

Female visual displays affect the development of male song in the cowbird

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The role of social stimulation in avian vocal learning is well documented¹. The separate contribution of social, as opposed to vocal, stimulation has been difficult to address, however, because in almost all cases both tutor and pupil sing. The opportunity to isolate such effects arose in cowbirds (*Molothrus ater ater*) after discovering that males housed with non-singing female cowbirds made vocal changes which related directly to the female preferences for native song²⁻⁴. Here we report how females communicate with males about songs. We describe a visual display by females, a wing stroke, that is elicited by specific vocalizations. The songs that trigger wing strokes are in turn highly effective releasers of copulatory postures, and thus this previously unnoticed female display has biological significance. The data not only provide the first evidence of the tutorial role of male-female interactions during song ontogeny, they also clearly implicate visual stimulation in song learning, a process that has until now been assumed to be affected only by auditory information⁵.

The study was conducted in two parts. First, interactions between captive male-female pairs were videotaped in March and April, the time when cowbirds are found on their breeding habitat in the wild. Second, playbacks of songs that had elicited specific reactions from females during the videotaped sessions were presented one year later to females in breeding condition, thus testing the songs' effectiveness as releasers of copulatory postures.

We examined social interactions in eight male-female pairs in the first part of the study. The males were wild-caught juveniles captured when under 50 days of age; the females were adults captured the year before. Individual pairs were housed in sound-attenuating chambers and maintained according to previously described procedures⁶. The males were exposed to tape recordings of male songs for three months in the fall (autumn). Synchronized audio and video recordings were made in March and early April, at which point song types could be identified. These were stereotyped acoustic patterns differing from one another in the frequency contours of individual notes, note syntax, and the modulation of terminal whistles. Ten hours of singing were videotaped for four pairs and 12 hours for four other pairs. Audio taping continued into May to record the males' final repertoires as the pairs came into breeding condition.

The videotapes were analysed to mark the occurrence of every song and to record reactions by the female during each song. Only behaviours that began after the song's onset and before its finish, an interval of less than one second, were coded. In 88 hours of recording, 18,837 songs (range per male 1,320-4,614) were recorded. On average, 94% of the songs (range 86-98%) produced no visible change in the females' behaviour. When females did respond however, a striking display was a wing stroke, that is, rapid lateral movements of one or both wings away from the body (Fig. 1). It was called to our attention in part by the alert behaviour of the males, who frequently interrupted the pace of their singing after a wing stroke to approach and inspect the subsequent behaviour of the female (Fig. 2).

Wing strokes occurred during 237 of the 18,837 songs, ranging in frequency from 2 to 70 across the eight pairs, yielding a mean of 29.6 per female and a ratio of 1.1 wing strokes to every 100

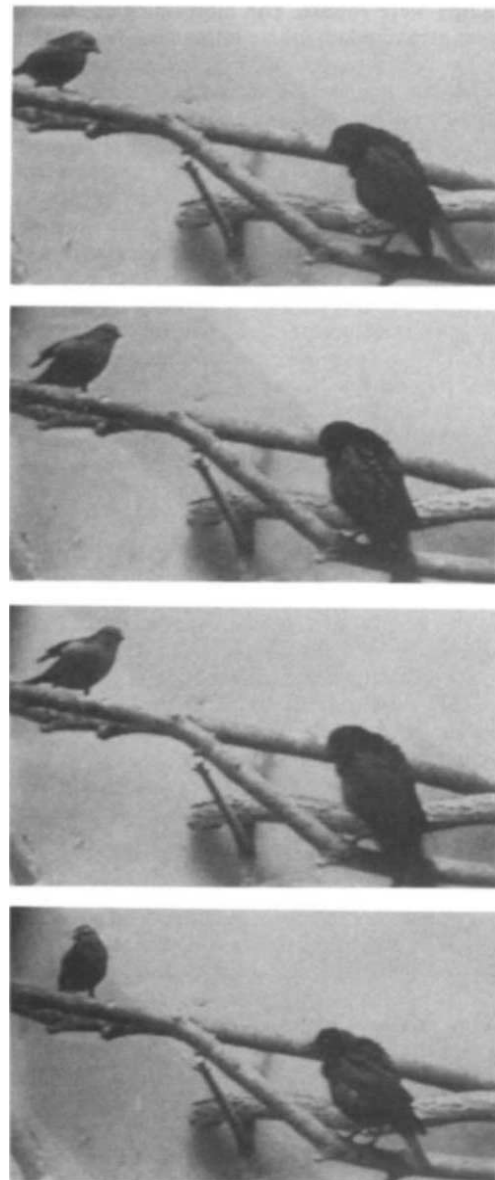


Fig. 1 A wing stroke is shown in the middle two panels. The male began his song 400 ms before the wing stroke. The upper panel shows the female's behaviour at song onset and the bottom panel shows her behaviour 900 ms later, just before the finish of the song. The photographs were made from the videotaped sequence used in experiment 4.

