Social context modulates singing-related neural activity in the songbird forebrain

Neal A. Hessler and Allison J. Doupe

Keck Center for Integrative Neuroscience and Departments of Physiology and Psychiatry, University of California, San Francisco, California 94143 USA

Correspondence should be addressed to N.A.H. (neal@keck.ucsf.edu)

Birdsong is a complex vocal behavior learned during early life in a process similar to the learning of human speech[1]. Like speech, singing is a social behavior—male songbirds often use it in courtship or in territorial defense[2]. Here we show that, in the adult zebra finch, the pattern of singing-related neural activity in several high-level brain areas specialized for song learning is dependent on whether a bird sings by itself or to another bird; thus, this activity can indicate not only that a bird is singing but also the social context of the song.

Zebra finch singing can be divided behaviorally into two types: 'directed' singing is highly aroused, is aimed at another bird, and often occurs during the courtship dance, whereas 'undirected' singing occurs when a finch is either alone or not orienting toward any other bird in particular[3,4]. In these experiments, we recorded song motifs (stereotyped sequences of four to seven syllables that constitute the basic repeated phrase of zebra finch song) and associated neural activity in the anterior forebrain song nuclei L-MAN and Area X, during sequential epochs of directed and undirected singing. These two nuclei are part of a specialized dorsal forebrain–basal ganglia loop that is crucial for song learning[5–7] (**Fig. 3a**), but can be lesioned in adult birds with no apparent effect on normal song production. In this respect they differ greatly from the song system motor nuclei HVc and RA

(**Fig. 3a**), which are necessary for song production throughout life[8]. Recently, however, we found that, as do HVc and RA[9,10], L-MAN and Area X of adult birds increase their firing rate immediately before the onset of song as well as during singing (N.A.H. & A.J.D. *Soc. Neurosci. Abstr*. **23**, 245, 1997), consistent with some function, albeit more subtle, in adult song. In this study, we recorded neural activity in L-MAN and Area X during directed and undirected singing. Directed song was elicited by presenting, in a separate cage, another zebra finch (male or female) to the adult bird being recorded; the recorded bird moved to the edge of its cage and sang while facing the visitor. Undirected song was recorded while the bird was alone in various locations in its cage.

