Social Modulation of Sequence and Syllable Variability in Adult Birdsong

Jon T. Sakata, Cara M. Hampton, and Michael S. Brainard
Keck Center for Integrative Neuroscience, University of California, San Francisco, California
Submitted 28 November 2007; accepted in final form 23 January 2008

INTRODUCTION

Variability is inherent to the expression of all behaviors, but its function and regulation have received little attention. In the context of well-learned skills, such variability is often construed as biological “noise” that is below the threshold for behavioral importance or that the nervous system is unable to eliminate. However, theoretical studies suggest that trial-by-trial behavioral variability potentially plays an important role in learning: variation in motor output could reflect active “motor exploration” required for the reinforcement of motor commands that produce less desired outcomes and the weakening of motor commands that produce less desired outcomes (Doya and Sejnowski 2000; Sutton and Barto 1998). Understanding whether and how variability is regulated can lend insight into the importance of behavioral variability and its neural sources.

In many species of songbird, adult song, once learned, is extremely stereotyped from one rendition to the next, and there may be little detectable change in acoustic structure over periods of months or even years (reviewed in Brainard and Doupe 2002; Doupe and Kuhl 1999; Tchernichovski et al. 2001). Hence adult “crystallized” song provides an example of an extremely well-learned motor skill. Nevertheless, there is small but measurable residual variation in adult song. Recent studies in the zebra finch have provided support for the hypothesis that residual variability in adult song might reflect a form of motor exploration in which the nervous system actively generates variation as a part of vocal practice required to continuously maintain and optimize song (Jarvis et al. 1998; Kao and Brainard 2006; Kao et al. 2005; Ölveczky et al. 2005).

Support for the idea that a component of adult song variability might be actively generated as part of vocal practice derives primarily from behavioral and neural investigations of zebra finch song. At a behavioral level, it has been observed that adult male zebra finches subtly alter the variability of their songs between social contexts in which males sing alone (“undirected” or UD song) and those in which they sing to females (“female-directed” or FD song) (Kao and Brainard 2006; Kao et al. 2005). The acoustic structure of individual song elements, or “syllables,” is consistently more variable from one rendition to the next for UD song than for FD song. At a neural level, several lines of evidence suggest that the excess variability present in UD song may be actively generated by the anterior forebrain pathway (AFP) (Kao and Brainard 2006; Kao et al. 2005; Ölveczky et al. 2005), an avian homologue of a cortical-basal ganglia circuit (Perkel 2004; Reiner et al. 2004). This circuit specifically projects to premotor nuclei and is required for normal song learning and adult song plasticity (Bottjer et al. 1984; reviewed in Brainard 2004; Brainard and Doupe 2000; Scharff and Nottebohm 1991; Williams and Mehta 1999). Neural activity within the AFP is greater and more variable for UD song than FD song, suggesting that signals arising from the AFP might actively drive variability in UD song (Hessler and Doupe 1999; Jarvis et al. 1998; Kao et al. 2005). Artificial manipulation of activity arising from the AFP further supports this possibility. Lesions of the lateral nucleus of the anterior nidopallium (LMAN), the output nucleus of the AFP, prevent the context-dependent modulation of syllable variability by reducing the variability present in UD song to the level present in FD song (Kao and Brainard 2006). Conversely, introduction of variable neural activity into LMAN, by microstimulation during singing, can drive increased variability in syllable structure (Kao et al. 2005). Hence signals from LMAN are both necessary and sufficient to account for the increased variability in syllable structure in UD song relative to FD song.
Collectively, these data are consistent with a model in which, despite the great stereotypy of adult song, there is nevertheless a subtle but active regulation of song variability. More specifically, it has been hypothesized that brain and behavior switch between a state of vocal “practice” (during UD song), in which variation is actively introduced to premotor song structures, to a state of “performance” (during FD song), in which neural sources of variability are reduced to emphasize features of the current “best” song as part of effective courtship (Jarvis et al. 1998; Kao and Brainard 2006). This practice versus performance hypothesis has been systematically investigated only in the zebra finch and derives almost exclusively from measurements of the modulation of the acoustic structure of individual song syllables. The active regulation of syllable sequencing, a hierarchically distinct aspect of song organization from syllable structure, has not been systematically investigated in part because the sequencing of syllables in adult zebra finch song is often invariant (Kao and Brainard 2006; Zevin et al. 2004; but see also 2 birds in Sossinka and Böhner 1980). Current models of song production suggest that separate components of song premotor circuitry contribute differentially to the sequencing versus structure of syllables (reviewed in Fee et al. 2004; Hahnloser et al. 2002; Margoliash 1997; Vu et al. 1994; Yu and Margoliash 1996), and, consequently, syllable sequencing may be subject to different forms of regulation from syllable structure.

