Social Context Rapidly Modulates the Influence of Auditory Feedback on Avian Vocal Motor Control

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Sakata JT, Brainard MS. Social context rapidly modulates the influence of auditory feedback on avian vocal motor control. J Neurophysiol 102: 2485–2497, 2009. First published August 19, 2009; doi:10.1152/jn.00340.2009. Sensory feedback is important for the learning and control of a variety of behaviors. Vocal motor production in songbirds is a powerful model system to study sensory influences on behavior because the learning, maintenance, and control of song are critically dependent on auditory feedback. Based on previous behavioral and neural experiments, it has been hypothesized that songs produced in isolation [undirected (UD) song] represent a form of vocal practice, whereas songs produced to females during courtship interactions [female-directed (FD) song] represent a form of vocal performance. According to this “practice versus performance” framework, auditory feedback should be more influential when birds engage in vocal practice than when they engage in vocal performance. To directly test this hypothesis, we used a computerized system to perturb auditory feedback at precise locations during the songs of Bengalese finches and compared the degree to which feedback perturbations caused song interruptions as well as changes to the sequencing and timing of syllables between interleaved renditions of UD and FD song. We found that feedback perturbation caused fewer song interruptions and smaller changes to syllable timing during FD song than during UD song. These data show that changes in the social context in which song is produced rapidly modulate the influence of auditory feedback on song control in a manner consistent with the practice versus performance framework. More generally, they indicate that, for song, as for other motor skills including human speech, the influence of sensory feedback on activity within vocal premotor circuitry can be dynamically modulated.

INTRODUCTION

Sensory feedback is used to learn and guide the performance of a wide range of behaviors, including vocal behavior (Brainard and Doupe 2000a; Doupe and Kuhl 1999; Houde and Jordan 1998; Pearson 2004; Sabes 2000; Smotherman 2007). During motor learning, sensory feedback is used to reinforce motor commands underlying desired outcomes and weaken motor commands that produce undesired outcomes (Doya and Sejnowski 2000; Doyon and Benali 2005; Sutton and Barto 1998; Todorov and Jordan 2002; Troyer and Doupe 2000; Tumer and Brainard 2007). With regard to motor control, sensory feedback provides information about the degree to which the execution of a motor plan achieves a desired behavioral outcome and about the adjustments to the motor plan needed to improve behavioral performance. Sensory feedback is integrated into the motor plan for many behaviors, and perturbations of feedback can lead to acute changes in performance. For example, delaying auditory feedback during speech production causes acute vocal alterations such as interruptions, slowing of speech, and mis-sequencing of syllables (Howell and Archer 1984; Howell and Powell 1987; Lee 1950).

The degree to which sensory feedback contributes to motor control can be affected by factors such as the strength of learning and behavioral variability. In general, the degree to which feedback contributes to motor control is more pronounced when a behavior is being learned than when that behavior is already well established. For example, hearing loss leads to a more rapid and severe degradation of speech in young individuals who are developing language than in older adults (Cowie and Douglas-Cowie 1992; Lane and Webster 1991). Furthermore, in adults, auditory feedback perturbations lead to fewer speech errors when producing a more recently acquired language than when producing the native language (Borden 1979; Howell and Dworzynski 2001; Howell et al. 2006; MacKay 1968; Van Borsel et al. 2005). Vocal training not only improves vocal accuracy but also the ability to ignore aberrant feedback (Wyke 1974; Zatare and Zatorre 2008). This latter finding is particularly interesting because it indicates that the influence of auditory feedback is subject to top-down processes (i.e., in response to instruction to ignore feedback) and that training can enhance this top-down control. Taken together, these studies show that the influence of auditory feedback on motor control can be dynamically modulated.

Songbirds provide an excellent opportunity to study dynamic changes in the influence of auditory feedback. Like speech, the development of song is critically dependent on auditory feedback (reviewed in Brainard and Doupe 2000a, 2002; Konishi 1965, 2004). However, as in humans, the dependence on auditory feedback changes with age: whereas birds deafened as juveniles exhibit a relatively rapid deterioration of song, birds that are deafened as adults can continue to sing relatively normal song for weeks after deafening (Brainard and Doupe 2001; Konishi 1965; Konishi and Nottebohm 1969; Lombardino and Nottebohm 2000; Nordeen and Nordeen 1992; Price 1979). Even on reaching adulthood, the dependence on auditory feedback continues to decline with age such that deafening-induced changes take longer to develop in older adult birds than in younger adult birds (Brainard and Doupe 2001; Lombardino and Nottebohm 2000).

As for speech, dynamic changes in the contribution of auditory feedback to song control can be studied by reversibly perturbing auditory feedback during ongoing vocalizations. This method has been used to show contributions of auditory feedback to real-time song control and song plasticity (Cynx and von Rad 2001; Kochevnikov and Fee 2007; Leonardo 2004; Leonardo and Konishi 1999; Sakata and Brainard 2006,
M E T H O D S

A n i m a l s

Fifteen adult male Bengalese finches (median: 6 mo; range: 4–33 mo) were used in the study. During the experiments, birds were housed individually in sound-attenuating chambers (Acoustic Systems), and food and water were provided ad libitum. Photocycles (14:10 light:dark) were maintained during development and throughout all experiments. All procedures were performed in accordance with established animal care protocols approved by the University of California, San Francisco Institutional Animal Care and Use Committee (IACUC).