The neural activity in L-MAN and Area X associated with the production of song motifs differed markedly between directed and undirected singing. Multi-unit activity level in L-MAN during 280 successive motifs of song from a representative bird was clearly dependent on the social context in which
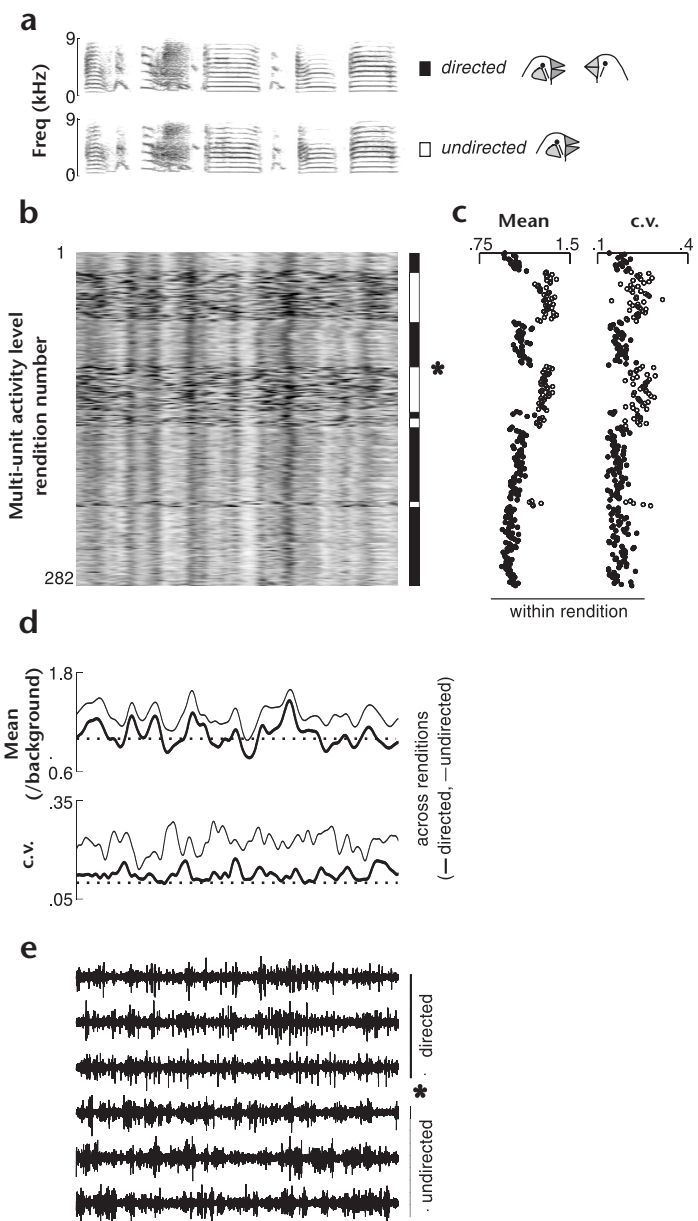
Fig. 1. Distinct pattern of L-MAN activity during singing in two social contexts. **(a)** Similar song motifs produced during directed and undirected singing. **(b–d)** Population neural activity levels (recorded with an insulated tungsten electrode carried in a lightweight microdrive), quantified by rectifying and smoothing (10-ms Gaussian window) raw activity waveforms, and aligned so that the amplitude envelopes of the first three syllables of each motif maximally overlapped (alignment of later motif elements remained good; the standard deviation of offset time of all motifs was 19 ms). (b) L-MAN activity level during 280 successive renditions of a song motif; level of activity is proportional to darkness of shading. Vertical bar to right of (b) indicates social stimuli present during each motif rendition; filled bar, another bird present; empty bar, isolation. Directed singing was performed in the first two epochs to two different males, in the last two to a female. (c) Mean and c.v. of activity level for each motif rendition (filled circles, directed; empty circles, undirected). (d) Mean and c.v. of activity level were calculated in successive one-millisecond bins across all renditions of directed and undirected motifs. Dotted lines represent background (non-singing) mean and c.v. of activity level. **(e)** Neural activity waveforms recorded during production of six consecutive song motifs spanning a change from directed to undirected singing (waveforms were drawn from the epoch marked by the asterisk in b). Duration of (a), (b), (d) and (e), 950 ms.
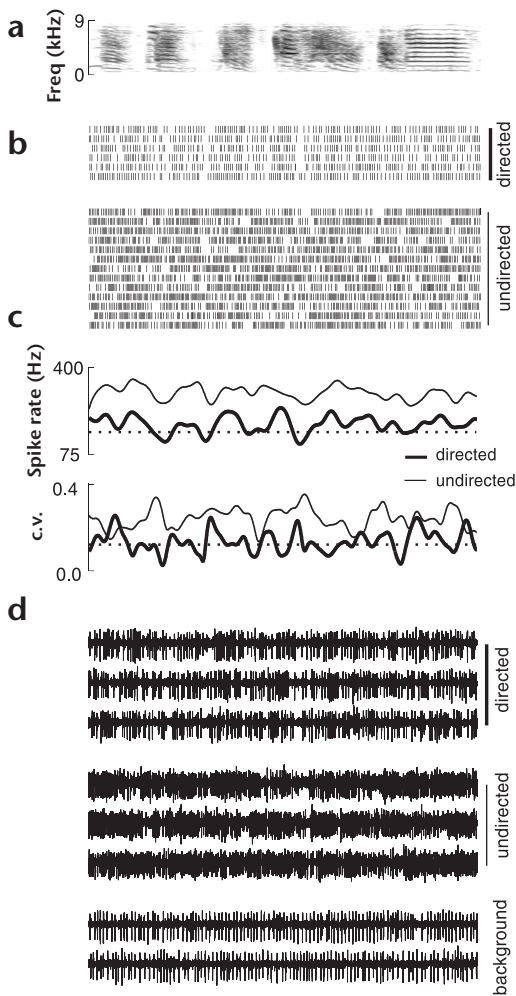
Fig. 2. Singing-related firing of an Area X single neuron is modulated by social context. **(a)** Spectrogram of a typical song motif during directed singing. Note differences in structure from the motif sung by the bird in Fig. 1, which reflect differences between individual birds' songs due to learning. **(b)** Spike arrival times for a single neuron recorded during multiple renditions of the motif in directed and undirected singing contexts. In both behavioral conditions, these data represent activity of a single unit by the criteria of interspike intervals (only 1% were less than 1.5 ms) and by waveform shape. **(c)** Mean and c.v. of spike rate across multiple motif renditions for directed and undirected singing. Dotted lines represent background (non-singing) mean and c.v. **(d)** Representative neural activity waveforms recorded during directed and undirected singing of the song motif, along with two waveforms of equal length drawn from a non-singing period. (a–d), duration, 700 ms .

