Lesions in a songbird vocal circuit increase variability in song syntax

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Abstract

Complex motor skills like speech and dance are composed of ordered sequences of simpler elements, but the neuronal basis for syntactic ordering of individual actions into sequences is poorly understood. Birdsong is a learned vocal behavior composed of syntactically ordered sequences of individual syllables. Activity in song premotor nucleus HVC (proper name) has been linked to the control of syllable sequencing, but sequencing may also be affected by its recurrent inputs. We here test the contribution of one of HVC's inputs, mMAN (*medial magnocellular nucleus of the anterior nidopallium*), to the variable songs of adult male Bengalese finches (*Lonchura striata domestica*). The syntax of Bengalese song includes several patterns: 1) *chunks*, where syllables follow stereotypical order 2) *branch points*, where a given syllable can be followed by two or more different syllables in a probabilistic manner and 3) *repeat phrases*, where an individual syllable is repeated a variable number of times. We found that after bilateral lesions of mMAN, the acoustic structure of syllables remained largely intact, but sequencing became more variable for each of these patterns, seen by 'breaks' in previously stereotyped chunks, increased uncertainty at branch points and increased variability of repeat numbers. This increase in sequencing variability highlights the potential importance of regions projecting to HVC in the ordering of vocal elements. Previous studies on zebra finches found little effect of mMAN lesions on their relatively stereotyped adult song. In contrast, our results suggest that mMAN contributes to sequencing the variable songs of Bengalese finches and highlight the utility of species with more complex song syntax in investigating neuronal control of ordered motor sequences.

Introduction

Complex behaviors are composed of sequences of simpler motor elements. For example, in human speech, sentences are formed by syllables that are flexibly sequenced according to syntactic rules (Berwick *et al.*, 2011), which define the order of individual elements. Production of motor sequences according to syntactic rules is important not only for speech, but also for most other natural movements. Despite the ubiquity of syntactically organized sequential behaviors, little is known about how the brain produces flexible motor sequences (Lashley, 1951; Tanji, 2001; Aldridge and Berridge, 2003; Wiltschko *et al.*, 2015; Veit *et al.*, 2021).

Birdsong is a learned behavior that is well suited for the study of the neural control of motor sequences. Song is composed of discrete elements, called syllables, that are organized according to syntactic rules. The complexity of song syntax varies widely across different songbird species - from the simple and repetitive songs of owl finches (Wang *et al.*, 2019) and zebra finches (Zann, 1996) to the variable songs of Bengalese finches (Honda and Okanoya, 1999) and canaries (Nottebohm, Stokes and Leonard, 1976; Cohen *et al.*, 2020) up to the immense repertoire of nightingales (Hultsch *et al.*, 2004; Costalunga *et al.*, 2023). The neuronal mechanisms of birdsong production have been primarily studied in the zebra finch, partly by leveraging the structure of zebra finch song, in which syllables are produced in a relatively stereotyped and linear sequence. Consequently, we have a detailed understanding of the neuronal mechanisms for producing individual song syllables in this species, but we know little about how syllables are organized into the more complex and variable sequences that are characteristic of many other species (Ivanitskii and Marova, 2022).

Song production is controlled by neuronal activity in a set of brain nuclei called the song motor pathway, including premotor nucleus HVC (proper name). HVC neurons projecting to primary motor nucleus RA (*robust nucleus of the arcopallium*) encode syllable identity and have been shown to play a role in controlling syllable timing (Simpson and Vicario, 1990; Vu, Mazurek and Kuo, 1994; Hahnloser, Kozhevnikov and Fee, 2002; Long and Fee, 2008; Aronov *et al.*, 2011; Ölveczky *et al.*, 2011; Lynch *et al.*, 2016; Picardo *et al.*, 2016; Zhang *et al.*, 2017). HVC activity in Bengalese finches (Sakata and Brainard, 2008; Fujimoto, Hasegawa and Watanabe, 2011) and canaries (Cohen *et al.*, 2020) has also been shown to encode syllable identity, and can additionally influence sequencing and reflect the sequential context in which syllables are produced (Fujimoto, Hasegawa and Watanabe, 2011; Zhang *et al.*, 2017; Cohen *et al.*, 2020). The nuclei of the motor pathway are essential for song production (Nottebohm, Stokes and Leonard, 1976; Scharff and Nottebohm, 1991), but the extent to which these sequence-selective

activity patterns in HVC reflect internal neural dynamics or are shaped by recurrent inputs is poorly understood.

Although HVC is often considered to be at the top of a hierarchy in the song control circuit, it is part of a network of brain nuclei involved in song production and receives multiple inputs that might also contribute to premotor activity (McCasland, 1987; Hosino and Okanoya, 2000; Jin, 2009; Hamaguchi, Tanaka and Mooney, 2016; Vyssotski *et al.*, 2016). One prominent source of input to HVC is mMAN (*medial magnocellular nucleus of the anterior nidopallium*) which is part of a recurrent loop that bilaterally connects the two hemispheres through RA and bilateral connections from thalamic nucleus DMP (*dorsomedial nucleus of the posterior thalamus,* Fig. 1E) and could thus be ideally positioned to convey sequence-related activity across hemispheres (Vates, Vicario and Nottebohm, 1997; Schmidt, Ashmore and Vu, 2004; Williams, Nast and Coleman, 2012). A parallel recurrent pathway, which projects to RA through nucleus lMAN (*lateral magnocellular nucleus of the anterior nidopallium*, Fig. 1E) has been shown to contribute variability to syllable pitch, a parameter that is controlled by RA premotor activity (Kao, Doupe and Brainard, 2005; Sober, Wohlgemuth and Brainard, 2008; Miller, Cheung and Brainard, 2017). lMAN-guided variability can serve as a form of motor exploration which is essential for learning (Sober and Brainard, 2012; Dhawale, Smith and Ölveczky, 2017), regulating social context-dependent changes in pitch variability (Kao and Brainard, 2006; Hampton, Sakata and Brainard, 2009), and biasing pitch in the direction of improved motor performance during learning (Andalman and Fee, 2009; Warren *et al.*, 2012; Tian and Brainard, 2017). This raises the question of whether mMAN, as the output of a parallel recurrent pathway that projects onto HVC, might have a similar role in contributing to premotor activity in its projection target, with the potential to influence syllable identity or sequencing variability (Kubikova, Turner and Jarvis, 2007; Seki and Okanoya, 2008; Ali *et al.*, 2013)