songs (wing strokes h^{-1} /songs h^{-1}). Two features of the wing stroke suggest it may be a precursor to a full copulatory posture, a display seen only in the breeding season. First, the wing stroke resembles the initial wing movement of a copulatory posture. Second, and most striking, the wing stroke occurs extremely rapidly while the male's one-second song is still in progress, a defining feature of the release of the copulatory posture, in this species.

The second part of the study, the playback tests, was carried out one year later. The subjects were six of the females studied in part one and two additional females which had been housed identically with males. In the time between coding of wing strokes and conclusion of playback testing, the females resided in same-sex pairs in sound-attenuating chambers.

Five song series were obtained from five of the eight videotaped male-female pairs. Each song series was composed



Fig. 2 The male's orientation and approach towards the female after producing a song that had elicited a wing stroke. The song had begun at 8:09:45:6 and ended at 8:09:46:1. The wing stroke began at 8:09:46:0 and ended at 8:09:46:2, shown in the upper panel. The female is now stretching her wing. This sequence is part of the playback series comprising experiment 3.

of 16 songs, the eight songs that had preceded a song eliciting a wing stroke, the wing-stroke song itself, and the seven songs following it. The major criterion for selecting sequences was audio recording quality. Each song series was run as a separate experiment. In each, the 16 songs were presented one at a time in six daily trials, separated in time by a minimum of 90 minutes. Each experiment took about two weeks to complete and the songs were played back an average of five times (range 4–8). The songs were not presented in their original order (hence the wing-stroke song was not necessarily the ninth song) but were ordered differently each day with the constraints that no song should occur twice in one day and that all songs should be played an equal number of times at different times of day.

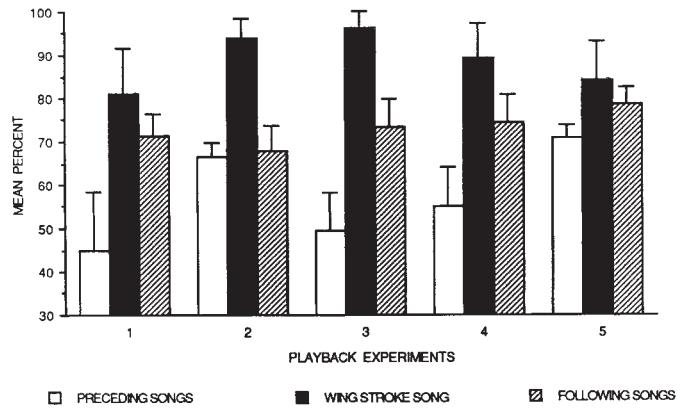


Fig. 3 The mean per cent and standard error of copulatory responses by the eight females for each experiment. Differences in potency among the 16 songs were tested by Friedman analyses of variance of ranks. Significant differences were obtained for each. The χ^2 values were as follows (df = 15): Exp. 1, 78, $P < 0.001$; Exp. 2, 31.2, $P < 0.01$; Exp. 3, 36.0, $P < 0.01$; Exp. 4, 38, $P < 0.01$; and Exp. 5, 25, $P < 0.05$. In each experiment, the wing-stroke song, song nine, received the highest mean rank. Wilcoxon signed rank tests were used to test for two differences: differences in potency between the wing-stroke song and the mean potency of songs 1–8 and differences between the mean potency of songs 1–8 and songs 10–16. For the first comparison, the Wilcoxon T value was 0, $P < 0.01$ for experiments 1–4 and $T = 2$, $P < 0.02$ for experiment 5. For the second comparison, T was 1 or 0 for experiments 1, 3, and 4, $P < 0.02$ and the T value was > 6 (n.s.), for experiments 2 and 5.

Playback testing began when the females came into breeding condition as judged by egg-laying. Song potency was defined as the percentage of trials in which a song elicited a copulatory posture, that is, the female lowered and spread her wings, arched her neck, and separated the feathers around the cloacal area.

The females responded differently to the 16 songs. In each experiment, the wing-stroke song yielded the highest mean potency, 89% (range 81–96%; Fig. 3). In contrast, the mean percent of responses to the preceding eight songs was 57% (range 0–100%) whereas the seven songs following the wing stroke elicited a mean percentage of responses of 72% (range 0–100%), producing statistically significant differences in each experiment (Fig. 3). Thus, although males sang other effective songs, only the wing-stroke song produced consistently high levels of copulatory responses.