S o n g r e c o r d i n g

After \( \geq 24 \) h of habituation to the sound-attenuating chamber, a computerized, song-activated recording system was used to detect and digitize song [Observer: A. Leonardo (California Institute of Technology) and C. Roddy (University of California, San Francisco); Evtaf: E. Tumer (University of California, San Francisco)] for later off-line analysis using software written in the Matlab programming language (Mathworks).

We recorded and analyzed songs produced in two social contexts. UD songs are produced in isolation, whereas FD songs are produced during courtship interactions with females. During experimental sessions, FD song was elicited by placing a cage with a female next to the experimental male’s cage. As in other songbirds, FD songs of Bengalese finches are readily distinguishable from UD songs because they 1) are produced after a male approaches or faces another individual, 2) are accompanied by a courtship dance (e.g., pivoting body from side to side), and 3) are associated with the fluffing of the male’s plumage (Morris 1954; Zann 1996). Behavior of birds was monitored remotely by video camera, and only songs that were accompanied by at least two of the above behaviors were categorized as FD songs. When FD songs were produced, they were almost always produced within 15 s after the introduction of the female. Females were removed after \(< 1 \) min regardless of whether the males produced FD song. This design ensured that FD songs in our study reflected those that were elicited at a short latency after exposure to a female (Hessler and Doupe 1999; Kao and Brainard 2006; Sakata et al. 2008; Teramitsu and White 2006). We separated exposures to females by 4–5 min (modal interval), which allowed for the collection of UD song between female exposures. Because UD and FD songs were interleaved in this manner, we could compare songs that were temporally proximal to each other, and hence, our results emphasize the rapid influence of social context on song control. In most instances (\( n = 12 \)), experimental sessions were restricted to 1 day; however, in a few instances (\( n = 3 \)), we collected data over 2 experimental days to increase sample sizes and statistical power. Our manipulations of social context caused significant changes to song organization, including changes to syllable sequencing and timing, thereby confirming the efficacy of our context manipulation (Hampton et al. 2009; Sakata et al. 2008).

Previous studies have shown circadian variation in several features of song during song learning (Derégnaucourt et al. 2005; Tchernichovski et al. 2001). Consequently, variation in the time of day in which experiments were conducted could lead to variation in the degree to which social context and auditory feedback perturbations affect song control. For this study, experimental sessions were initiated in the morning (8:30–11:00 AM) and lasted between 1 and 7 h (median = 3 h). To assess circadian influences on the degree to which social context modulated the effects of feedback perturbation, we compared the degree to which social context altered the effect of feedback perturbation on song control between experiments that were restricted to the morning and experiments that spanned the morning and afternoon. For none of the features there was a significant difference between experiments that were restricted to the morning and experiments that spanned the morning and afternoon in the social modulation of the effect of feedback perturbation. Consequently, data were combined regardless of the time of day in which testing occurred.

P e r t u r b a t i o n of auditory feedback

We used a computerized system to detect specific syllables as they were produced and to administer localized perturbations of auditory feedback during ongoing song. Syllable identification and targeting were identical to those of Sakata and Brainard (2006, 2008). Targeted syllables were detected based on the pattern of spectral features present in the syllable (Fig. 1). We targeted syllables at “branch point sequences,” portions of song in which the sequencing of syllables varies from rendition to rendition. For the songs of six birds, the targeted syllable was produced in more than one branch point sequence, and for the songs of five birds, the targeted syllable was produced not only in a branch point sequence but also within a stereotyped sequence.

After the detection of the targeted syllable, a prerecorded sound (single syllable from the male’s repertoire) was played back at a short and fixed latency via a free-field speaker (\( \sim 70–100 \) dB) so that the singing bird experienced a superposition of extraneous feedback onto his own normal feedback (altered feedback trials). The range of feedback intensities approximates the intensity of the bird’s own vocalizations during song production measured within 10 cm of the bird (Cynx and von Rad 2001). For most experiments (20 of 28), the syllable used as the disruptive stimulus was the same as the targeted
FIG. 1. Altering auditory feedback. Templates to spectral features of targeted syllables were made, and a computerized system was used to detect specific syllables as they were being produced and to transiently disrupt auditory feedback. Depicted is a spectrogram of the sequence “abcdefg” for a bird. In this representation of song, power is plotted as a function of frequency (y-axis; 0–8 kHz) and time (x-axis; ~700 ms of song is depicted). For this experiment, the syllable d was targeted, and a delayed version of the syllable d was superimposed on the sound of the bird’s own song during altered feedback trials. On the altered feedback trial depicted here, the bird continued to sing despite the feedback perturbation, but the timing of syllables was delayed because of the perturbation. Such changes to song tempo caused by feedback perturbation were common (see Sakata and Brainard 2006, 2008). On randomly interleaved control trials, the syllable was detected but feedback remained normal. Altered feedback and control trials were administered with equal probabilities. Additionally, we collected interleaved renditions of song produced in isolation [undirected (UD) song] or to females [female-directed (FD) song]. This design allowed us to directly analyze the real-time contributions of auditory feedback to song control (Sakata and Brainard 2006) and to compare the degree to which feedback perturbations differentially affected the control of UD and FD song.

