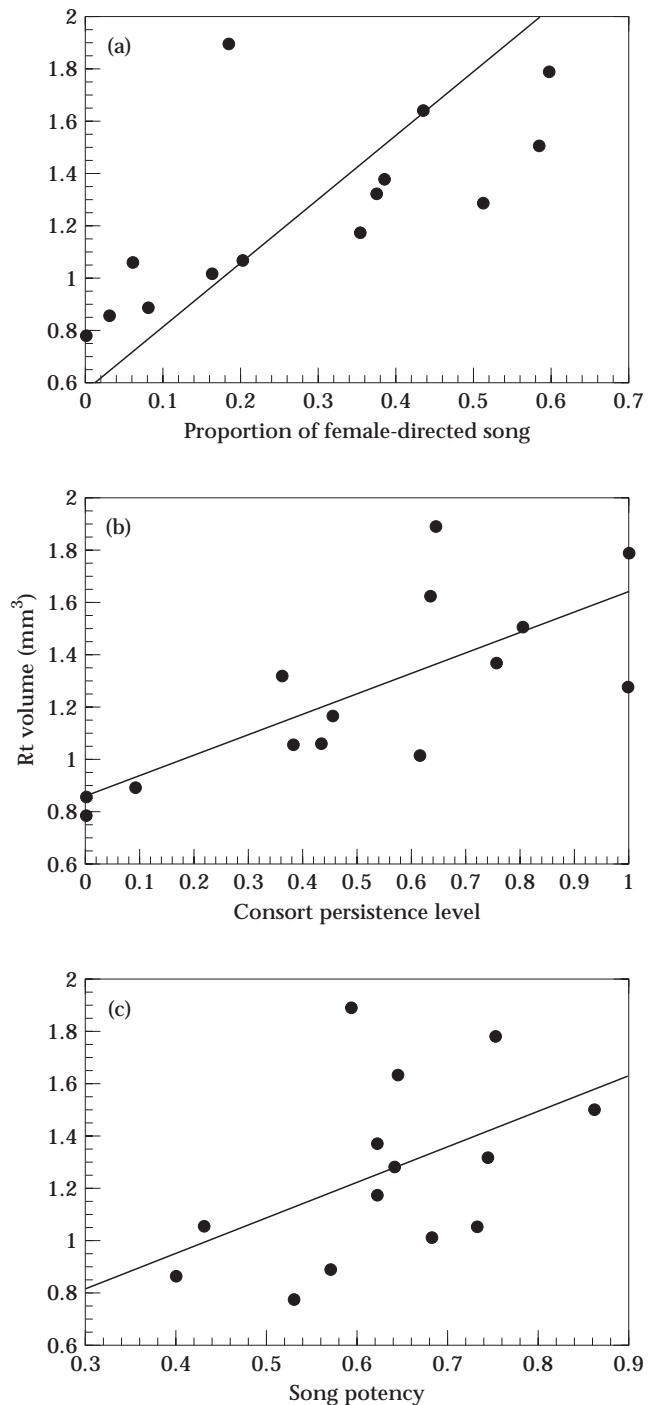


Figure 2. Scatterplots of the relationship between volume of nucleus rotundus and (a) proportion of female-directed vocalizations, (b) consort persistence level and (c) song potency.

males than did the distant females, and the males housed with the local females developed song sooner and sang more potent songs. Thus, the possibility exists that a more stimulating social environment that engages the males' visual and vocal attention may be associated with a different pattern of Rt growth.

A mammalian thalamic nucleus implicated in attending to the salience of visual cues is the pulvinar (reviews

in Robinson & Peterson 1992; Robinson 1993). Nucleus rotundus (Rt) in the avian thalamus is homologous to the inferior/caudal portion of the pulvinar (Shimizu & Karten 1993). The overall structure of the mammalian and avian visual systems are quite different in the relative volume of retinal projections of the tectofugal and thalamofugal visual pathways (Karten 1969). Thus, it is likely that, as the main visual pathway in avians, the tectofugal pathway is involved more closely with fundamental form, colour and motion processing in birds than it is in mammals.