We tested the hypothesis that mMAN contributes to syllable sequencing by studying the effect of bilateral mMAN lesions on song production in adult Bengalese finches. We found that lesions had little effect on the acoustic structure of individual syllables but led to an increase in the variability of multiple aspects of syllable sequencing. These results highlight the potential importance of recurrent inputs such as mMAN in shaping the syntactical structure of adult birdsongs.

Methods

Subjects and sound recordings

Experiments were carried out on seven adult male Bengalese finches (*Lonchura striata domestica*) obtained from the Brainard lab's breeding colony at University of California, San Francisco (median age 640 days post-hatch, range 149-1049 at start of experiment). Birds were raised with their parents and then housed in same-sex group cages. For the experiments, birds were placed in individual sound-attenuating boxes (Acoustic Systems, Austin, TX) and maintained on a 14:10 hour light: dark period. Song was recorded using an omnidirectional microphone above the cage using custom LabView software for continuous monitoring of song output (Tumer and Brainard, 2007). All procedures were performed in accordance with animal care protocols approved by the University of California, San Francisco Institutional Animal Care and Use Committee (IACUC).

mMAN lesions

After several days of sound recording, birds were anesthetized using ketamine and midazolam with isoflurane. mMAN was localized using stereotaxic coordinates and was destroyed bilaterally using current injections of 100uA current for 80s at four sites per hemisphere. After conclusion of the study, birds were deeply anesthetized and perfused with paraformaldehyde. 40um sections were cut on a microtome and processed with either Nissl stain (cresyl violet) or calcitonin gene-related peptide (CGRP) (Hampton, Sakata and Brainard, 2009). mMAN lesion size was estimated by experienced observers and estimated to be either complete (bird 1, birds 3-6) or greater than 75% (bird 2 and 7) for all birds used in this analysis (Sup. Fig. 3).

Data analysis

Bengalese finch songs were recorded for several days before and after lesions. Singing activity resumed on average 3.8 days post lesions (range 2-6 days, Sup. Fig. 1), and we analyzed songs starting on average 5.3 days post lesions (range 3-11 days) up to 7.4 days post lesions (range 5-12 days). We analyzed at least two days pre and post lesions, and subsampled the larger of the two datasets pooled over all days such that the pre and post datasets were equal in size for each bird (average of 299 song bouts, range 102-601, for all analyses). Sup. Figure 1 includes additional song bouts which were excluded by the subsampling procedure.

Annotation

We annotated the entire syllable sequence in an automated way. We first used a subset of the data to generate a training set for classification. Separate classifiers were trained for each bird. In the training data, syllable onsets and offsets were determined by amplitude thresholding. We then performed dimensionality-reduction and unsupervised clustering of the spectrograms of detected syllables using UMAP (McInnes *et al.*, 2018; Sainburg, Thielk and Gentner, 2020), in order to determine the number of different syllables in an objective way (mean 13, range 11-14). On average, we used 181 files (range 70-505) for creating this training dataset, which was then used to train a deep neural network (TweetyNet) for the annotation of birdsong (Cohen *et al.*, 2022). TweetyNet was used to segment and annotate all songs, followed by semi-automated

hand-checking using custom-written software in MATLAB R2021b to ensure quality annotation.

Simplification of the syllable sequence

Song bouts were defined as continuous sequences of syllables separated by at least 2s of silence. The syllable sequence was then simplified by merging introductory notes into a single introductory state, i.e., a single node '*I*' in the transition diagram (Fig. 1A,F). Introductory notes were defined as up to three syllable types occurring at the start of bouts which were quieter, and more variable in timing and structure than other syllable types (Rajan and Doupe, 2013; Veit *et al.*, 2021). Similarly, syllables which were repeated a variable number of at least two times were reduced to a single state (e.g. '*bbbbbbbbb*' to '*B*', Fig. 1A,F) in the syllable sequence, and the number of repetitions within these repeat phrases was analyzed separately (Hampton, Sakata and Brainard, 2009; Zhang *et al.*, 2017; Jaffe and Brainard, 2020). Only repeat phrases with variable numbers of syllable repetitions were analyzed in this way, therefore, cases in which a syllable was repeated an exact number of times on each occurrence (occurring with repeat numbers of 2 or 3 in our dataset, see Fig. 3A) were not considered a variable repeat phrase.