Here we take advantage of the intrinsic variability of syllable sequencing in another songbird, the Bengalese finch, to investigate whether syllable sequencing is subject to active modulation in adult songbirds. Bengalese finches, like zebra finches, produce an adult song that remains stable over time (Clayton 1987; Immelmann 1969). However, unlike zebra finch song, the songs of adult Bengalese finches retain a significant degree of moment-by-moment variability in the sequencing of syllables (reviewed in Okanoya 2004). Hence, it is possible to quantify the variability of syllable sequencing in adult Bengalese finch song as well as the modulation of that variability in response to alterations of social context. We report that in addition to the forms of song modulation previously observed in the zebra finch, there is a rapid social modulation of syllable sequencing in adult Bengalese finch song. The nature of this modulation is analogous to that previously reported for syllable structure with greater variability in sequencing present in UD song than in FD song. Hence, even in the crystallized songs of adult birds, there is nevertheless a capacity for active and rapid modulation of multiple, hierarchically distinct song features.

**Methods**

**Animals**

Adult Bengalese finch males (n = 19; range: 4–18 mo; median = 6 mo) were raised in our colony. Birds were housed with their parents and siblings until ≥60 days of age, then housed with other males on a 14L:10D photoperiod. Birds were isolated and housed individually in a sound-attenuating chamber (Acoustic Systems, Austin, Texas) for ≥1 day prior to testing, and food and water were provided ad libitum. 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).

**Data collection**

Sound was recorded using an omnidirectional microphone (Countryman Associates, Menlo Park, CA) positioned above the male’s cage. A computerized, song-activated recording system was used to detect and digitize song. Observer, A. Leonardo, Caltech; C. Roddey, UCSF; digitized at 32 kHz; or Sound Analysis Pro v.1.04 (http://offer.sci.ccny.cuny.edu/html/sound_analysis.html); digitized at 44.1 kHz. Recorded songs were digitally filtered at 0.3–8 kHz for off-line analysis using software written in the Matlab programming language (The MathWorks, Natick, MA).

Undirected songs are produced in isolation, whereas FD songs are produced during courtship interactions with females. During the day of the experiment, FD song was elicited by placing a cage with a female adjacent to the experimental male’s cage. As in the zebra finch, FD songs of Bengalese finches are readily distinguishable from UD songs because they are produced after a male approaches or faces another individual, are accompanied by a courtship dance (e.g., pivoting body from side to side), and are associated with the fluffing of the male’s plumage (Morriss 1954; Zann 1996). Behavior of birds was monitored by experimenters either remotely by video camera or through a small observation window in the sound boxes, and only songs that were accompanied by at least two of the preceding 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 <2 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 following exposure to a female (Hessler and Doupe 1999; Kao and Brainard 2006). The median interval between exposures to females was 5 min (range: 2–19 min). This allowed for the collection of UD song between exposures to females. 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 rapid changes to song by social context. Although our experiment was designed to collect UD and FD song in an interleaved manner, males did not always sing UD song between exposures to a female. Therefore ≥30 min of UD songs were also recorded before the first exposure and after the last exposure to a female.

**Song parameters and definitions**

An example of a Bengalese finch song is provided in Fig. 1. For purposes of description and analysis, we use the term syllable to refer to individual acoustic elements of Bengalese finch song that are separated from each other by ≥5 ms of silence (Okanoya and Yamaguchi 1997). Syllables that are simple in structure and repeated at the beginning of song are referred to as “introductory notes” (e.g., syllable ‘i’ in Fig. 1). Within song, syllables are organized into stereotyped or variable sequences, and syllables that are followed by variable transitions are referred to as “branch points” (e.g., syllable ‘a’ in Fig. 1). Some syllables are repeated a variable number of times and are referred to as “repeats” (e.g., syllable ‘f’ in Fig. 1). Following amplitude-based syllable segmentation (Matlab), we labeled syllables manually based on visual inspection of spectrograms and analyzed syllable and sequence differences across social contexts.

To analyze changes to the variability of syllable structure, we compared the fundamental frequency (FF) of syllables that had distinct and stable harmonic structure (see Kao et al. 2005). For each syllable we calculated the autocorrelation of a segment of the sound waveform. We focused on these syllables because the calculation of FF is less sensitive to changes in syllable duration (e.g., relative to syllables with frequency sweeps). The FF was defined as the distance, in Hertz, between the zero-offset peak and the highest peak in the autocorrelation function. Each example of a syllable was visually screened to ensure that only examples devoid of sound artifacts that could affect FF calculation (e.g., sound of movement, female calls in

---

**J Neurophysiol • VOL 99 • APRIL 2008 • www.jn.org**
background) were used in the analysis. To improve the resolution of frequency estimates, we performed a parabolic interpolation of the peak of the autocorrelation function (de Cheveigne’ and Kawahara 2002). We found that the FF of syllables varied from rendition to rendition and characterized this variation using the coefficient of variation (CV: 100*σ/μ).

To quantify changes to the variability of syllable sequencing, we analyzed changes in the probability of sequence transitions at branch points across UD and FD songs. An example of a branch point is presented in Fig. 1 in which the syllable ‘a’ could be followed by syllables ‘b’, ‘c’, ‘i’, or ‘l’.