Song analysis

After amplitude-based syllable segmentation (Matlab), we labeled syllables manually based on visual inspection of spectrograms. These labels were used to measure the effect of feedback perturbation on the frequency of song interruptions, on syllable sequencing, and on song tempo for UD and FD song.

Song interruptions

The superposition of extraneous feedback during song can lead to terminations of song (‘song interruptions’; Cynx and von Rad 2001; Sakata and Brainard 2006). Song interruptions were defined as instances in which song did not resume within 300 ms after the onset of the feedback perturbation (Sakata and Brainard 2006). Interruptions have been measured to assess sensory influences on vocal behavior in songbirds as well as primates (Cynx 1990; Cynx and von Rad 2001; Miller et al. 2003; Riebel and Todt 1997; Seki et al. 2008; ten Cate and Ballintijn 1996). Therefore we compared the degree to which localized auditory feedback perturbations caused song interruptions between FD and UD song.

To maximize our ability to detect such differences, we controlled two aspects of our experimental design. First, we targeted sequences in which abrupt song terminations were rarely observed when feedback was normal (3.7 ± 1.0% of the control trials during UD song and 5.1 ± 1.1% of the control trials during FD song; UD vs. FD: paired t-test; P > 0.15). Second, because the frequency of song interruptions can attenuate with repeated exposure to feedback perturbation (Sakata and Brainard 2006), we controlled for potential attenuation effects across UD and FD song by interleaving the production of UD and FD song (as described above) and by randomly designating whether feedback perturbation was initiated during UD (n = 10) or FD song (n = 18) across experiments.

Sequencing and timing of syllables

We also analyzed the effects of feedback perturbation on the sequencing and timing of syllables for UD and FD song. To quantify changes to syllable sequencing caused by feedback perturbation, we analyzed the probability of different syllable transitions immediately after the targeted syllable (1st-order transitions) during interleaved control and altered feedback trials. Typically, there were two to five first-order transitions at a branch point. In a previous study, we report that localized feedback perturbations identical to those used here lead to significant increases in the variability of sequence transitions (i.e., transition entropy) at branch points (Sakata and Brainard 2006). Here we used an identical analysis, and for each branch point, we quantified the transition entropy of the branch point as
transition entropy = \sum_i - p_i \times \log_2(p_i)

where the sum is over all possible transitions, and \( p_i \) is the probability of the \( i \)th transition across all songs (Gil and Slater 2000; Sakata and Brainard 2006; Sakata et al. 2008). Branch points with transitions that are more variable (i.e., closer to uniform transition probabilities) have higher transition entropies. Sequences in which the dominant transition was produced on \( \geq 95\% \) of the trials were considered stereotyped sequences and were not included in the sequence analysis. Instances in which song was terminated immediately after the branch point were treated as song interruptions and not included in the calculation of entropy.

In principle, a change in sequence transitions can occur without a corresponding change in transition entropy. For example, a branch point with two possible transitions, a and b, could have transition probabilities of 0.25 and 0.75, respectively, under one condition and transition probabilities of 0.75 and 0.25, respectively, under another condition. Whereas there is clearly a change in the transition probabilities across conditions, the transition entropy would be 0.81 for both conditions. Consequently, feedback perturbation could lead to differential changes to syllable sequencing across UD and FD song that are not manifest as changes to transition entropy. To analyze this possibility, we calculated, for UD and FD songs separately, the average difference in transition probabilities between control and altered feedback trials. For this calculation, we computed the absolute difference in transition probabilities across control and altered feedback trials for each transition at the branch point and then calculated the average of these differences.

To quantify changes to song tempo caused by feedback alteration, we first determined syllable boundaries using an automated, amplitude-based segmentation algorithm (Matlab). Durations of specified sequences were measured from the start of the targeted syllable to the start of the first syllable after the termination of the feedback perturbation (Sakata and Brainard 2006, 2008). We measured sequence durations for only the most prevalent transition within the branch point sequence.

The termination of song caused by feedback perturbation precludes the analysis of features such as syllable sequencing and timing. We capitalized on the fact that the frequency of song interruptions diminishes with repeated exposure to feedback perturbations (see Sakata and Brainard 2006) and exposed 11 birds to the altered feedback paradigm for at least 1 day before the experimental session; this allowed us to more readily analyze changes to syllable sequencing during the experimental session. During these exposure sessions, the birds remained in isolation and produced only UD song. For all song features, prior exposure to feedback perturbations did not affect how social context modulated the degree to which feedback perturbation influenced song control, so the data for birds with and without prior altered feedback sessions were combined in the analysis.

Statistical analysis

In this experiment, we explicitly examined how the social context in which song is produced modulates the degree to which perturbations of auditory feedback influenced song control. Therefore for each song feature (song interruptions, syllable sequencing, and song tempo), we compared the magnitude of change caused by feedback perturbation between UD and FD song using a paired t-test.

For the analysis of song interruptions, we calculated the percent of altered feedback trials in which feedback perturbation caused song interruptions and compared the magnitude of this effect between UD and FD songs. We restricted our analysis of song interruptions to sequences in which \( \geq 10 \) trials were obtained for both control and altered feedback conditions within UD and FD song. Using this criterion, 28 sequences from the 15 birds were included in the analysis. Median sample sizes for control and altered feedback conditions were 44 and 37 trials, respectively (range: control, 11–219; altered feedback, 10–198).