Separating different syllable states based on sequential context

Transition probabilities between syllables in Bengalese finch song can depend not only on the identity of the syllable, but on the identity of at least one preceding syllable (Jin and Kozhevnikov, 2011; Katahira *et al.*, 2011; Morita *et al.*, 2021). In order to consider this sequential context-dependency of transition probabilities, we followed the procedure of Katahira et al. 2011 to classify spectrally similar syllables (i.e., with the same labels) into different 'states' based on the identity of the preceding syllable. For example, in Figure 1A, the syllable *'g'* is preceded by *'I'* or *'f'* and is followed by *'h'* or *'a'*. If *'g'* is preceded by *'I'* it is always followed by *'h'* and if it is preceded by *'f'*, it can be followed by *'h'* or *'a'*. We would therefore like to introduce two separate states g_1 and g_2 in the transition diagram representing this sequential context. The input for this analysis was the syllable sequence with introductory and repeat phrases already condensed into single states. We compared the distribution of transition probabilities from these syllables by themselves (e.g., syllable *'g'*) to the distribution of transition probabilities from these syllables considering the context of one preceding syllable (e.g., syllable *'f-g'*) using the chi-square goodness of fit test. We split the syllable into a new state (e.g. *'g_1'*) if $p < 0.01/n$ where n is the number of comparisons made (Bonferroni correction for multiple comparisons) (Jin, 2009; Katahira *et al.*, 2013). After completing this for all syllables of a given type (e.g., *'g_1'*, *'g_2'*, *'g_3'*), we compared the different output states to each other using chi-square goodness of fit test and merged them back together if $p > 0.01/n$ (mean 15, range 6-21 additional states/bird).

Determining chunks and branchpoints

To determine chunks (Suge and Okanoya, 2010), we used the modified syllable sequence after chi-square analysis. We calculated a syllable-to-syllable transition diagram, while eliminating any syllables occurring with less than a threshold frequency of 0.9% and omitting branches with a probability of less than 5%. From the resultant graph, we merged all non-overlapping

linear sequences (which consisted of nodes with only a single input and output branch) into chunks. The transition probabilities within resultant chunks were typically higher than 90% (range 87-100%). Values smaller than 100% for non-branching paths result from the omission of one or several small branches of less than 5%. We then re-calculate the transition probabilities between chunked nodes. We defined branchpoints as the set of single syllables that are retained after this processing and the end syllables of the newly defined chunks. To simplify diagrams for visualization, we eliminated any syllables which occurred less than once per bout and branches with transition probabilities of less than 5%.

Transition entropy

Transition entropy is a measure of uncertainty of sequence at a given syllable (Sakata and Brainard, 2006). With c different outputs from the given syllable *'a'* and $P(i)$ the probability of the ith outcome, we calculate the entropy H_a, as:

$$H_a = -\sum_{i=1}^{c} P(i) \log P(i)$$

We call this value 'transition entropy per branchpoint' in Figure 3A.

To determine the overall variability of song before and after mMAN lesions, we calculated total transition entropy TE, over all syllables b as:

$$TE = -\sum_{b=1}^{n} H_b * P(b)$$

where H_b is the transition entropy at b and $P(b)$ is the frequency of syllable b (Chatfield and Lemon, 1970; Katahira *et al.*, 2013).

History dependence

History dependence is a previously established metric that measures the extent to which the most common transition at a given syllable is influenced by the transition at the last occurrence of this syllable (Warren *et al.*, 2012). It has been used to characterize instances of apparent sequence variability, where seemingly variable transitions are always strictly alternating. For example, if the possible transitions from syllable *'a'* are *'ab'* or *'ac'* but these strictly alternated (ab…ac…ab…ac, and so on) then the seemingly variable branchpoint *'a'* is perfectly predictable based on its history (Warren *et al.*, 2012). Such apparent variability should be largely eliminated in our sequence analysis by the introduction of context dependent states (i.e., in this example, the *'a'* would be re-labelled as a_1 or a_2 depending on the context in which it occurs) and identification of chunks. However, if higher-order dependencies in the song determine the order of chunks, we might still expect some variable transitions to be governed by history dependence. If ab is the most frequently occurring transition from a, and ac is the collection of all other transitions from a, we define history dependence D of a as:

$$D = |P(ab_n|ab_{n-1}) - P(ab_n|ac_{n-1})|$$

where $P(ab_n|ab_{n-1})$ is the conditional probability of *ab* transition given that *ab* transition occurred at the previous instance of *a* and $P(ab_n|ac_{n-1})$ is the conditional probability of *ab* transition given that *ac* transition occurred at the previous instance of *a*.

Chunk consistency

As defined above, a chunk is defined by a single dominant sequence, but may have a small amount of variability across different instances. To quantify the stereotypy of chunks, we used a measure based on sequence consistency previously defined for relatively stereotyped zebra finch songs (Scharff and Nottebohm, 1991). Across all instances of a given chunk, we identified the syllable sequence that occurred most often as the 'dominant sequence'. We then defined '*n_dominant*' as the number of instances of the dominant sequence, and '*n_other*' as the number of instances of other sequence variants for the chunk.

We quantified chunk consistency *C* as the proportion of total instances that were the dominant sequence:

$$C = \frac{n_dominant}{n_dominant + n_other}$$

To compare a chunk before and after mMAN lesions, the dominant sequence for the pre lesion chunk was used as a reference, regardless of whether the same sequence qualified as a chunk post lesion. To quantify chunk consistency post lesion, the most dominant sequence post lesion was used (even if that was not the same as the most dominant sequence pre lesion).

Repeat number variability

To study the influence of mMAN on repeat phrases, we examined the distribution of repeat numbers before and after lesions. We quantified the variability of these distributions as their coefficient of variation (*CV = standard deviation/mean*).

