Behavioral and Neural Signatures of Readiness to Initiate a Learned Motor Sequence

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Summary

All movements are thought to be “prepared” in the brain before initiation [1–3], and preparation can be impaired in motor diseases [4, 5]. However, little is known about what sort of preparation precedes self-initiated, naturally learned sequences of movements. Here we took advantage of a canonical example of a precisely timed learned motor sequence, adult zebra finch song, to examine motor preparation. We found that the sequences of short vocalizations, or introductory notes (INs), preceding song gradually increased in speed and converged on an acoustic endpoint highly similar across renditions, just before song initiation. The more the initial IN differed acoustically from the final IN, the greater the number of INs produced presong. Moreover, the song premotor nucleus HVC exhibited IN-related neural activity that progressed to a distinctive endpoint immediately before song. Together, our behavioral and neural data suggest that INs reflect a variable period of preparation during which the brain attains a common “ready” state each time sequence generation is about to begin.

Results

Preparation in the brain before movement [1–3, 6, 7] is believed to be important for movement initiation, and diseases like Parkinson’s disease and speech apraxia include initiation defects [4, 5]. Thus, understanding preparation could provide insight into how movements initiate or fail to do so. Preparation before cue-triggered movements and movement sequences has been investigated extensively in primates. Early studies showed that disrupting preparatory neural activity delayed movements [1, 2] and suggested that this activity was subthreshold movement-related activity [8–11]. Recent studies propose instead that preparatory activity is best explained as motor circuit dynamics converging onto an internal state required for movement generation, without a clear relationship to movement parameters [12–14].

In addition to triggered movements, there exist naturally learned movement sequences, like the dives of Olympians or basketball players’ free throws, that are self-initiated without explicit external triggers. These movement sequences are highly stereotyped and almost habit-like as a result of extensive practice. The preparation before such self-initiated, naturally learned movement sequences remains poorly understood. The songs of adult zebra finches are also precise learned motor sequences, with similarities to human speech [15–17]. Although birds sing to court females, they sing when alone as well, showing that song can begin without external triggers. Thus, adult zebra finch song provides a readily quantifiable example of a self-initiated, naturally learned movement sequence.

A consistent feature of virtually all zebra finch songs is the production of short introductory notes (INs) prior to singing one or more repetitions of the core learned portion of song (the “motif”; Figure 1A). Here, to test whether INs might represent motor preparation, we analyzed the properties of INs and how they transition to song, as well as the accompanying IN-related neural activity from the song premotor nucleus HVC.

Analysis of Undirected Song

We recorded and analyzed self-initiated, “undirected” song bouts from 11 adult male zebra finches (median song bouts per finch: 153; range: 69–175). Bouts typically began with a variable number of INs preceding the motif (Figure 1A). Although motif sequence was highly stereotyped, motif initiation could be preceded either by a variable number of INs or by the last syllable of the previous motif (Figure 1B). Motifs at bout onsets were almost always preceded by INs (Figure 1C; see also Figure S1A available online), but the number of these INs was not correlated with bout length (Figure 1E) (p > 0.05, 9 of 11 birds). However, the number of INs within bouts was strongly correlated with the duration of silence following the end of the previous motif (Figures 1D and 1F) (p < 0.05, 10 of 11 birds; mean r = 0.70, range: 0.37–0.90; Figure 1G), suggesting that the next motif requires more INs if it is sung after a longer silence.

Sequences of INs Speed Up and Reach a Similar Acoustic Endpoint across Renditions

Because the number of INs before each motif varies, we hypothesized that sequences of INs reach a similar last IN before motif initiation (Figure 2A). To evaluate this, we characterized IN sequences using two measures: (1) IN sequence timing as measured by the gap between successive INs, and (2) the acoustic properties of individual INs.