![Transition Entropy](attachment:transition_entropy.png)  

**FIG. 1.** Organization of Bengalese finch song. A: spectrogram of an example of an undirected song from a Bengalese finch male. Power (gray scale) is plotted as a function of frequency and time. Above the spectrogram are distinct labels for each syllable to aid in sequence analysis. The song element ‘i’ is an introductory note, which predominantly occurs prior to song initiation but also can be produced within song. There are 13 distinct syllables for this male, and the syllable ‘m’ (from song in A) is repeated a variable number of times within song. B: oscillogram showing amplitude of the sound trace for the song in A. C: left: spectrogram of the syllable ‘m’; right: histogram depicting the variation in the fundamental frequency of the syllable across renditions of undirected song. D: transition diagram describing the variability in syllable sequencing for undirected songs from this male. Thicker arrows represent more prevalent transitions. Syllables such as ‘a’, ‘c’, ‘i’, and ‘n’ are branch points where the transitions following the syllable are variable as opposed to syllables ‘b’, ‘d’, ‘e’, ‘g’, ‘h’, ‘l’, ‘m’, ‘o’, and ‘p’ where the transitions are stereotyped.

The variability in syllable sequencing was quantified using the transition entropy:

$$\text{transition entropy} = \sum -p_i \cdot \log_2(p_i)$$

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). Branch points with transitions that are more variable (i.e., closer to uniform probability) have higher transition entropy scores. Sequences in which the dominant transition occurred >95% of the time were not considered branch points. Instances in which song was terminated immediately following the branch point were not included in the calculation of entropy. Repeated syllables can also be construed as branch points (i.e., repeat syllable or transition to a different syllable), but we analyzed the entropy of repeats separately from other branch points. Context-dependent sequence changes within stereotyped sequences were not observed.

Additionally, we analyzed context-dependent differences in the number of introductory notes, song length, and song tempo, all features previously reported to be modulated by social context in zebra finches (Cooper and Goller 2006; Kao and Brainard 2006; Sossinka and Böhmer 1980). We counted the number of introductory notes preceding each song by starting at the first introductory note prior to the first (nonintroductory) syllable of the song and then counting backward in time until there was >500 ms of silence (Kao and Brainard 2006). If there were more than one type of introductory note, all were counted in the analysis. The song of one male did not have introductory notes and, hence, was not included in this analysis. Song length was defined as the interval from the onset of the first (nonintroductory) syllable to the offset of the last syllable of the song. We defined the end of a song as the offset of a syllable that was followed by >500 ms of silence. For comparisons of song tempo across social contexts, we measured the duration of matched sequences of syllables that occurred commonly. The interval from the onset of the first syllable to the onset of the last syllable in the sequence was computed. Onsets were selected as boundaries be-
cause the change in amplitude is sharper and less variable for onsets than for offsets, allowing for a more accurate estimate of duration.

Data analysis

Before all analyses, the distributions of data for all behavioral parameters were assessed for violations of normality using the Shapiro-Wilk W test, and only when distributions did not violate normality were parametric tests used. For comparisons within an individual, t-test were used to assess whether song parameters were significantly different between UD and FD song (see examples in RESULTS and figures).

The songs of many birds contained multiple distinct examples of a measured song feature (FF, transition entropy, song tempo). Songs from a single Bengalese finch could contain multiple distinct syllables with flat acoustic structure for FF measurement, multiple unique branch points, and multiple distinct sequences in which song tempo could be measured. Data for each unique example were recorded (e.g., FF for each unique syllable in a bird), and for each example, we calculated the percent change using the following formula

\[
\text{percent change} = 100 \times \frac{\mu_{\text{FD}} - \mu_{\text{UD}}}{\mu_{\text{UD}}}
\]

where \(\mu_{\text{FD}}\) and \(\mu_{\text{UD}}\) refer to the sample means for FD and UD songs, respectively. To avoid pseudoreplication in population analyses of social context, we computed the weighted average of the percent changes for males with multiple examples of a specific feature. The weighted average was computed using the following equation

\[
\text{percent change per male} = \frac{\Sigma \Delta_i \times n_i}{\Sigma n}
\]

where \(\Delta_i\) represents the percent change of the \(i\)th example of a feature, \(n_i\) represents the sample size for \(i\)th example, and \(\Sigma n\) represents the sum of \(n\)'s across all examples of that feature. For example, for a single male with two examples of branch points in his song, if there was a 4% decrease in entropy at a branch point that was sung 100 times across both contexts and a 2% decrease in entropy at a branch point that was produced 300 times across both contexts, the overall percent change in entropy would be a decrease of 2.5% (4%*100/400 + 2%*300/400 = 2.5%). Thereafter, we tested whether the mean of the weighted differences was significantly different from zero using a t-test or Wilcoxon signed-ranks test.

Paired t-tests were used to analyze differences in introductory notes and song length between UD and FD songs.

For all tests, we set \(\alpha = 0.05\) (2-tailed). Analyses were done using JMP 5.0.1 (SAS Institute, Cary, NC) for the Macintosh.