For the analysis of syllable sequencing, we summarized the effect of feedback perturbation both as the percent change in transition entropy from control to altered feedback trials and as the mean change in transition probabilities from control to altered feedback trials. We compared the magnitude of these effects across UD and FD songs for each branch point. We restricted our analysis to branch point sequences in which there were \( \geq 15 \) transitions for both control and altered feedback conditions within UD and FD song. Using this criterion, we analyzed sequence changes for 14 branch point sequences from 12 birds. The number of birds included in the analysis of syllable sequencing is less than that used for the analysis of song interruption because, for three birds, feedback perturbation resulted in too many interruptions and too few song continuations for analysis. Median sample sizes for control and altered feedback conditions were, respectively, 49 and 42 trials (range: control, 18–211; altered feedback, 18–184).

For the analysis of song tempo, we summarized the effect of feedback perturbation as the percent change in sequence duration from control to altered feedback trials. We compared the magnitude of this effect across UD and FD song. We restricted our analysis to datasets in which there were \( \geq 10 \) examples of a defined sequence (i.e., the most prevalent transition of the branch point) for both control and altered feedback conditions within UD and FD song. Using this criterion, we analyzed 11 sequences from nine birds. Median sample sizes for control and altered feedback conditions were, respectively, 46 and 34 trials (range: control, 13–117; altered feedback, 11–99).

For all song features, we also analyzed the effect of social context on a per individual level. This set of analyses was incorporated to ensure that the observed results were not a product of pseudoreplication (i.e., in some instances, more than 1 example for a song feature was analyzed for an individual). For these analyses, we calculated, for each feature, the average effect for each sequence weighted by the prevalence of that sequence relative to all other sequences for that individual (i.e., weighted average). In all instances, the analysis at the individual level corroborated the analysis at the sequence level.

Unless otherwise indicated, the criterion for significance for all tests was set at \( \alpha = 0.05 \) (2-tailed). All statistical analyses were conducted using JMP 5.0.1 (SAS, Cary, NC) for the Macintosh.

RESULTS

In this study, we assessed how the social context in which song was produced affected the degree to which perturbations of auditory feedback influenced song control. To this end, we used a computerized system to acutely perturb auditory feedback at precise locations in song (Fig. 1; Sakata and Brainard 2006, 2008; Tumer and Brainard 2007) and measured the degree to which song organization was affected. Trials in which auditory feedback was perturbed (altered feedback trials) were randomly interleaved with trials with normal feedback (control trials), thereby allowing for the analysis of moment-by-moment feedback contributions to vocal motor control. Furthermore, we elicited production of UD and FD song in an interleaved manner by presenting females at regularly spaced intervals during the experimental period. This ensured that any slow and uncontrolled variation in susceptibility to the influence of feedback perturbation (such as circadian changes in song structure) would contribute equally to effects observed in both social contexts. We specifically analyzed how social context modulated the degree to which feedback perturbations caused song interruptions, changes to song tempo, and changes to syllable sequencing.
Song interruptions

Superposition of an extraneous syllable during ongoing song can cause abrupt terminations of song (song interruptions; Cynx and von Rad 2001; Sakata and Brainard 2006). An example of a song interruption caused by localized feedback perturbation is depicted in Fig. 2A. In this example, we targeted the syllable c in the sequence abbc... and played back a delayed version of c during altered feedback trials. The top spectrogram depicts an example in which song was terminated after feedback perturbation (song interruption), whereas the bottom spectrogram depicts an example in which song continued after feedback perturbation (song continuation). In this experiment, feedback perturbation during UD song led to an increase in song interruptions [Fig. 2B; undirected; control (C) vs. altered feedback (AF)]. Similarly, across all experiments, there were significantly more song interruptions during altered feedback trials than during control trials when birds produced UD song (paired t-test; P < 0.0001), consistent with data from a previous report (Sakata and Brainard 2006).

The practice versus performance framework predicts that the influence of auditory feedback will be attenuated when birds produce FD song. Consequently, we anticipated that the degree to which feedback perturbations caused song interruptions would be reduced when males produced FD song relative to UD song. The results from the experiment depicted in Fig. 2 are consistent with this prediction: feedback perturbation caused fewer song interruptions when the bird produced FD song than when he produced UD song (Fig. 2B). In this experiment, feedback perturbation caused song interruptions on 29.8 ± 6.1% (SE; 17 of 57) of altered feedback trials for UD song but on only 12.0 ± 4.3% (3 of 25) of altered feedback trials for FD song (song was never terminated after the c on control trials with normal feedback).

Such a difference between UD and FD songs in the degree to which feedback perturbation caused song interruptions was consistent across experiments. For 26 of the 28 experiments, perturbation of feedback caused more song interruptions during UD song than during FD song. Across all experiments, the percent of altered feedback trials with song interruptions was significantly smaller for FD song than for UD song (Fig. 2C; percent of altered feedback trials with interruptions: FD = 15.5 ± 2.8%; UD = 36.7 ± 4.2%; per sequence: t27 = 6.3, P < 0.0001; per bird: t14 = 6.5, P < 0.0001). These data indicate that the effect of auditory feedback perturbations on song production was attenuated when birds produced FD song relative to when they produced UD song.