Intervals between Successive INs Become Shorter and More Stereotyped Close to Motif Onset

We found that gap duration within each song sequence decreased as the sequence progressed toward the last gap, which we called G−1 (p < 0.05 for 10 of 11 birds, Wilcoxon sign-rank test; mean decrement across consecutive gaps: 23.8%; range: 11.4%–46.7%; Figures 2A and 2B, left). Moreover, in each bird, despite a variable number of INs before individual motifs, the gap at a particular position relative to G−1 was highly similar in duration (Figure 2B, left). Across all birds, median gap duration became shorter closer to motif onset, and variability of gap duration across renditions decreased (Figures 2C and 2D, left).

Acoustic Properties of INs Converge onto a Highly Similar Last IN across Motif Renditions

We next asked whether the acoustic properties of INs changed as each IN sequence progressed to the last IN, which we called IN−1 (Figure 2A). To characterize acoustic properties, we used four different features [18] (see Supplemental Experimental Procedures): duration, log amplitude, mean frequency, and
A spectrogram of one song bout (top) with five introductory notes (INs, “i”) before the bird’s characteristic motif comprising four syllables (“a,” “b,” “c,” and “d”) separated by gaps. To visualize INs across multiple bouts, we generated an IN/non-IN representation (bottom), where syllables and gaps were replaced with appropriately timed colored bars: black (INs), gray (non-INs), and white (silent periods).

Analysis of Similarity between INs and Song
In addition to being motif preparation, INs might influence the quality of subsequent song. To test this, we used the acoustic distance between pairs of syllables as a similarity measure and asked whether similar last INs preceded similar song. In all birds, we found significant, albeit weak, correlations between the similarity of last IN pairs and the similarity of subsequent first motif syllables (n = 11 birds, p < 0.05, mean r = 0.25 ± 0.04; range: 0.1–0.4). Thus, whereas INs are not obligatory for motif production, as evidenced by motifs lacking INs within bouts, when present they not only prepare for singing but may also influence subsequent song. Further support for this idea will require experiments disrupting the last IN state.

Analysis of INs during Courtship Song
To test further our hypothesis that IN sequences represent preparation, we asked whether the large number of INs seen before female-directed song [19] (Figure S1C) might reflect “preparation” before motif initiation.
Figure 2. Intervals between INs Become Shorter and INs Converge on Similar Acoustic Structure across Trials

(A) Gap and IN position (white and black bars) in each sequence was represented relative to a common endpoint, the gap between the last IN and the motif, called $G_{-1}$, and the last IN before motif onset, called IN$_1$.

(B) Median gap duration (one bird) versus gap position across IN sequences of different lengths; undirected song, left, and directed song, right. Each color represents IN sequences of a particular length, and circles and bars represent median values and interquartile range. Values have been slightly offset along the x axis for clarity.

(C and D) Median gap duration (C) and variability of gap duration (D) are negatively correlated with gap position for both undirected, left (n = 11 birds), and directed song, right (n = 6 birds). Squares and error bars represent mean and SEM. Black circles and gray lines represent individual birds; red circles represent bird in (B).

(E) Two acoustic features (mean log amplitude versus mean Wiener entropy) of INs illustrate the convergence on a similar end state for IN sequences of different lengths (data from one bird; undirected, left; directed song, right). Blue, red, and black symbols represent IN$_1$, IN$_2$, IN$_3$, respectively, and ellipses represent one SD from the mean.

(F) Acoustic distance (Mahalanobis distance) from the last IN is negatively correlated with IN position, for both undirected (left) and directed song (right). Conventions as in (C) and (D); bird in (E) shown as red circles here and in (G).

(G) Acoustic distance of the first IN in a sequence from the last IN was positively correlated with the number of INs in that sequence (undirected, left; directed song, right). Black circles represent individual sequences from all birds; squares represent mean and one SD across all birds.
Figure 3. Neural Activity of HVC Interneurons Progresses toward a Distinct Common State before the Last IN

(A) Schematic of the song neural pathways, including a motor pathway (black) and a basal ganglia-thalamus-"cortex" loop (red) specialized for song. HVC contains at least three different types of interconnected neurons: HVC<sub>RA</sub> and HVC<sub>X</sub> projection neurons, and interneurons. Other important feedback loops through HVC are also shown.