The song sequences were examined with respect to acoustic content and organization. The five males sang four to seven different song types before the wing-stroke song. In four of the sequences (experiments 1–4, see Fig. 3), no new song types occurred after the wing-stroke song. The song type eliciting the wing stroke accounted for an average of 19% (range 0–38%) of the singing during the first eight songs in contrast to 63% (range 43–86%) of all singing after the wing stroke, a non-overlapping difference in each experiment. The song type eliciting the wing stroke was also sung more repetitively after the wing stroke: the mean number of immediate repetitions before the wing stroke was 1 (range 0–2) compared to 3.75 (range 2–6) after it. These changes, in addition to the higher potencies of songs following wing strokes, highlight the conspicuous nature of the wing stroke to males who immediately attempted to make use of the information contained in the female's display.

Thus, the present study underscores the multi-modal nature of the stimulation available to males as they learn to sing, and adds credence to a multi-phasic view of song learning whereby it is influenced by both natal and juvenile experiences⁷. Most importantly, the data bring into focus visual dimensions of song learning that have not been described before. Males must not

only listen to the nature of the sounds they produce, but also look at the reactions their sounds provoke.

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1. Petrinovich, L. in *Social Learning: Psychological and Biological Perspectives* (eds Zentall, T. & Galef, B. G.) 255-278 (Lawrence Erlbaum, New York, 1988).
2. King, A. P. & West, M. J. *Nature* **305**, 704-706 (1983).
3. West, M. J. & King, A. P. *Ethology* **70**, 225-235 (1985).
4. King, A. P. & West, M. J. *Anim. Behav.* (in the press).
5. Kroodsma, D. E. in *Acoustic Communication in Birds* Vol. 2 (eds Kroodsma, D. E. & Miller, E. H.) 1-28 (Academic Press, New York, 1982).
6. West, M. J. & King, A. P. *J. comp. Psychol.* **100**, 296-303 (1986).
7. McGregor, P. K. & Krebs, J. P. *Behaviour* **79**, 127-147 (1982).

Acetylcholine inhibits identified interneurons in the cat lateral geniculate nucleus

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The transmission of visual information from retina to cortex through the dorsal lateral geniculate nucleus (LGNd) is controlled by non-retinal inputs^{1,2}. Enhanced visually evoked responses in cat LGNd relay cells during periods of increased alertness³ have been attributed in large part to increased rate of acetylcholine (ACh) release by fibres ascending from the brainstem reticular formation⁴⁻⁷. ACh can modulate geniculate visual responses *in vivo*⁵⁻⁸, but comparatively little is known about the underlying ionic mechanisms of these cholinergic actions. Although direct excitation of LGNd relay neurons has been shown *in vitro*⁹, the situation is complicated because cholinergic axons form numerous and complex synapses not only with relay cells, but also with inhibitory interneurons¹⁰, and electrical activation of the brainstem cholinergic neurons reduces inhibitory postsynaptic potentials in the LGNd¹¹⁻¹³. We report here that morphologically characterized interneurons in the cat LGNd possess distinctive electrophysiological properties in comparison with those of relay cells and are inhibited by ACh through a muscarinic receptor-mediated increase in potassium conductance. Together the direct excitation of relay cells and inhibition of intrageniculate interneurons allow the ascending cholinergic system to exert a powerful facilitatory influence over the transfer of visual information to the cerebral cortex.

Intracellular recordings from cells in lamina A or A₁ of the cat LGNd revealed two distinct classes of neurons, which we tentatively term principal (P) and I neurons. P neurons display electrophysiological properties similar to those reported previously for thalamocortical relay cells¹⁴⁻¹⁶, including the presence of a low threshold Ca²⁺ spike which underlies burst discharges (Fig. 1e, arrow head), anomalous rectification in both the hyperpolarizing (Fig. 1e) and depolarizing ranges (data not shown), a delayed onset to firing during depolarization (Fig. 1c, arrow head), and a general lack of pronounced spike frequency accommodation (Fig. 1c).