RESULTS

Organization of Bengalese finch song

As in many songbird species, songs of the Bengalese finch consist of both simple and complex song elements called ‘syllables’ the structure and sequencing of which are learned. Upon reaching sexual maturation, the song of an adult Bengalese finch is considered to be ‘crystallized’ because the composition of song remains stable across time (Clayton 1987; Immelmann 1969; Okanoya and Yamaguchi 1997; Woolley and Rubel 1997). Song is typically preceded by low-amplitude, spectrally simple song elements called introductory notes, and song itself is composed of a number of spectrally distinct syllables (typically 5–10) arranged into both stereotyped and variable sequences (Fig. 1, A and B). In the example provided in Fig. 1, there was one type of introductory note followed by 13 distinct syllables. Introductory notes can also be interspersed within song; in this example, the introductory note ‘i’ could be produced following the syllable ‘a’. The acoustic structure of syllables varies from rendition to rendition, and we characterized this variability by measuring the distribution of FFs of syllables with flat harmonic structure (Fig. 1C). The sequencing of syllables also varies from rendition to rendition, and we characterized this variability by measuring the probability of each possible transition over a large set of songs. This sequence variability can be illustrated using a “transition diagram” (Fig. 1D), where each node corresponds to a unique syllable and the thickness of arrows connecting each node reflects the probability of transitions between syllables. For this example, transitions from some syllables were stereotyped (e.g., syllables ‘b’, ‘d’, ‘e’, ‘g’, ‘h’, ‘l’, ‘m’, ‘o’, and ‘p’), whereas transitions from other syllables were variable (e.g., syllables ‘a’, ‘c’, ‘f’, and ‘n’). Sequences followed by variable transitions are referred to as branch points. For example, the syllable ‘a’ is a branch point that could be followed by the syllables ‘b’, ‘c’, ‘i’, and ‘l’. The syllable ‘f’ was repeated a variable number of times (4–6 times in this example), and we refer to these types of syllables as repeats. These types of variability in syllable structure and sequencing make adult Bengalese finches a useful model to study the modulation of vocal motor behavior.

Variability of syllable structure

To test the generality of previous observations in the zebra finch and to confirm that our manipulation of social context was effective in altering song, we first examined changes to the variability with which individual syllables were produced in the Bengalese finch. In the adult zebra finch, the structure of syllables is less variable during songs produced to females relative to songs produced for a male (Kao and Brainard 2006; Kao et al. 2005). Here we similarly found a dramatic reduction in the variability of syllable structure during FD song in the Bengalese finch. We quantified the variability in syllable structure using the coefficient of variation (CV) for FF of individual syllables (see METHODS). Figure 2A provides an example from an adult Bengalese finch from the type of syllable with clear harmonic structure that we measured. The songs of many birds contained multiple distinct examples of a measured song feature (FF, transition entropy, song tempo). Songs from a single Bengalese finch could contain multiple distinct syllables with flat acoustic structure for FF measurement, multiple unique branch points, and multiple distinct sequences in which song tempo could be measured. Data for each unique example were recorded (e.g., FF for each unique syllable in a bird), and for each example, we calculated the percent change using the following formula

\[
\text{percent change} = 100 \times \frac{\mu_{\text{FD}} - \mu_{\text{UD}}}{\mu_{\text{UD}}}
\]

where \(\mu_{\text{FD}}\) and \(\mu_{\text{UD}}\) refer to the sample means for FD and UD songs, respectively. To avoid pseudoreplication in population analyses of social context, we computed the weighted average of the percent changes for males with multiple examples of a specific feature. The weighted average was computed using the following equation

\[
\text{percent change per male} = \frac{\Sigma \Delta_i \times n_i}{\Sigma n}
\]

where \(\Delta_i\) represents the percent change of the \(i\)th example of a feature, \(n_i\) represents the sample size for \(i\)th example, and \(\Sigma n\) represents the sum of \(n\)'s across all examples of that feature. For example, for a single male with two examples of branch points in his song, if there was a 4% decrease in entropy at a branch point that was sung 100 times across both contexts and a 2% decrease in entropy at a branch point that was produced 300 times across both contexts, the overall percent change in entropy would be a decrease of 2.5% (4%*100/400 + 2%*300/400 = 2.5%). Thereafter, we tested whether the mean of the weighted differences was significantly different from zero using a t-test or Wilcoxon signed-ranks test.

Paired t-tests were used to analyze differences in introductory notes and song length between UD and FD songs.

For all tests, we set \(\alpha = 0.05\) (2-tailed). Analyses were done using JMP 5.0.1 (SAS Institute, Cary, NC) for the Macintosh.
Overall, mean FF was greater during the production of FD song for 14 of 20 measured syllables, and this difference was significant for 10 of the 14 examples (t-test, \( P < 0.05 \)). Across individuals, FF was significantly increased during FD song (mean \( \pm SE: 0.46 \pm 0.2\% \); t-test: \( t_{12} = 2.34, P = 0.0371 \); Fig. 2, E and F).