Results

Structure of Bengalese finch song

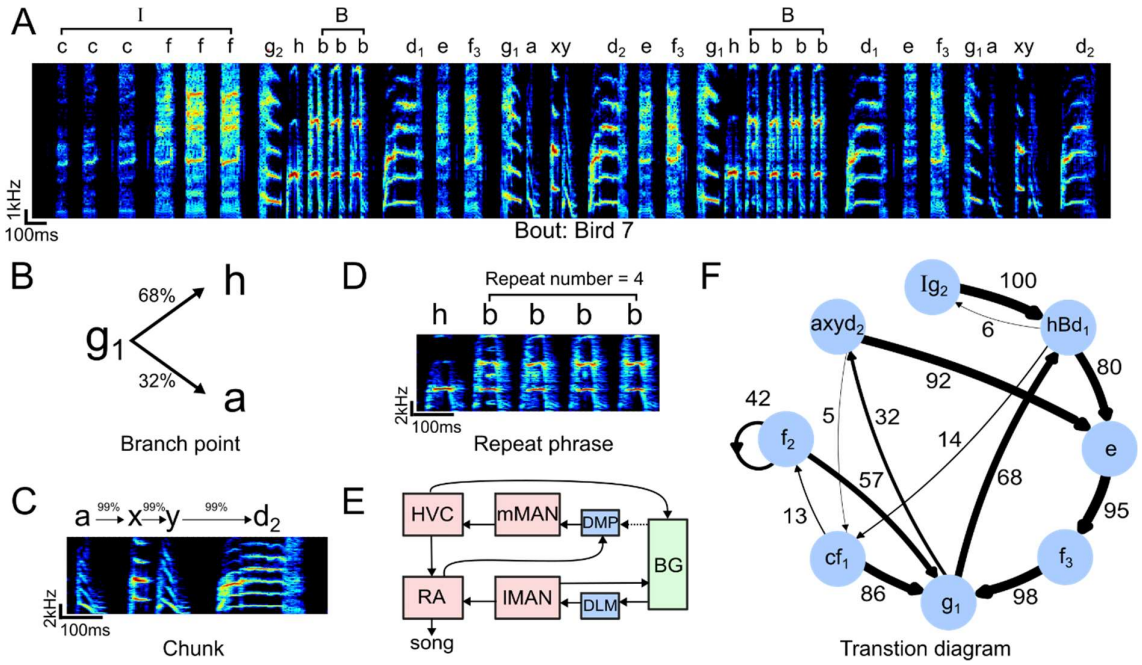

Figure 1: Structure of Bengalese finch song (A) Example spectrogram (bird 7) depicting an entire song bout with introductory state *'I'*, repeat phrase '*B*' and individual syllables. (B) Example transition diagram depicting a branchpoint with variable sequencing. Numbers above the arrows denote transition probabilities in percent. (C) Example spectrogram of a chunk. Chunks are defined as highly stereotyped sequences of syllables which only have a single in- and output branch and are condensed into one state in the transition diagram (see Methods). (D) Example spectrogram of a repeat phrase, summarized by capital letter '*B*' in the transition diagram. The repeating syllable (here, syllable *'b'*) repeats a variable number of times across different instances of the repeat phrase. (E) Schematic showing recurrent pathways projecting onto motor pathway nuclei through lMAN and mMAN. Red: pallial nuclei, blue: thalamic nuclei, green: basal ganglia. Dotted line indicates suspected connection by Kubikova et al. 2007. Abbrev: mMAN: *medial magnocellular nucleus of the anterior nidopallium*, DMP: *dorsomedial nucleus of the posterior thalamus*, BG: *basal ganglia*, DLM: *medial portion of the dorsolateral thalamus*, lMAN: *lateral magnocellular nucleus of the anterior nidopallium*, RA: *robust nucleus of the arcopallium*. (F) Example of a transition diagram. Nodes denote chunk or syllable labels, numbers denote transition probabilities (in percent, % symbol omitted for clarity), d_1/d_2, g_1/g_2 denote different states of syllables *d* and *g* respectively based on different sequential contexts, capital letters denote repeat phrases. Edges at each node may not sum to 100% because branches smaller than 5% are omitted for clarity.

Bengalese finch songs are composed of a fixed number of syllable types, which are arranged into variable sequences following syntactical rules. In this study, we sought to first characterize the different features of Bengalese finch song, before investigating their change following mMAN lesions. For each bird, we first annotated all songs with labels for each of the syllable types, and then defined a transition diagram for analysis according to the following procedure (see Methods):

1) We identified introductory notes (Rajan and Doupe, 2013; Veit *et al.*, 2021) and grouped them together into a single introductory state *'I'* in the transition diagram. This reduced the overall transition entropy of song for 7 birds from 0.95 to 0.85 (Fig. 1A).
2) Repeat phrases, in which the same syllable is repeated a variable number of times, were also reduced to a single state in the transition diagram (indicated by capital letters) and the distribution of repeat numbers was analyzed separately (Hampton, Sakata and Brainard, 2009; Zhang *et al.*, 2017; Jaffe and Brainard, 2020) (Fig. 1D).
3) In Bengalese finch song a single spectrally defined and labelled syllable can occur in different fixed sequences (for example, syllable *'b'* in the sequences *a-b-c* and *d-b-e*) and therefore reflects distinct states in the song sequence (Wohlgemuth, Sober and Brainard, 2010; Katahira *et al.*, 2011). We followed the procedure of Katahira et al. to determine if any initially identified syllable types corresponded to more than one state based on whether the preceding syllable carried information on transition probabilities (see Methods, Katahira *et al.*, 2013). After identifying and splitting these 'hidden states' (for example b_1, b_2) for each bird, overall transition entropy was further reduced from 0.85 to 0.54 (Fig. 1F).
4) In the new syllable sequence, we identified 'chunks' (Suge and Okanoya, 2010), sub-sequences of high-probability transitions. We defined these as paths of continuous, non-branching sequences in the transition diagram, after omitting branches that occurred with probability < 5% (Fig. 1C). Lastly, we identified branch points in the transition diagram as the set of single syllables and endpoints of chunks, i.e., all states where the syllable sequence proceeds in a probabilistic manner (Fig. 1B,F).