Song Potency, Singing Rates and Area X

Here we found that vocalizing rate and song potency were negatively correlated with the volume of area X. In cowbirds, recent work has revealed that achievement of song stereotypy is activity-dependent with a strong positive correlation between singing rate and the time at which song stabilizes (King et al. 1996). Vocal activity and the amount of resulting acoustic, proprioceptive or social feedback may lead to volumetric regression in area X or conversely, males with a smaller area X may be likely to sing more regardless of the type of feedback. We cannot differentiate these two possibilities with the present data. Thus, in future studies, an important goal will be to look for possible seasonal or experiential changes in the volume of area X and possibly other song nuclei as a function of social context (Rausch & Scheich 1982; Brenowitz et al. 1991; Wallhauser-Franke et al. 1995b). The lack of correlations between any of the five behavioural measures and HVc, 1MAN and RA suggest specificity of function within the song system, as has been found with other songbirds.

We are intrigued by the possibility of experientially based changes in the volume of area X given that a comparison of song use in the same birds studied here and in the previous breeding season using identical methods (West et al. 1996) showed that neither song potency nor consort persistence level measured the previous year correlated with volumes of area X or Rt measured 1 year later (present study). These data indicate changes in adulthood in some courtship skills, especially song potency, and leave open the possibility of a corresponding plasticity of area X.

A smaller area X was found to be associated with more successful songs. Most studies of songbirds correlating measures of volume and measures of singing have found positive relationships between volume and behaviour, or no relationship. Clearly more data are needed in cowbirds and other species to place the present finding in perspective. At present, studies of brain-behaviour correlations relating to the song system have been limited to very few species (Brenowitz & Kroodsma 1996). Given the extraordinary diversity found in how songbirds acquire and use song, it is highly likely that diverse patterns will be found in neural investigations. Thus, the present finding of a negative correlation should not be viewed as anomalous. There are simply too few data in too few species using too few behavioural measures to render a judgement at this point.

HVc and Repertoire Size

The preceding arguments are also relevant to our findings with respect to HVc. We found that HVc volume was not correlated with repertoire size in these cowbirds. A closer examination of the mode of learning in various species (e.g. improvisation or imitation) is needed to analyse the diversity of findings regarding the relationship between repertoire size and HVc (DeVoogd et al. 1993; Brenowitz & Kroodsma 1996; Kroodsma 1996). Because cowbirds have small repertoires (one to seven song types), it may be that males use social and vocal feedback to improve the quality of particular structures within song types, such as note clusters. Female discrimination of song, as tested by playback, suggests that fine structure within song is far more important to a song's potency than global features such as number of phrases or presence or absence of the song's terminal whistle (West et al. 1979). Thus, for cowbirds, repertoire quality or choice of song types, not repertoire size, may correlate with success. This conclusion reinforces the need to expand behavioural assays of song learning to include measures of performance in many species.

Conclusions

This study represents only the second investigation of interrelationships between song-related behaviour and song-related nuclei in cowbirds (see Hamilton et al. 1997 for information on female cowbirds). As such, we must be cautious in our conclusions. All the more so in that we measured a number of brain areas and a number of behaviours in a small sample of birds. We provided the individual data on all measures to allow careful evaluation of the relationships being reported. We intentionally cast a wide behavioural net because many previous studies in our laboratory indicated that too narrow a focus on song structure or song playback can be misleading. For example, male cowbirds, with very potent songs are not always successful. They must also direct songs frequently to females during the breeding season (West et al. 1981).

The interrelationships among courtship behaviours and their associations with both area X and Rt, a song nucleus and a visual nucleus, do suggest, however, that the brain is using multiple systems to coordinate courtship as a unitary, multimodal behaviour. Of special interest is the neural indication here of the role of visual attention. This finding has wide implications for the methods used to study vocal learning, most importantly, the need to manipulate and document access to visual as well as vocal sources in instruction. Abundant evidence exists documenting the role of acoustic experience in avian vocal learning. However, some of the evidence must now be re-examined to ascertain whether young learners were deprived not only of species-typical sounds, but also of species-typical visual stimulation. The latter source of information may especially aid songbirds in learning not what to sing, but when to sing and to whom.

Acknowledgments

The work was supported by grants from the NSF and NICHD. All birds were collected under permit No. PRT 767881. All protocols were approved by the Indiana University Institutional Care and Use Committee. We thank three anonymous referees and M. D. Beecher for comments.

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