**Variability of syllable sequencing: branch points**

Branch points provide an opportunity to investigate whether variability in syllable sequencing is actively regulated. By definition, branch points reflect the locations within song at which syllable transitions are variable (Fig. 1). Here we measured how this variability at branch points is influenced by social context. In our dataset, there were 35 examples of branch points in the songs of 16 Bengalese finches. Figure 3A illustrates each occurrence of the branch point 'ab' in one UD song (top) and one FD song (bottom) from an individual bird. For this branch point, FD song had more transitions to ab and fewer transitions to 'i' and 'ccc' than did UD song. The probability of each type of transition at this branch point is quantified in Fig. 3B. Transitions from 'ab' to the sequences 'ab', 'ccc', and 'i', respectively, were produced 36.1, 49.6, and 14.3% of the time during UD song (\( n = 252 \) transitions in 40 songs) and 66.4, 31.4, and 2.2% of the time during FD song (\( n = 223 \) transitions in 33 songs). For each branch point, we characterized the variability in sequence transitions by quantifying transition entropy (Gil and Slater 2000; Sakata and Brainard 2006); stereotyped sequences have entropy scores of zero, and increased variability translates into increased entropy (see METHODS). This example illustrates a case in which transition entropy was reduced by 27.4% during FD song relative to UD song. The reduced entropy indicates that the variability of sequencing at this branch point decreased during the production of FD song. Across the 35 examples of branch points, there was a decrease in transition entropy from UD song.
to FD song in 27 cases (Fig. 3C), and across individuals, the mean decrease in entropy from UD to FD song was significant (mean ± SE: −16.7 ± 3.7%; t-test: $t_{15} = 4.53$, $P = 0.0004$).

In principle, decreases in transition entropy could reflect the consequence of birds consistently “choosing” some types of transition more often at branch points. To examine this possibility, we analyzed whether social context had systematic influences for three types of transitions.

First, strings of repeated syllables are present in Bengalese finch song, and in songbird species such as the canary, such repeats can play an important role in social interactions (Kreutzer et al. 1999; Vallet and Kreutzer 1995; Vallet et al. 1998). Therefore, we asked whether UD and FD songs differed in the prevalence of transitions to repeats at branch points. In the example provided in Fig. 3A, the sequence ‘ab’ could be followed by repeats of the syllable ‘c’ or by other transitions. In this case, the prevalence of transitions to the repeated syllable ‘c’ decreased from 49.6% during UD song to 31.9% during FD song. Across the 10 examples (in 8 males) of branch points with repeats as possible transitions, there was not a consistent difference between UD and FD songs in the prevalence of transitions to repeats and, consequently, no quantitative difference between social contexts in the probability of such transitions (paired t-test: $t_9 = 0.06$, $P = 0.9543$).

Second, a fixed sequence of syllables is sometimes repeated following the branch point (e.g., transition from ‘ab’ to ‘ab’ in Fig. 3A). In our dataset, there were 14 examples (in 8 males) of branch points with such transitions, and in 10 cases, the frequency with which sequences were repeated was higher for FD song. Correspondingly, the proportion of times a sequence was repeated at these branch points was significantly greater during the production of FD song relative to UD song (paired t-test: $t_{13} = 3.05$, $P = 0.0093$).

**FIG. 3.** Effect of social context on transition entropy at branch points for Bengalese finch song. **A:** an example of the effect of social context on transition entropy. **Top:** a spectrogram of an example of UD song; **bottom:** an example of FD song. Arrows highlight instances of the branch point sequence ‘ab’. Black arrows indicate transitions from ‘ab’ to ‘ab’; gray arrows indicate transitions from ‘ab’ to the repeated syllable ‘c’; and white arrows indicate transitions from ‘ab’ to ‘i’. Scale bar = 1 s. **B:** transition probabilities during UD and FD songs for this male. **Left:** spectrograms of the 3 distinct transitions following the sequence ‘ab’. **Right:** summary of the percent of times the male transitioned to each of these possible sequences for UD and FD song as well as the transition entropies for the 35 branch points analyzed in 16 males. Values for 27 of the 35 branch points fall below the line of unity, indicating that overall transition entropy was reduced during FD song relative to UD song. **D:** distribution of percent change values (per male) for transition entropy. The mean was significantly less than 0 (mean ± SE: −16.7 ± 3.7%; t-test: $t_{15} = 4.53$, $P = 0.0004$).
Third, introductory notes can be produced within a song, and such syllables can potentially follow branch points. For example, in Fig. 3A, one of the potential transitions is to the introductory note ‘i’, and for this branch point, the prevalence of transitions to syllable ‘i’ was reduced from 14.3% during UD song to 2.2% during FD song. Across the 11 examples (in 7 males) of branch points with introductory notes as potential transitions, there were 10 cases in which the prevalence of transitions to introductory notes was reduced during FD song relative to UD song, and this reduction was significant (paired t-test: \( t_{10} = 2.56, P = 0.0285 \)). This suggests that birds bias transitions away from introductory notes during the production of FD song.

In summary, changes in the prevalence of transitions to introductory notes and the prevalence with which sequences were repeated at branch points contributed to the overall change in transition entropy across social contexts.

Variability of syllable sequencing: repeats

Repeated syllables can be considered nodes with variable sequence transitions, and, consequently, we applied the same analysis of transition entropy to repeats. In the example provided in Fig. 4A, the syllable ‘e’ could be followed by either the syllable ‘e’ or ‘f’. During UD song, the bird transitioned to ‘e’ and ‘f’, respectively, 88.4 and 11.6% of the time, whereas during FD the bird transitioned to ‘e’ and ‘f’, respectively, 90.5 and 9.5% of the time. This change in transition probability translated into a \(-13\%\) decrease in transition entropy from 0.518 to 0.451. For 19 of 21 repeated syllables in the songs of 14 Bengalese finches, transition entropy decreased from UD to FD song (Fig. 4B), and across all individuals, entropy was significantly reduced during FD song (Fig. 4C; mean ± SE: \(-12.2 ± 2.0\%\); t-test: \( t_{15} = 6.13, P < 0.001 \)). This decrease in entropy was caused by a reliable increase in the mean number of times a syllable was repeated from UD to FD song (Fig. 4D).