each motif was produced (**Fig. 1b**). The average level of neural activity during motifs was consistently lower during singing to another bird (**Fig. 1c**, left; **Fig. 1d**, top), as was the amount of modulation of activity within renditions (**Fig. 1c**, right). Furthermore, the variability in the pattern of activity across renditions was different: the distinct pattern of firing in relation to song syllables was more evident across successive motifs for directed than for undirected singing (**Fig. 1b**). This is shown quantitatively by the much lower coefficient of variation (c.v. = s.d./mean) of activity levels across all renditions for directed singing (**Fig. 1d**, bottom). Nonetheless, averaged across renditions, there was a similar pattern of activity related to motifs in the two conditions (**Fig. 1d**, top); the greater magnitude and variability of activity during undirected singing appeared to be due primarily to unpatterned burstiness throughout the motif (**Fig. 1e**).

A similar influence of social context on neural activity was seen in Area X, evident in the activity of both small clusters of neurons and well-isolated single neurons. **Fig. 2** shows a single neuron whose firing rate was lower when the bird sang to another bird than when it sang alone (**Fig. 2b, c** top and **d**). As in L-MAN, the pattern of firing was more consistent across renditions for directed than for undirected singing (**Fig. 2c**, bottom). Note also that, although firing during directed singing was just slightly above background non-singing levels (**Fig. 2c,** top and **2d**), there was a consistent pattern of increas-

es and decreases in spike rate related to particular parts of the motif (**Fig. 2b** top and **d** top).

We observed similar effects in 11 of 11 multi-unit L-MAN recordings from 6 birds, in 2 of 2 small neuronal cluster recordings from Area X of 2 birds and in 2 of 2 single-neuron recordings from Area X of 2 birds. Average activity levels were lower in all recordings during song motifs directed to females than during undirected motifs, and for three of four birds during song directed to a male (**Fig. 3b**, $p < 0.01$, *t*-test, for all birds except one Area X recording, indicated by filled star, and one recording from L-MAN, indicated by symbol +a; see **Fig. 3** legend for details). Variability of the pattern of activity across multiple renditions, as quantified by c.v., was significantly higher during undirected singing than during singing directed to a female for all recordings from L-MAN and X. In all but one (see above), the c.v. for activity during undirected singing was also higher than that during male-directed song (**Fig. 3c**; sign test, $p < 0.01$). These differences did not seem to require auditory feedback; two birds showed similar effects before and after deafening (**Fig. 3b** and **c**). The social modulation effect was specific to singing; background non-singing activity levels did not differ between isolated and accompanied contexts ($p > 0.01$, *t*-test).

Despite these clear differences in neural activity during singing, song motifs produced in both behavioral contexts were similar in structure (**Fig. 1a**), timing and amplitude. Directed song motifs tended to be of slightly shorter duration than motifs from undirected song, although the maximum mean difference observed in any bird was less than 2% (766 ± 11 ms, mean ± s.d. for 366 undirected motifs versus 754 ± 10 ms for 60 directed motifs), and such differences were significant ($p < 0.01$, *t*-test) in only 4 of 13 recording sessions from 8 birds (for all recordings, the ratio of undirected:directed motif durations ranged from 0.992 to 1.02; mean number directed motifs per session, 85, mean number undirected motifs per session, 154). As was true for the other production parameters, there were no large differences in amplitude between the two conditions; for five of eight birds, the ratio of average directed:undirected motif amplitude ranged from 0.97 to 1.05. An apparently lower amplitude of directed versus undirected motifs in three of eight birds could be explained by consistent differences in the bird's orientation relative to the microphone (song broadcast from speakers mimicking the birds' position and orientation in the two conditions reproduced the recorded amplitude differences).