(B) Activity of a single HVC interneuron during the production of an IN sequence: top, spectrogram; bottom: extracellular voltage trace; right, 100 example spike waveforms (black), average spike waveform (red). Signal-to-noise ratio: 6.89. Syllable durations are highlighted in pink.

(C) Raster plots of spiking activity for interneuron in (B) during IN sequences of different lengths. The 200 ms analysis window was centered on IN onset (t = 0, IN durations in pink). Each row of raster plots represents activity during INs from an IN sequence of a specific length, with peri-IN time histograms of neural activity in red overlaid on each plot.

(D) Correlation to mean last IN firing pattern as a function of IN position for the neuron in (C). Black traces and gray shading represent mean and one SD for the correlations at a particular IN position. The black dashed line represents a measure of random correlation; IN durations are in pink.

(E) Correlation to mean last IN firing pattern as a function of time within the 200 ms analysis window is shown for different IN positions for all neurons. Each trace represents the mean and SEM of the correlation between the firing pattern of INs at a particular position and the mean firing pattern across all last INs. Different colors represent different IN positions, as shown in the legend. The red dashed lines before IN onset and after IN offset represent, respectively, the...
We recorded from HVC, because it is required for normal motif production in adult male zebra finches during undirected singing (mean SNR = 6.84; range: 4.93–9.92; and n = 23 multiunit sites from 6 birds) in the premotor nucleus HVC of adult male zebra finches during undirected singing (Figure 3A). We recorded from HVC, because it is required for normal motif production [20–22], and asked how its activity related to the progression of INs presong.

One class of HVC neurons projects to motor nucleus RA (HVCra neurons) and is critical to motif production. These neurons are difficult to record, and only a small fraction are active during IN production [23, 24], so our data set did not include HVCra recordings with IN-related activity. Instead, we analyzed recordings from single neurons projecting to area X (HVCx neurons; n = 30 of 46) and from putative interneurons (n = 16 of 46; see Supplemental Experimental Procedures), which are thought to represent a population readout of the activity of HVCra neurons. Multiunit sites (n = 23), which are likely to be dominated by high interneuron firing rates, were included with single interneurons.

**HVC Interneurons**

Interneurons were active during IN sequences (Figure 3B), with a significantly higher mean firing rate than baseline before an IN (Figure S2B; p < 0.05, Kruskal-Wallis ANOVA, post hoc Tukey-Kramer criterion).

We examined the progression of neural activity (in a 200 ms window centered on IN onset) over the course of IN sequences (Figure 3C). We first calculated the similarity between activity patterns of all pairs of last INs and found that they were strongly correlated with each other (R value; mean ± SEM = 0.68 ± 0.02; 39 sites; correlation significantly different from that expected by chance: R value; mean ± SEM = 0.01 ± 0.01; p < 0.05, Mann-Whitney test for each neuron). Neuronal activity patterns during earlier INs at a given position relative to IN−1 were also strongly correlated with each other (R value; mean ± SEM = 0.68 ± 0.03; 39 sites; p < 0.05). Thus, neural activity patterns are stereotyped at each position, including a highly similar state during each last IN, regardless of the number of preceding INs.

We then asked how neuronal activity during earlier INs compared to activity during last INs, by calculating the similarity between the firing pattern during each IN and the mean firing pattern during all last INs (see Supplemental Experimental Procedures). Starting 40 ms before IN onset (approximate HVC premotor latency), the firing pattern during the last IN was significantly different from the firing patterns at earlier INs (Figures 3D and 3E; p < 0.005, Kruskal-Wallis ANOVA, post hoc Tukey-Kramer criterion; see Supplemental Experimental Procedures for details of statistics used). Thus, for many HVC interneurons, activity reached a distinct state for the last IN and was highly similar across renditions.