Transition entropy increased after bilateral mMAN lesions

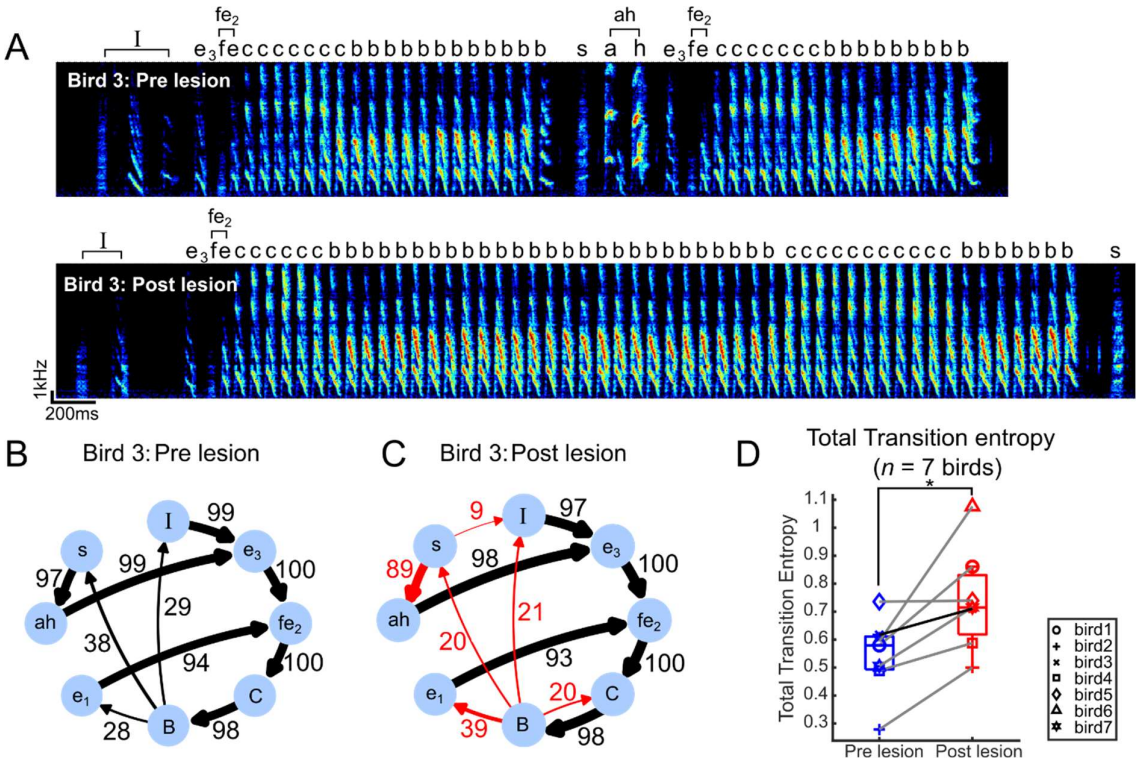

Figure 2: Transition entropy increased after bilateral mMAN lesions (A) Example spectrogram (bird 3) pre lesion (above) and post lesion (below). *'I'* denotes introductory state, and *'fe$_2$'* and *'ah'* denote chunks, shown as single nodes in the transition diagram. (B) Pre lesion transition diagram, as in 1F. Note that *'fe$_2$CB'* is also a chunk before the lesions but is shown as three separate nodes in order to align with the post lesion diagram in C. (C) Post lesion transition diagram. Arrows in red mark example nodes with relatively high increase in transition entropy, including the introduction of new branches after mMAN lesions. (D) Total transition entropy for 7 birds before and after mMAN lesions (* p<0.05, n=7, Wilcoxon signed-rank test). Example bird from A-C is shown as darker line.

After bilateral mMAN lesions, all syllable types were preserved (Sup. Fig. 2), but the sequencing of syllables became more variable. In the example in Figure 2B,C, we show the transition diagrams of one example bird before and after mMAN lesions. We observed a change in transition probabilities at existing branch points, as well as the appearance of novel branches. We quantified changes in sequencing variability for all syllables using transition entropy (Sakata and Brainard, 2006; Katahira *et al.*, 2013). Total transition entropy increased significantly after mMAN lesions, indicating that song syntax overall became more variable (Fig. 2D). These effects appeared as soon as the bird resumed singing after the lesions and were stable for the time which the birds were followed (Sup. Fig. 1).

An analysis of changes at individual branch points revealed that transition entropy increased for most branchpoints after mMAN lesions (Sup. Fig. 4A). To investigate the source of differences in the magnitude of changes across branch points, we considered the history-dependence of each of these branchpoints. History-dependence captures apparent variability governed by long-range dependencies in the sequence and history-dependent transitions previously have been found to be difficult to modify in a sequence modification training protocol (Warren *et al.*, 2012). They might therefore be less affected by lesions as well. Alternatively, we might expect lesion effects to be stronger for these transitions, if mMAN contributes specifically to long-range dependencies. Consistent with the first possibility, we observed that transitions that had high history dependence tended to show smaller changes in transition entropy while transitions that had low history dependence tended to show larger changes after mMAN lesions (Sup. Fig. 4B).