Therefore, the effect of social context on the sequencing of repeated syllables is congruent with the effect on sequencing at branch points.

Modulation of other song features

Context-dependent modulations of song unrelated to variability have also been noted in other songbirds (Cooper and Goller 2006; Eens 1993; Kao and Brainard 2006; Sossinka and Böhner 1980). Therefore, we examined changes to three other song features—the number of introductory notes, song length, and song tempo.

In the zebra finch, the number of introductory notes preceding the onset of song is significantly higher when males sing to females than when they sing in isolation (Kao and Brainard 2006; Sossinka and Böhner 1980). We found that the number of introductory notes preceding song was also significantly higher for Bengalese finch FD song (paired t-test: \( t_{17} = 2.14, P = 0.0469 \)). On average, there were 16.9% more introductory notes preceding FD song than UD song.

Songs produced in the presence of a female have been found to be significantly longer than those produced in isolation (Eens 1993; Kao and Brainard 2006; Sossinka and Böhner 1980). However, we did not find a significant difference in song length (exclusive of introductory notes) between UD and FD songs in the Bengalese finch (paired t-test: \( t_{18} = 0.86, P = 0.3996 \)).

Songs produced to females are faster than songs produced in isolation in zebra finches (Cooper and Goller 2006; Kao and Brainard 2006; Sossinka and Böhner 1980), and we found a
similar increase in tempo from UD to FD song in Bengalese finches. Figure 5A depicts an example where the tempo of a fixed sequence was significantly faster during FD song than UD song. In this example, the interval from the onset of the first syllable ‘a’ of the sequence ‘abcdede’ to the onset of the last syllable ‘e’ was significantly reduced during FD song relative to UD song (1.56% reduction; \( t_{62} = 4.90, P < 0.0001 \); Fig. 5A). Across all males, this effect was very reliable, with mean sequence durations being lower for FD song than UD song in 29 of the 33 sequences measured (Fig. 5B). Overall, sequence durations were 1.31 ± 0.3% shorter during FD song than UD song, a difference that was statistically significant and comparable to tempo differences observed in zebra finches (Cooper and Goller 2006; Kao and Brainard 2006; Sossinka and Böhner 1980) (Fig. 5C; Wilcoxon signed-ranks test: \( T = 92.0, n = 19, P < 0.001 \)).

**Rapidity of song modulation**

We collected interleaved bouts of UD and FD song, which allowed us to examine how rapidly the structure of song changed between contexts. Assessing the degree to which songs changed rapidly or gradually across the course of the experiment lends insight into the types of mechanisms controlling this social modulation. In four birds, we plotted, as a function of time, three features that could be analyzed for each rendition: repeat number, FF, and song tempo. As in all birds examined, these birds produced FD songs quickly (<1 min) following the introduction of a female. In Fig. 6A, we depict an example and plot the median number of times a syllable was repeated for each song produced during the experimental period. Two things are evident from this figure. First, the number of times a syllable was repeated during a particular song was generally higher during FD song than UD song even when UD and FD songs were temporally proximal to each other. Second, although there was variability across songs in repeat number, there was no trend for a systematic change in repeat number across the experimental period. From data like these, we also calculated mean repeat numbers for UD songs produced within the two minutes prior to the introduction of the female and for FD songs produced within a minute after the introduction of the female (Fig. 6B); the values are normalized to the mean repeat number across all UD songs. Plotted are the means for three repeats that were significantly increased during FD song relative to UD song (\( t\)-test, \( P < 0.05 \); Fig. 6B). Similar data for FF and song tempo are summarized in Fig. 6, C and D. Because FD songs were produced within a minute of exposure to a female, these data highlight the rapidity with which social context affects song organization.

**DISCUSSION**

The display of all behaviors, even well-learned behaviors, is variable. One prevalent view is that this variability is uncontrollable biological noise that is unimportant to the organism. Alternatively, a component of such variability might enable motor learning or assist in the active maintenance of motor performance (Doya and Sejnowski 2000; Kao and Brainard 2006; Kao et al. 2005; Ölveczky et al. 2005; Sutton and Barto 1998). Indeed, it has recently been demonstrated that the residual variability in adult song can be used to guide vocal
plasticity in a songbird (Tumer and Brainard 2007). Although the precise function of behavioral variability remains unknown, some experimental studies indicate that variability is actively regulated, suggesting that variability could serve a function. For example, in the zebra finch, the acoustic structure of vocal motor elements (i.e., syllables) is less variable when males sing to females (UD song) than when males sing in isolation (UD song) (Kao and Brainard 2006; Kao et al. 2005).