Song tempo

Real-time manipulations of auditory feedback can affect the tempo at which song is produced (Sakata and Brainard 2006, 2008). An example of changes to song tempo caused by feedback perturbation is shown in Fig. 3A. In this experiment...
ments, feedback perturbation led to a significant increase in sequence durations affected sequence durations would be attenuated when confirming the efficacy of our feedback manipulation.

We anticipated that the degree to which feedback perturbations affected sequence durations would be attenuated when birds produced FD song. The results from the experiment depicted in Fig. 3B are consistent with this prediction. Whereas feedback perturbation during UD song caused the duration of the sequence cde to significantly increase (t-test; $P < 0.05$), feedback perturbation during FD song caused a nonsignificant increase in the duration of cde (Fig. 3B, bottom; 1.1% increase for FD song vs. 3.4% increase for UD song). Across all sequences analyzed, the effect of feedback perturbation on song tempo was significant for both UD and FD song (paired t-test; $P < 0.01$ for both UD and FD song), indicating that feedback perturbations were influential in both social contexts. However, the effect of feedback perturbations on song tempo was smaller during FD song than during UD song for every sequence measured (Fig. 3C). Consequently, across all experiments, the magnitude of tempo change was significantly smaller for FD song than for UD song (3.1% for UD song vs. 5.4% for FD song; per sequence: $t_{10} = 5.8$, $P = 0.0002$; per bird: $t_b = 6.1$, $P = 0.0003$). This indicates that, as for song interruptions, the effect of auditory feedback perturbations on song tempo was attenuated during FD song relative to UD song.

Syllable sequencing

Manipulations of auditory feedback acutely influence syllable sequencing at branch point sequences, and this influence can be summarized as an increase in the variability of sequence transitions, or transition entropy (see METHODS; Sakata and Brainard 2006). Figure 4A shows an example of how feedback perturbation affects transition entropy for UD and FD song. Consistent with a previous report (Sakata and Brainard 2006), feedback perturbation caused an increase in transition entropy when this bird produced UD song (Fig. 4A; 54% increase in entropy), and across all experiments, transition entropy was significantly higher on altered feedback trials than on control trials for UD song (paired t-test; $P = 0.0025$).

**FIG. 3.** Social context modulated the degree to which feedback perturbations caused changes to song tempo. A: an experiment in which the syllable c was targeted. On altered feedback trials, a delayed version of the syllable c was played back to the bird during ongoing song (same example as in Fig. 2A). Plotted are spectrograms of a control trial (top) and altered feedback trial (bottom) (scale bar = 100 ms); both plots are aligned by the onset of syllable c. The onsets of the c and the 2nd e are marked with yellow dashed lines for the control trial and with white dashed lines for the altered feedback trial. During the altered feedback trial, the onset of the 2nd e was delayed relative to the control trial, leading to a longer duration for the sequence cde for the altered feedback trial. B: plotted are the distributions of sequence durations for cde for all control (C: blue) and altered feedback (AF: yellow) trials during UD and FD song for the experiment depicted in A. Three differences are evident. First, sequence durations were on average longer during altered feedback trials than during control trials. Second, sequence durations were on average shorter for FD song than UD song. Third, the magnitude of increase in sequence duration caused by feedback perturbation was smaller for FD song (1.1% increase) than for UD song (3.4% increase; $P < 0.05$). Data are means ± SE (ms). Blue triangles indicate means for control trials, and yellow triangles indicate means for altered feedback trials. C: across experiments, we summarized the effect of feedback perturbation as the percent change in sequence duration between control and altered feedback trials (positive values indicate increases in sequence duration caused by feedback perturbations). For both UD and FD song, this percent change was always positive, indicating that feedback perturbation significantly increased sequence durations. Moreover, across all experiments, the magnitude of time change caused by feedback perturbation was significantly smaller for FD song than for UD song ($P = 0.0002$). These data indicate that the effect of auditory feedback perturbations was attenuated when males sang to females relative to when males sang in isolation.
We anticipated that feedback perturbations would cause smaller changes to syllable sequencing during FD song than during UD song. In the experiment summarized in Fig. 4A, feedback alteration caused only a modest 4% increase in transition entropy (from 0.871 to 0.904) for FD song, relative to a 54% increase (from 0.991 to 1.522) during UD song. Hence, in this case, there was indeed an attenuation of the influence of feedback perturbation on syllable sequencing for FD song relative to UD song. However, this pattern of difference was not consistent across experiments (Fig. 4B). Consequently, there was not a significant difference between UD and FD song in the degree to which feedback perturbation affected transition entropy.

To potentially identify sequence changes not captured by changes to transition entropy, we also quantified the mean (absolute) change in transition probabilities (see METHODS). In the example provided in Fig. 4A, feedback perturbation caused a mean (absolute) change in transition probabilities of 0.05 vs. 0.23, respectively) during FD song than during UD song. However, just as for transition entropy, this pattern of difference was not consistent across experiments, and consequently, there was not a significant effect of social context on the degree to which feedback perturbation affected transition probabilities. These data suggest that, in contrast to song interruptions and tempo, we did not detect a significant social modulation of the effect of auditory feedback perturbations on syllable sequencing.