Chunks became more variable after bilateral mMAN lesions

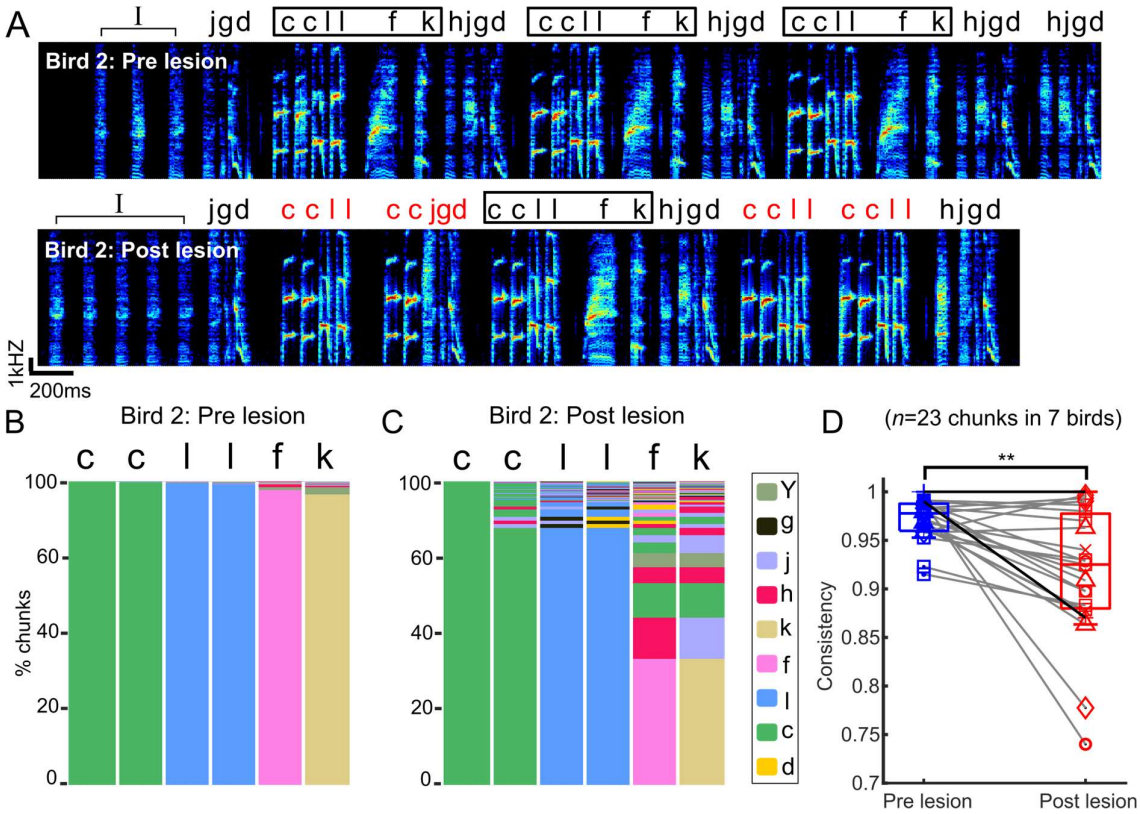

Figure 3: Chunks became more variable after bilateral mMAN lesions. (A) Example spectrogram (bird 2) before and after bilateral mMAN lesions. Atypical chunk sequences are highlighted in red. (B,C) Transitions following the first '*c*' of the *'ccllfk'* chunk from (A) before and after mMAN lesions. Different column colors represent different syllables. (D) Chunk consistency before and after bilateral mMAN lesions (** p<0.01, n=23, Wilcoxon signed rank test). Example bird is shown as darker line.

322 Bengalese finch songs contain short, relatively stereotyped sequences of syllables, here called
323 'chunks' (Okanoya and Yamaguchi, 1997; Suge and Okanoya, 2010; Isola, Vochin and Sakata,
324 2020; Veit *et al.*, 2021). We speculated that transitions within chunks might be differently
325 affected by mMAN lesions, if for example the relatively fixed sequences within chunks are
326 determined within premotor song nucleus HVC and inputs to HVC are only relevant at variable
327 branch points. We found that the relatively fixed sequences within chunks were altered after
328 mMAN lesions. An example bird (Fig. 3A-C) exhibited one prominent chunk which was
329 occasionally observed to 'break' after lesions, i.e., branching within the chunk increased to a
330 degree where it would no longer be considered a chunk after the lesions. The branching
331 increased most noticeably in the *'l-f'* transition (Fig. 3C), which is characterized by a longer
332 gap than other transitions within the chunk (Fig. 3A). We therefore wondered whether
333 sequencing changes were related to gap durations and found that indeed gap durations within
334 chunks were weakly correlated with the increase in transition entropy at the corresponding
335 transitions (Sup. Fig. 5A). Overall, we found that changes within chunks were approximately
336 the same magnitude as changes at branch points (Sup. Fig. 5B).

337 We quantified changes in within-chunk transitions using sequence consistency (Scharff and
338 Nottebohm, 1991), a measure previously used to describe the relatively consistent sequence of
339 zebra finch song. Sequence consistency measures how consistently the most probable sequence
340 is followed (see Methods). Sequence consistency within chunks significantly decreased across
341 23 chunks from all birds, indicating that this aspect of song structure was affected by mMAN
342 lesions (Fig. 3D).

Repeat numbers became more variable after mMAN lesions.