For adult zebra finches, syllable sequencing is usually stereotyped, and there is limited evidence for social modulation of sequence (Kao and Brainard 2006; Zevin et al. 2004; but see 2 birds in Sossinka and Böhner 1980). Here we studied the social modulation of sequencing in the Bengalese finch, a species with a high degree of variability in syllable sequencing in adult crystallized song. We found that the variability of syllable sequencing is rapidly modulated by social context with less variable sequencing produced during FD songs than UD songs (Figs. 3, 4, and 6). Moreover as in the zebra finch, we found that the variability of syllable structure was also rapidly modulated by social context with reduced variability in acoustic structure produced during FD song (Fig. 2). These observations indicate that variability in both syllable sequence and structure is actively and rapidly regulated by the CNS and is consistent with the idea that control of variability could serve a function.

Because song variability is greater when males sing in isolation, it has been suggested that UD song represents a state of motor practice in which motor space is “explored,” whereas FD song reflects a state of motor performance of the “best” renditions of learned song (Jarvis et al. 1998; Kao and Brainard 2006; Kao et al. 2005). This motor exploration provides greater opportunity for vocal plasticity by differential reinforcement of motor commands for “desired” versus “undesired” outcomes.

The possibility of increased plasticity during UD song is also supported by the observations that, relative to FD song, neural activity and immediate early gene (IEG) expression in song control nuclei are greater during UD song, features that have been associated with heightened plasticity (Hessler and Doupe 1999; Jarvis et al. 1998). Our results extend the finding of variability regulation from syllable structure to syllable sequencing and suggest that plasticity of syllable sequencing could also be heightened during UD song. Furthermore, our results are consistent with the possibility that species differences in the intrinsic variability of syllable sequencing are correlated with differences in the plasticity of syllable sequencing in adult song; syllable sequencing in adult Bengalese finch song is more variable than in zebra finch song, and adult Bengalese finches demonstrate more rapid plasticity of sequencing following manipulations of auditory feedback than adult zebra finches (Brainard and Doupe 2001; Nordeen and Nordeen 1992; Okanoya and Yamaguchi 1997; Sakata and Brainard 2006; Scott et al. 2000; Woolley and Rubel 1997).

Converging evidence suggests that a component of song variability is actively introduced into the vocal motor pathway by neurons in a forebrain-basal ganglia circuit specialized for song, the anterior forebrain pathway (AFP) (Boettjer et al. 1984; Hessler and Doupe 1999; Kao and Brainard 2006; Kao et al. 2005; Ölveczky et al. 2005; Scharf and Nottebohm 1991). In particular, the lateral nucleus of the nidopallium (LMAN), which projects to the robust nucleus of the arcopallium (RA) in the vocal motor pathway, has been found to influence vocal motor variability. Lesions of LMAN in adult zebra finches reduce the variability of syllable structure (Kao and Brainard 2006; Kao et al. 2005), and lesions and inactivations of LMAN in juvenile zebra finches decrease the variability of both syl-
lable structure and sequencing (Bottjer et al. 1984; Kao et al. 2005; Ölveczky et al. 2005; Scharff and Nottebohm 1991). Furthermore, the variability of spiking activity in LMAN is reduced during renditions of FD song, which could serve to reduce the variability of firing in RA and, consequently, syllable structure (Hessler and Doupe 1999; Kao et al. 2005; Leonardo and Fee 2005; Ölveczky et al. 2005). This causal relationship is supported by the finding that introducing variable amounts of activity into LMAN increases the variability of syllable structure (Kao et al. 2005).

The observation that the variability of both syllable structure and sequence was reduced when Bengalese finches produced FD song suggests that the regulation of both types of variability could be controlled by shared neural substrates. A recent report demonstrating that inactivation of LMAN reduces syllable and sequence variability in juvenile zebra finches supports this notion (Ölveczky et al. 2005). Alternatively, independent mechanisms could drive context-dependent changes in syllable structure versus syllable sequencing in adult songbirds. For example, lesions of LMAN in adult zebra finches affect the variability of syllable structure but not of syllable sequencing, although this could be because adult zebra finches already produce songs with very stereotyped sequences (e.g., Kao and Brainard 2006). Separate medial and lateral basal ganglia circuits project to different areas of the vocal motor pathway, and it has been proposed that the lateral pathway, which includes LMAN and projects to RA, regulates syllable variability, whereas the medial pathway, which includes the medial nucleus of the nidopallium (MMAN) and projects to HVC (proper name), regulates sequence variability (Foster et al. 1997; Jarvis et al. 1998; Kubikova et al. 2007; Reiner et al. 2004). Mounting evidence indicates that HVC and its afferents are critically involved in sequence generation for song (reviewed in Fee et al. 2004; Hahnloser et al. 2002; Hosino and Okano 2000; Vu et al. 1994; Yu and Margoliash 1996) and that RA is important for the acoustic structure of syllables (Leonardo and Fee 2005; Vu et al. 1994; Yu and Margoliash 1996); therefore, the distinct connectivity of the medial and lateral basal ganglia circuits to the vocal motor pathway is consistent with such differential contributions to syllable sequencing versus structure. Further support for this notion comes from the observation that IEG expression in the lateral but not the medial pathway is modulated by social context in the zebra finch, a species in which there is substantial evidence for context-dependent changes in adult syllable structure but not in syllable sequencing (Jarvis et al. 1998; Kao and Brainard 2006; Kao et al. 2005; but see Sossinka and Böhner 1980). In this regard, because changes to both syllable structure and sequencing were observed in Bengalese finches from UD to FD song, it would be interesting to assess whether both medial and lateral basal ganglia circuits display context-dependent changes in IEG expression.