**Contributions of song stereotypy to the influence of feedback perturbations**

FD songs are structurally distinct from UD songs in that both syllable structure and sequencing are more stereotyped during
FD song (Kao and Brainard 2006; Kao et al. 2005; Olvecky et al. 2005; Sakata et al. 2008; Sossinka and Böchner 1980). In a previous study, we reported a relationship between the stereotypy of syllable sequencing and the sensitivity to feedback perturbation (Sakata and Brainard 2006); we observed that the degree to which auditory feedback perturbations affected song tempo was significantly smaller when feedback was perturbed during stereotyped sequences than during variable sequences. This is consistent with the possibility that differences in the structure of song (i.e., sequence stereotypy) contribute to differences in the degree to which song can be disrupted (by perturbations of feedback). Correspondingly, because FD song is more stereotyped than UD song, some of the observed differences in the influence of feedback perturbation between social contexts might be a consequence of differences in song structure.

To assess this possibility, we first examined the relationship between sequence stereotypy (as measured by transition entropy) and the magnitude of the effects of feedback perturbation. We focused on features in which the effects of feedback perturbation were significantly different between UD and FD song (song interruptions and tempo). We analyzed whether the effect of social context remained significant after controlling for context-dependent differences in sequence variability. To this end, we calculated the residuals from the regression of the effects of altered feedback (on song interruptions and tempo) against the stereotypy of song structure (transition entropy). These residuals reflect the effects of feedback perturbation that cannot be accounted for by differences in song stereotypy. We therefore compared the residuals for UD and FD song to determine the degree to which context-dependent differences in the effects of feedback perturbation persisted after taking into account context-dependent differences in song stereotypy.

For song interruptions, context-dependent differences in song stereotypy could not account for context-dependent differences in the effects of feedback perturbation. Across all experiments, the prevalence of song interruptions during altered feedback trials was not correlated with the transition entropy at the corresponding branch points (Fig. 5A). The slope of the regression between song interruptions and transition entropy was not significantly different from zero ($\beta = 7.5 \pm 8.3$; $P = 0.3701$). Consistent with this finding, the social context in which song was produced continued to have a significant effect on the prevalence of song interruptions in response to feedback perturbation after taking into account variation in song stereotypy (Fig. 5B; paired $t$-test: $P = 0.0004$). These analyses indicate that variation in the structural stability of song (as quantified by transition entropy) cannot
account for the reduced effect of feedback perturbation on song interruptions for FD song relative to UD song.

In contrast, we found that there was a significant relationship between the effects of feedback perturbation on song tempo and transition entropy. The slope of the regression between the percent change in sequence duration caused by feedback perturbation and transition entropy was significantly greater than zero ($\beta = 7.2 \pm 2.8$; $P = 0.0223$), indicating that smaller effects of feedback perturbation on song tempo were found at more stereotyped sequences (i.e., those with lower transition entropy). This positive relationship was observed for both UD and FD song and is consistent with a previous finding that feedback perturbations within stereotyped sequences (transition entropy $> 0$) cause smaller changes to song tempo than feedback perturbations at branch points (transition entropy $< 0$; Sakata and Brainard 2006). Moreover, the residuals from this regression were not significantly different between UD and FD song (paired $t$-test: $P = 0.1629$). This suggests that context-dependent differences in the effects of feedback perturbation on song tempo may be the result of context-dependent differences in the structural stability of song.

**Discussion**

Sensory feedback is important for behavioral control for a wide range of behaviors. Previous studies have shown that factors such as the strength of learning and behavioral variability can influence the degree to which sensory feedback contributes to behavioral control. For example, sensory feedback is more influential for behavioral control when learning or practicing a new motor skill than after the motor skill is well learned (Adams 1971; Borden 1979; Howell and Dworzynski 2001; Howell et al. 2006; Pratt et al. 1994; Schmidt 1975; Van Borsel et al. 2005; Webster and Dorman 2001). Furthermore, behaviors that are more stereotyped in their expression are often more “feed-forward” and less affected by perturbations of feedback (Berridge and Fentress 1987; Sakata and Brainard 2006). Here, we analyzed how social context, a variable that influences the stereotypy of vocal behavior in songbirds (Kao and Brainard 2006; Kao et al. 2005; Őlveczky et al. 2005; Sakata et al. 2008; Sossinka and Böhner 1980; Teramitsu and White 2006; Woolley and Doupe 2008), modulates the degree to which perturbations of auditory feedback affect song control in the Bengalese finch.