This study demonstrates a marked, consistent and rapid-onset influence of social context on singing-related electrophysiological activity. In the anterior forebrain nuclei L-MAN
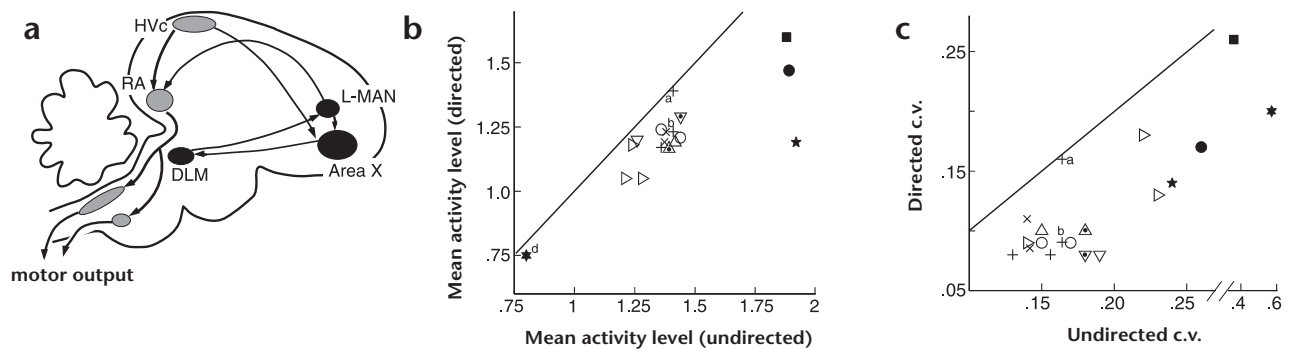
Fig. 3. A social effect on singing-related firing was seen in all recordings from L-MAN and Area X. **(a)** The song nuclei Area X and L-MAN (black) form part of an anterior forebrain loop though basal ganglia and thalamus that indirectly connects the song motor nuclei HVc and RA (gray). **(b)** Mean activity levels (normalized to background non-singing levels; for L-MAN, population activity was quantified as in Fig. 2 legend; for Area X, small-cluster or single-neuron activity rates were determined) were significantly higher during undirected than directed singing for 10 of 11 recording sessions from L-MAN (open symbols, 6 birds represented by different symbols) and for 3 of 4 birds from Area X (filled symbols; single n.s. case, *d). In one recording session in L-MAN, singing to a male caused firing indistinguishable from singing alone ($p > .01$; symbol, +a), whereas singing to a female resulted in significantly less activity than did undirected singing ($p < .01$; symbol, +b). In other recording sessions for this bird, only singing to a female was performed (symbol +). Recording sessions before and after deafening individual birds are indicated by symbols with and without center dots, respectively. **(c)** Variability of firing pattern across motif renditions was significantly lower for 10 of 11 recording sessions from L-MAN (as discussed above, +b depicts female-directed signing in the one exception) and 4 of 4 for Area X. Symbols represent the same birds as in (b). Average c.v. across all renditions for a social context was divided into ten equivalent epochs; comparisons between mean values in each epoch were made with a sign test. Dotted lines in (b) and (c) indicate where symbols would lie if values for directed and undirected singing were equal.

and Area X (**Fig. 3a**), activity during directed singing is lower in magnitude and more consistent in pattern across renditions than activity during undirected song. It is striking that song motif structure is nonetheless so little changed, but it is not yet clear whether singing-related activity of the song motor nuclei HVc and RA[9,10] is similarly altered by social cues. Strong modulation of neural activity in HVc and RA would be likely to result in changed song output; lack of a gross difference in motif structure between directed and undirected singing thus raises the possibility that social modulation of neural activity is weaker in these motor nuclei than in the anterior forebrain nuclei, although this will need to be tested directly. Strong effects in the anterior forebrain with smaller effects on its output motor nucleus RA would be consistent with numerous studies suggesting that the influence of anterior forebrain nuclei on motor pathway output is developmentally regulated, becoming more subtle in adult birds singing stable song[5–7,11]. The effect of social context on anterior forebrain neural activity could reflect a varying level of attention or arousal of the singing bird in different settings, or may be related to the communicative function of singing to another bird (cowbirds, for example, visually monitor the song recipient for its response[12]) versus singing alone. Clearly, further studies should also include an analysis of the song recipient's behavior in the two conditions that we use. In light of our results, it is intriguing that the song nuclei we studied receive strong dopaminergic inputs[13], which are proposed to mediate reinforcement, and could be responsible for the difference in the level and precision of neural firing between the two social conditions. Molecular events downstream of electrophysiological activity can depend on the type or pattern of neuronal firing: our observations that activity during directed singing, though still patterned and related to song, is of lower magnitude and less bursty than that during undirected singing, may also explain the recently reported lower levels of immediate-early gene induction in L-MAN, Area X, and RA during directed compared to undirected singing[14]. Finally, social and attentional factors profoundly affect behavior and learning in many animals[15], including humans. The social modulation of neural activity described in this report suggests that the study of birdsong, a well-characterized learned behavior involving discrete neural circuitry, could shed light on the neural mechanisms underlying social influences on behavior.

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