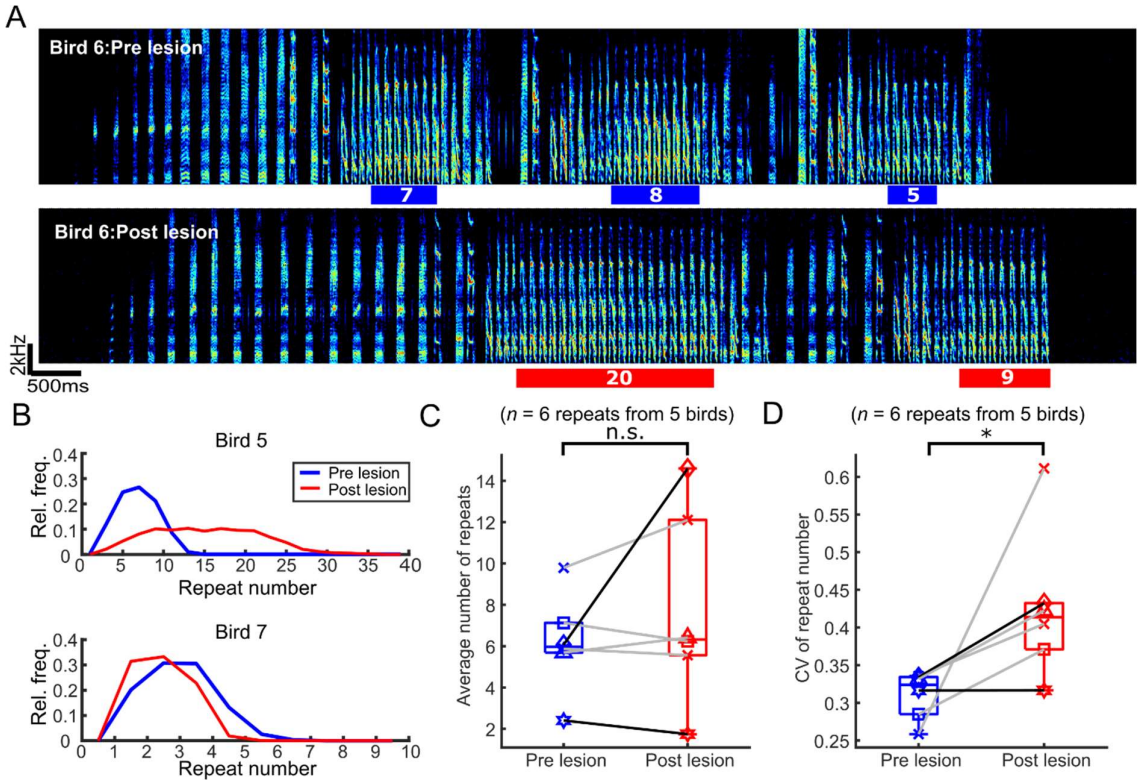

Figure 4: The number of syllables per repeat phrase (repeat number) became more variable after bilateral mMAN lesions. (A) Example spectrogram (bird 6) highlighting one repeating syllable before (blue) and after (red) mMAN lesions. (B) Repeat numbers for two additional example birds before (blue) and after (red) mMAN lesions. (C) Average repeat numbers before and after mMAN lesions for all repeat phrases. (D) Coefficient of variation for distribution of repeat numbers before and after mMAN lesions for all repeat phrases. Example birds from B are shown as darker lines.

We next tested how mMAN lesions affected repeat phrases. Repeat phrases might be governed by separate neural mechanisms than branch points (Fujimoto, Hasegawa and Watanabe, 2011; Jin and Kozhevnikov, 2011; Wittenbach *et al.*, 2015; Zhang *et al.*, 2017).

In the example bird in Figure 4A,B, the average repeat number for the indicated syllable increased after mMAN lesions. Across birds, the mean repeat number did not change consistently, although individual birds could exhibit quite dramatic effects on repeat number, (Fig. 4B,C), ($p>0.05$, $n=6$, Wilcoxon signed rank test). The distributions of repeat numbers became wider and the coefficient of variation for repeat numbers increased significantly after mMAN lesions (Fig. 4D), (* $p<0.05$, $n=6$, Wilcoxon signed rank test).

360 **Discussion**

361 Bird songs are vocal sequences composed of individual syllables which appear in sequential
362 order dictated by syntactic rules, but the neuronal control of song syntax is largely unknown.
363 Previous studies in zebra finches showed that mMAN lesions in juveniles disrupt normal song
364 learning, while lesions in adults affected song initiation, but not subsequent production of song
365 (Foster and Bottjer, 2001; Horita, Wada and Jarvis, 2008; Ali *et al.*, 2013). We speculated that
366 the modest effect of lesions in adult zebra finches might be due to the relatively stereotyped
367 nature of zebra finch song. Consistent with this possibility, our results demonstrate that lesions
368 of pallial nucleus mMAN affect the more complex and variable song syntax in Bengalese
369 finches. We showed that while mMAN lesions did not abolish the production of song itself or
370 grossly disrupt the acoustic structure of individual syllables, they increased the variability of
371 syllable sequencing; the transition probabilities between syllables became less predictable
372 (increased transition entropy), new branches were introduced into previously stereotyped
373 chunks, and the number of repetitions of repeated syllables (repeat number) became more
374 variable. These results indicate that mMAN is involved in controlling aspects of song syntax
375 in a songbird species that exhibits complex syllable sequencing and suggest that mMAN might
376 play a similar or prominent role in additional species with even greater song complexity, such
377 as canaries or nightingales.

378 Song in passerine birds relies on a network of recurrently connected nuclei, with different
379 pathways for song production and learning. The song motor pathway consists of primary motor
380 nucleus RA, which projects directly to vocal motor nuclei in the brainstem, and is involved in
381 the moment-by-moment control of the structure of individual syllables (Vu, Mazurek and Kuo,
382 1994; Sober, Wohlgemuth and Brainard, 2008; Miller, Cheung and Brainard, 2017). Neurons
383 in premotor song nucleus HVC that project to RA have been shown to encode syllable identity
384 and contribute to syllable timing (Simpson and Vicario, 1990; Vu, Mazurek and Kuo, 1994;
385 Hahnloser, Kozhevnikov and Fee, 2002; Long and Fee, 2008; Aronov *et al.*, 2011; Ölveczky
386 *et al.*, 2011; Zhang *et al.*, 2017). Consistent with a possible role of HVC in also controlling
387 variable syllable sequencing, cooling HVC influences syntax in Bengalese finches (Zhang *et*
388 *al.*, 2017). However, lesions of one input to HVC – the nucleus Nif (*nucleus interfacialis of the*
389 *anterior nidopallium*) – can also influence syllable sequencing in the Bengalese finch by
390 making the sequence more stereotyped (Hosino and Okanoya, 2000; Cardin, Raksin and
391 Schmidt, 2005; Otchy *et al.*, 2015) raising the question of how much of the control of syntax

392 relies on the internal dynamics of HVC, versus activity relayed to HVC by its inputs (Okanoya
393 and Yamaguchi, 1997).