Auditory feedback is critical for the acquisition, maintenance, and moment-by-moment control of song in songbirds (Brainard and Doupe 2000a; Cynx and von Rad 2001; Konishi 2004; Kozhevnikov and Fee 2007; Leonard and Konishi 1999; Lombardino and Nottebohm 2000; Nordeen and Nordeen 1992; Okanoya and Yamaguchi 1997; Sakata and Brainard 2006, 2008; Sober and Brainard 2005; Tumer and Brainard 2007; Woolley and Rubel 1997). Based on previous behavioral and neural observations, we predicted that the contribution of auditory feedback to song control might be rapidly modulated by social context. One prior study reported that the degree to which background noise levels influence the loudness of vocalizations is attenuated when birds produce songs to females relative to when birds produce songs in isolation (Kobayashi and Okanoya 2003). Moreover, previous studies have shown that bursting activity and immediate early gene expression in a circuit crucial for feedback-dependent song plasticity are reduced when males produce FD song relative to when they produce UD song (Hessler and Doupe 1999; Jarvis et al. 1998; Kao et al. 2008). Because bursting activity and the expression of immediate early genes have been hypothesized to enhance plasticity, these findings have led to the hypothesis that FD song constitutes a form of vocal performance wherein plasticity mechanisms are less engaged, whereas UD song represents a form of vocal practice wherein plasticity mechanisms are more engaged (Jarvis et al. 1998; Kao and Brainard 2006; Kao et al. 2005; Sakata et al. 2008). Because song plasticity is critically dependent on auditory feedback, this practice versus performance framework predicts that FD songs should show a decreased dependence on auditory feedback.

In this study, we directly tested this prediction. We compared the degree to which real-time perturbations of auditory feedback caused acute vocal motor changes to FD and UD song in adult Bengalese finches. Previous studies that document the acute effects of localized feedback perturbations on song control in the Bengalese finch have only examined the effect of feedback perturbation as birds produced UD song (Sakata and Brainard 2006, 2008). Consistent with the practice versus performance framework, in this study, we found that feedback perturbation caused smaller changes to FD song than to UD song. Specifically, we found that feedback perturbations caused fewer song interruptions (Fig. 2) and smaller changes to song tempo (Fig. 3) during FD song than during UD song. For both song interruptions and song tempo, the degree to which feedback perturbation altered song was approximately half as great for FD song as for UD song. Because we analyzed only FD songs that were produced within 1 min of the introduction of the female (see Methods), these data indicate that the neural circuits activated during courtship behavior can rapidly modulate the contribution of auditory feedback signals to vocal motor control. Correspondingly, these data support the possibility that the capacity for auditory feedback-based song plasticity is reduced during courtship (Jarvis et al. 1998; Kao and Brainard 2006; Kao et al. 2008; Williams et al. 2003).

Our data show that the social context in which song is produced modulates the degree to which auditory feedback perturbations influence activity in premotor circuits. Song is controlled by neurons in the vocal motor pathway, which includes the premotor nuclei HVC (proper name) and RA (the robust nucleus of the arcopallium; Reiner et al. 2004). Neurons in this pathway encode the motor commands for song as well as information about auditory feedback. For example, both song tempo and the starting and stopping of song are regulated by activity in the vocal motor pathway. HVC is required to initiate and produce learned song, and stimulation of neurons in HVC and RA can cause premature terminations of song (Ashmore et al. 2005; Nottebohm et al. 1976; Vu et al. 1994; Wang et al. 2008). Neurons in HVC that project to RA fire at precise times during song (Hahnloser et al. 2002; Kozhevnikov and Fee 2007), leading to the hypothesis that this neuronal population encodes and controls temporal aspects of song (reviewed in Fee et al. 2004; Long and Fee 2008). Furthermore, neurons in HVC are sensitive to auditory perturbations during song production, suggesting that HVC neurons integrate auditory feedback signals with song premotor activity (Sakata and Brainard 2008). Because we observed that the magnitude of song changes elicited by perturbations of auditory feedback was attenuated during FD song, our results indicate that feed-
back perturbations cause smaller changes to activity in the vocal motor pathway when birds produce FD song than when they produce UD song.

There are a number of ways in which social context could influence the degree to which perturbations of auditory feedback affect activity in vocal motor circuitry. For example, social context could modulate the sensitivity of auditory neurons to auditory feedback. The auditory responses of neurons in HVC and RA are influenced by activity within primary and secondary auditory areas (reviewed in Mooney 2004). Although the sensitivity of neurons in the ascending auditory pathway to auditory feedback has not been documented in adult songbirds, it has recently been shown that neurons in primary auditory areas (e.g., field L) of juvenile zebra finches are sensitive to auditory feedback perturbations during vocal production (Keller and Hahnloser 2009). Therefore it is possible that social context affects the sensitivity of neurons in primary auditory areas to perturbations of auditory feedback.

Social context might not only affect auditory processing but also the processing of other sensory signals. If this were the case, nonauditory perturbations (e.g., visual flashes or proprioceptive manipulations: Cynx 1990; Franz and Goller 2002; Riebel and Todt 1997; Seki et al. 2008; Suthers et al. 2002) would also differentially affect the control of UD and FD song, a possibility that remains to be tested. Midbrain areas like the ventral tegmental area and substantia nigra project to the vocal motor pathway and respond to salient, unexpected stimuli of a number of different modalities (Appeltants et al. 2000; Ritters and Alger 2004; reviewed in Schultz and Dickinson 2000). Consequently, it is possible that the effects of social context could be mediated by modulation in the sensitivity of midbrain neurons to salient stimuli.