Context-dependent changes in syllable variability and neural activity are hypothesized to arise from alterations in neuro-modulatory inputs to the AFP, such as catecholaminergic input from midbrain areas like the ventral tegmental area (VTA) or locus coeruleus (reviewed in Ball et al. 2003; Bharati and Goodson 2006; Castelino and Ball 2005; Castelino et al. 2007; Ding and Perkel 2002; Haral et al. 2007; Jarvis et al. 1998; Kao et al. 2005; Maney and Ball 2003; Perkel 2004; Sasaki et al. 2006; Yanagihara and Hessler 2006). The role of neuromodulatory systems in the social modulation of song structure, as opposed to classical effects of steroid hormones or other slower influences on gene transcription, is supported by the rapidity of changes to song variability (Fig. 6). Experimental data from songbirds suggest that dopaminergic and/or noradrenergic inputs to song system nuclei may be regulated by social context and could contribute to the social modulation of neural activity and IEG expression within the AFP (Castelino and Ball 2005; Ding and Perkel 2002; Hessler and Doupe 1999; Sasaki et al. 2006; Yanagihara and Hessler 2006). We propose that such changes in catecholamine concentrations in vocal motor circuits, including the AFP, underlie not only the decrease in the variability of syllable structure but also the decrease in the variability of syllable sequencing. Consistent with this perspective are reports that elevated dopamine levels, particularly in nigrostriatal circuits, can increase the stereotypy and repetitiveness of motor expression in rodents and primates (Berridge and Aldridge 2000; Berridge et al. 2005; Cromwell et al. 1998; reviewed in Ridley 1994; Saka et al. 2004).

In addition to changes to the variability of syllable structure and sequence, we observed that song tempo and FF were elevated during FD song relative to UD song in the Bengalese finch and suggest that these alterations could be mediated by changes in AFP activity. In zebra finches, songs produced to females are faster than songs produced in isolation (Cooper and Goller 2006; Kao and Brainard 2006; Sossinka and Böhner 1980), and lesions of IMAN lead to a gradual acceleration of song and an attenuation of context effects on song tempo (Brainard and Doupe 2001; Kao and Brainard 2006; Williams and Mehta 1999). Alterations in vocal quality are often reported in patients with Parkinson’s disease (Goberman et al. 2005; reviewed in Pinto 2004), and interference with dopamine function alters the bandwidth of ultrasonic vocalizations in rats (Ciucci et al. 2007). Therefore, context-dependent changes in activity within the AFP may be responsible for a suite of vocal motor modifications of adult song.

Given the importance of song for mating success in songbirds, social modulation of song features is likely to be relevant for courtship. It has been hypothesized that males emphasize more difficult, effortful, or attractive song features when singing to females to enhance their chances of successful reproduction (reviewed in Catchpole and Slater 1995; Gil and Gahr 2002; Lambrecht 1996; Nowicki and Searcy 2004; Searcy and Yasukawa 1996). Consequently, the observed differences between FD and UD song could reflect greater difficulty or energetic investment and highlight features that females might attend to during social interactions. The observed increases in song tempo (Fig. 5) and repeat number (Figs. 4 and 6) are consistent with increased difficulty or energetic investment. Faster songs can require shorter inspirations between syllables (Calder 1970; Cooper and Goller 2006; Glaze and Troyer 2006; Hartley and Suthers 1989; Suthers and Zollinger 2004; Suthers et al. 1999; Wild 1998), making them potentially more difficult to produce. Repeated syllables are produced with the shortest inter-syllable intervals in the Bengalese finch and are generally the loudest syllable in the repertoire (K. Bouchard, J. T. Sakata, and M. S. Brainard, unpublished observations), thereby making an increase in repeat number potentially more effortful. This perspective also suggests that reduced variability in syllable structure and sequencing could require greater motor control or effort and, therefore, that stereotypy itself...
could be a feature preferred by female Bengalese finches. The degree to which these features influence female choice remains to be addressed in this species, but in other songbird species, features that are influenced by social context have been found to affect female preferences (e.g., Kreutzer et al. 1999; Vallet and Kreutzer 1995; Vallet et al. 1998). One interesting example that highlights the potential function of changes to song variability is the chestnut-sided warbler: in this species, males produce more stereotyped songs during the time of day when interactions with females are more prevalent, and males with increased syllable stereotypy have been found to enjoy higher reproductive success (Byers 1995, 2007).

ACKNOWLEDGMENTS

We thank S. C. Woolley, A. J. Doupe, and M. J. Wohlgemuth III for critical readings of the manuscript. We also thank J. Wong and R. Mazumder for assistance with bird care.

GRANTS

Research was supported by National Institutes of Health Grants T32 NS-0707-25 and F32-MH-068055-01 to J. T. Sakata and T32 MH-20006 to C. M. Hampton, ARCS Scholar Award to C. M. Hampton, and funding from the McKnight Foundation, a Searle Scholars Award, the National Institute of Deafness and Other Communication Disorders, and National Institute of Mental Health Conte Center for Neuroscience Research to M. S. Brainard.

REFERENCES