**HVCx Neurons**

HVCx neurons typically burst only at specific times during song [23–26] and are thought to provide an efference copy of motor commands to song basal ganglia (area X). Given the importance of mammalian basal ganglia for movement initiation [27], we asked whether the firing of HVCx neurons (n = 30; 12 antidromically identified, 18 putative; see Supplemental Experimental Procedures) provided information about IN sequence progression and motif initiation.

A subset of our HVCx neurons (n = 12 of 30: 4 identified, 8 putative) produced single spikes and/or sparse high-frequency bursts during INs. Because these neurons only changed their firing rate without changing burst location, we used a window large enough to include the burst (a 100 ms window centered on IN onset) to analyze firing rate. HVCx neurons showed large firing rate changes for the last IN (Figures 4A–4D). Across all neurons, we found that the firing rate for the last IN was significantly different from the distribution of firing rates for all other preceding INs for 9 of 12 neurons (Figure 4E, red symbols; p < 0.05, Kruskal-Wallis ANOVA).

Further, in 4 of 9 neurons, the firing rate for the last IN was significantly different from the firing rate at each preceding IN position (p < 0.05, Kruskal-Wallis ANOVA, post hoc Tukey-Kramer criterion), reflecting a unique representation for the last IN. For the other five neurons, the firing rate during the last IN was significantly different from the firing rate at a subset of preceding IN positions (p < 0.05). Finally, a smaller subset of HVCx neurons (n = 3 of 12) had equal activity for all INs irrespective of position and sequence length (Figure 4E, black squares and Figure S3A). Thus, a majority of the neurons that we recorded represented the last IN differently from all other INs, providing song basal ganglia with a signal about a key behavioral transition from INs to song, independent of the number of INs. Similarly, recent work in Bengalese finches suggests that HVCx neuron firing rates can encode motif sequence-related information [26].

**Discussion**

Here, we show that IN sequences preceding the motif of an adult finch progress toward a common final acoustic state across motif renditions, with more INs if the initial IN is more acoustically distant from the final state. IN-related neural activity in a song premotor nucleus also reached
a distinct common final state before the last IN. These findings suggest that INs reflect preparation before motif initiation, ensuring that motifs always begin in the same state, regardless of initial conditions. Consistent with the preparation hypothesis, the greater number of INs before courtship song, sung in response to the unexpected appearance of a female, was associated with an initial state farther away from the premotif state and a slower process of converging on the final state. Although neural preparation before movements has been shown in many organisms [28, 29], this preparation remains poorly understood. Recent primate studies suggest that it reflects neural dynamics during the progression from resting to movement initiation state [12, 13]. Although this has been inferred from neural activity, our results extend these findings to show that both behavioral and neural properties converge on a highly similar state across renditions before initiation of naturally learned motor sequences. Unlike the hypothesized neural trajectory through space to a ready point [12, 13], the repetitive nature of INs suggests that preparation can involve repeating certain motor gestures until the state is ready.

What neural mechanisms might underlie “readiness”? Complex motor behaviors require the orderly and coordinated firing of multiple brain areas, within and across hemispheres. The songbird’s repeated introductory vocalizations may be a mechanism for achieving this coordination before song, by repeatedly activating loops between HVC and respiratory or auditory centers (Figure 3A) and across hemispheres. Consistent with this idea, both deafening and disruptions of interhemispheric coordination result in a marked increase in the number of INs, with failure to progress to song when bilateral synchronization is impeded [30–34].

Regardless of mechanism, songbirds, with their strings of repeated notes before a complex learned motor sequence, provide a highly tractable system for studying motor preparation. By analogy to our birds, the ball bouncing of basketball players before their free throws may represent not irrelevant habit or superstition but a useful set of movements by which the brain prepares to execute a practiced motor skill.

Experimental Procedures

Experiments were performed in accordance with NIH guidelines and were approved by the UCSF Institutional Animal Care and Use Committee.

Supplemental Information

Supplemental Information includes three figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.11.040.

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