In addition to the possibility that context-dependent changes in the degree to which auditory feedback perturbations affect song control arise because of an attenuation of the neural signals that represent sensory feedback, context-dependent changes could reflect modulation of the susceptibility of the vocal premotor pathway to disruption. Our results lend some support to this hypothesis because the magnitude of tempo change caused by feedback perturbation within both UD and FD song was significantly correlated with sequence stereotypy, a measure of the stability of the motor program (Fig. 5C) (see also Sakata and Brainard 2006). A reduction in the susceptibility of the motor program to disruption could be caused by a dampening of the effect of sensory information impinging on neurons in the vocal motor pathway or an increase in the strength of the connections within the vocal motor pathway. Both types of change could be mediated by neuromodulatory inputs. For example, the vocal motor pathway receives catecholaminergic inputs from midbrain areas that are active during the expression of social behaviors (Appeltants et al. 2000; Ball et al. 2003; Bharati and Goodson 2006; Castelino and Ball 2005; Castelino et al. 2007; Hara et al. 2007; Harding et al. 1998; Maney and Ball 2003; Sasaki et al. 2006; Soha et al. 1996; Yanagihara and Hessler 2006), and such inputs could be more active during the production of FD song than during UD song, resulting in higher levels of catecholamines in HVC and/or RA during FD song. High levels of norepinephrine in HVC or in areas that project to HVC dampen the auditory responsiveness of HVC neurons to the bird’s own song (Cardin and Schmidt 2004; Dave et al. 1998). Furthermore, increased dopaminergic input has been found to increase the stereotypy and resistance to disruption of some motor behaviors (Berridge 1989; Berridge and Aldridge 2000; Berridge et al. 2005; Cromwell et al. 1998). Consequently, changes in the levels of norepinephrine or dopamine in HVC or RA could alter the degree to which perturbations of auditory feedback affect activity in the vocal motor pathway. Furthermore, the basal forebrain sends cholinergic inputs into HVC and RA, and these inputs have been found to modulate the auditory responsiveness of neurons in the vocal motor pathway (Li and Sakaguchi 1997; Shea and Margoliash 2003). Hence, these inputs might also be differentially active during the production of FD and UD song, leading to changes in the sensitivity of vocal motor neurons to auditory feedback perturbations.

Our results may provide insight into discrepancies across previous studies in the degree to which feedback perturbations caused behavioral and neural changes. Cynx and von Rad (2001) examined the effects of delayed auditory feedback in zebra finches producing FD song and did not report a significant effect of feedback perturbations on song tempo. Our findings indicate that a more significant slowing of song might have been observed if the effects of auditory feedback were studied during UD song. With regard to feedback signals in the brain, Sakata and Brainard (2008) observed significant and consistent responses to feedback perturbation in the vocal premotor nucleus HVC, but several other studies did not observe responses to feedback perturbation in HVC or other song system nuclei (Kozhevnikov and Fee 2007; Leonardo 2004; Prather et al. 2008). Sakata and Brainard (2008) recorded neural activity in response to feedback perturbations only as birds produced UD song, whereas the other studies recorded activity primarily when males produced FD song or song in response to song playback or did not report the social context in which neural activity was recorded. Consequently, differences in the actual or perceived social environment in which the effects of feedback perturbation were studied, in addition to other experimental differences (including the subject species and the targeting of parts of songs with stereotyped or variable sequencing), may have contributed to differences in the effects of feedback perturbation observed across previous studies.

Whereas we observed a strong attenuation of the effect of feedback perturbation on song tempo and interruptions during FD song, the degree to which feedback perturbations affected syllable sequencing was not significantly modulated by social context (Fig. 4). This was unexpected because, like song tempo and the frequency of song interruptions, syllable sequencing is influenced by auditory feedback, social context, and activity in HVC and RA (Ashmore et al. 2005; Hampton et al. 2009; Sakata and Brainard 2006, 2008; Sakata et al. 2008; Sossinka and Böhner 1980; Vu et al. 1994; Wang et al. 2008; Yu and Margoliash 1996). Consequently, we anticipated that changes to social context would similarly affect the sensitivity of each of these song features to perturbations of auditory feedback. The lack of a significant social modulation of the effect of feedback perturbation for syllable sequencing might simply reflect a lack of sensitivity in our experimental design, although our data were sufficient to detect independent effects of feedback perturbation (increase in transition entropy) and of social context (decrease in transition entropy) that were consistent with previous studies (data not shown: Sakata and Brainard 2006; Sakata et al. 2008). The variation in the effects
of social context across song features, however, does suggest that social context does not globally affect attention and supports the possibility that social context influences behavior through multiple distinct processes (see also Hampton et al. 2009).

In summary, our results indicate that the degree to which perturbations of auditory feedback affect vocal motor control can be rapidly modulated by social context and support the notion that feedback-based plasticity mechanisms could be dampened during the production of courtship behaviors. These data are consistent with a previous study documenting that the influence of background noise levels on vocal amplitude (i.e., Lombard effect) is attenuated during FD song relative to UD song (Kobayashi and Okanoya 2003) and extend the social modulation of feedback to the real-time control of song tempo and song interruptions. The social modulation of acute influences of auditory feedback on song supports the possibility that feedback-based song plasticity might similarly be modulated by social context. This possibility is hinted at by a previous study that showed a trend for attenuated plasticity after syriegal deafferentation when male zebra finches were housed next to females relative to when they were housed in isolation (Williams et al. 2003). Experiments that further test the social modulation of feedback-dependent song control and plasticity should shed light on the interactions between the circuits controlling the expression of social behaviors and circuits regulating vocal learning and control.

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