In contrast, we found that I neurons either lack completely a typical low threshold Ca²⁺ spike (Fig. 1f; *n* = 4) or display only a small rebound exultation after a large hyperpolarization (data not shown; *n* = 4). Furthermore, I neurons possess action potentials which are on the average 28% shorter in duration than those of P cells (compare Fig. 1a and b), lack a delayed onset to firing (Fig. 1d), display more linear membrane potential versus injected current (*V*-*I*) relationships, and have a higher input resistance (I neurons: 89 ± 24 MΩ (mean ± s.d.), *n* = 9;

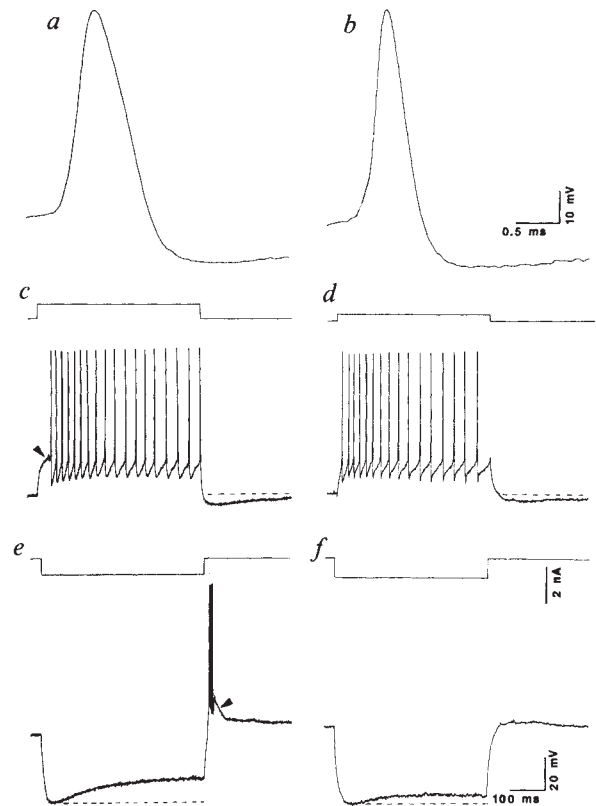


Fig. 1 Electrophysiological properties of LGNd P and I neurons. *a* and *b*, Single action potential of a typical P cell (*a*, 0.52 ms) is substantially broader at half peak amplitude than that of a typical I neuron (*b*, 0.31 ms) (average action potential duration ± s.d. for the two groups: P cells, 0.50 ± 0.20 ms, *n* = 20; I neurons, 0.36 ± 0.13 ms, *n* = 13; *t* = 2.27, *P* < 0.05). *c* and *d*, Responses to a depolarizing current pulse of a P cell (the same I neuron; resting membrane potential (*V*_m) = -64 mV; arrow head indicates delayed onset of firing) and an I neuron (*d*; resting *V*_m = -76 mV). *e* and *f*, Injection of hyperpolarizing current pulses into a typical P cell (*e*) and an I neuron (*f*). Note the lack of a rebound low threshold Ca²⁺ spike in the I neuron (*f*) versus the P cell (*e*, arrow head; burst contains four action potentials) despite the large hyperpolarization from -76 to -135 mV. All data in *a*, *c* and *e* are from the same P cell and those from *b*, *d* and *f* are from the same I neuron. Calibration bars in *f* for *c*-*f*, calibration bars in *b* for *a* and *b*. Top trace in *c*-*f* is injected current, whereas the bottom trace is the resulting deviation in *V*_m.

Methods. Eleven 3-month to 3-year old cats were deeply anaesthetized with ketamine (20 mg per kg, intramuscularly) and sodium pentobarbital (15 mg per kg, intravenously) and underwent a wide craniotomy. The animals were killed by decapitation and both hemispheres were rapidly removed and dissected to isolate the lateral geniculate nucleus which was then sectioned coronally on a vibratome as 400 μm slices. Slices were maintained in an interface chamber at 35 ± 1 °C and perfused with a solution containing (in mM) NaCl, 126; KCl, 2.5; MgSO₄, 2; NaHCO₃, 26; NaH₂PO₄, 1.25; CaCl₂, 2; glucose, 10; saturated with 95% O₂, 5% CO₂ to a final pH of 7.4. Epi-illumination of the coronal slices readily revealed the main laminae of the LGNd as well as the optic tract and optic radiation. Neurons were recorded only in the middle portions of laminae A and A₁ to avoid recording from perigeniculate or interlaminae cells. Microelectrodes were filled with 4 M KAc for normal recordings and 0.2 M LiCl saturated with lucifer yellow CH (LY) for intracellular labelling experiments. Only neurons having stable membrane potentials below -55 mV and overshooting action potentials were included in the electrophysiological analysis. Action potential durations were measured at half-peak amplitude. Acetylcholine (ACh, 10 mM) or acetyl-β-methylcholine (MCh, 10 mM) were applied from a broken micropipette (1-2-μm tip diameter) by pressure pulses in volumes of approximately 10 pl within 50-75 μm of the recorded cell. LGNd slices containing LY-labelled neurons were resectioned at 50-75 μm, mounted and cleared on microscopic slides, and photographed.