394 In this study, we were especially interested in the possibility that mMAN's inputs to HVC
395 might play an analogous role in modulating syllable sequencing to the role of lMAN's inputs
396 to RA in modulating syllable acoustic structure. In adult birds, lMAN's inputs to RA have been
397 shown to contribute variability to the pitch of individual syllables (Kao, Doupe and Brainard,
398 2005; Kao and Brainard, 2006; Stepanek and Doupe, 2010), regulate changes to pitch
399 variability between different social contexts (Kao and Brainard, 2006; Hampton, Sakata and
400 Brainard, 2009), and bias pitch in the direction of improved performance during learning
401 experiments (Andalman and Fee, 2009; Warren *et al.*, 2011; Tian and Brainard, 2017). The
402 variability contributed by lMAN may facilitate motor exploration which forms the basis of
403 pitch adaptations during learning (Andalman and Fee, 2009; Sober and Brainard, 2012;
404 Dhawale, Smith and Ölveczky, 2017). These effects are mediated by top-down influences of
405 lMAN on RA via a recurrent loop that passes through the songbird basal ganglia (Kao, Doupe
406 and Brainard, 2005; Andalman and Fee, 2009; Kojima *et al.*, 2018; Tian, Warren and Brainard,
407 2022), and are consistent with a role of RA in controlling aspects of syllable acoustic structure.
408 In contrast to these effects on syllable structure, lesions of lMAN in adults have not been found
409 to appreciably influence syllable sequencing (Hampton, Sakata and Brainard, 2009). However,
410 because mMAN forms the output of a parallel recurrent loop that passes through the basal
411 ganglia and projects to HVC, we hypothesized that analogous modulation of syllable
412 sequencing during production and learning of song might be mediated by mMAN. Our finding
413 that lesions of mMAN alter multiple aspects of syllable sequencing are consistent with such an
414 influence on song syntax.

415 The finding that mMAN can influence song syntax raises the question of whether and how this
416 pathway modulates syllable sequencing in the service of motor exploration, contextual
417 modulation, and learning, in a fashion that is analogous to lMAN's modulation of syllable
418 acoustic structure. That mMAN lesions consistently altered sequence variability indicates a
419 possible role in regulating variability for motor exploration. However, whereas lMAN lesions
420 consistently *decrease* the variability of acoustic structure, we found that mMAN lesions
421 generally *increase* the variability of syllable sequencing, suggesting that top-down
422 contributions to the control of syntax may be different or more distributed than for a syllable
423 feature such as pitch. One possibility is that several inputs to HVC could work synergistically
424 to modulate the syntactical structure of Bengalese finch songs. Consistent with this view, a

425 prior study in Bengalese finches found that lesions of Nif decreased sequence variability
426 (Hosino and Okanoya, 2000), suggesting that Nif and mMAN could be playing opposing roles
427 in regulating variability. Our findings also raise the question of whether the medial anterior
428 forebrain pathway including mMAN, plays a similar role also in the social-context dependent
429 regulation of sequence variability (Hampton, Sakata and Brainard, 2009; Jaffe and Brainard,
430 2020) and in learning to change syllable sequences during development (Foster and Bottjer,
431 2001; Lipkind *et al.*, 2013) or adulthood (Warren *et al.*, 2012; Veit *et al.*, 2021). Further studies
432 with careful manipulations of the basal ganglia-mMAN-HVC circuit will be required to
433 elucidate the mechanism with which this circuit affects sequencing in Bengalese finch song
434 and contributes to these behaviors.

435 Bengalese finch syllable sequences contain structure at multiple levels, mainly the organization
436 of individual stereotyped chunks, resembling the stereotyped motifs of zebra finch song, and
437 the variable branch points between chunks, repeat phrases, and other individual syllables. One
438 possibility to reconcile findings that both dynamics within HVC and its recurrent inputs affect
439 sequencing could be that the sequence within chunks is encoded predominantly in the synaptic
440 connections of HVC neurons (Jin, 2009), while the branching transitions found in Bengalese
441 finch song are influenced by input from other nuclei (Hosino and Okanoya, 2000). We here
442 show that mMAN lesions affect both transition entropy at previously variable branch points
443 and transitions within previously stereotyped chunks, indicating that such a simple split of
444 function between mMAN and HVC is not the case.

445 Besides these, other parts of the song circuit have been shown to affect syllable sequencing.
446 The basal ganglia are involved in selection and sequencing of motor elements in mammals, and
447 manipulations of songbird basal ganglia Area X have previously been shown to affect syllable
448 repetitions in zebra finches and Bengalese finches (Kobayashi, Uno and Okanoya, 2001;
449 Kubikova *et al.*, 2014; Tanaka *et al.*, 2016; Xiao *et al.*, 2021). These effects might be partly
450 mediated via connections through mMAN to HVC (Kubikova, Turner and Jarvis, 2007).
451 Additionally, the control of syllable sequencing must include bilateral coordination of the
452 motor pathways in the two hemispheres (Schmidt, Ashmore and Vu, 2004; Wang *et al.*, 2008).
453 As the two hemispheres in birds are not connected by a corpus callosum, the only pathways
454 mediating interhemispheric synchronization of the two HVCs are by bilateral connections
455 through thalamic nuclei. One such pathway involves a projection from thalamic nucleus Uva
456 to HVC (Coleman and Vu, 2005; Hamaguchi, Tanaka and Mooney, 2016; Moll *et al.*, 2023),
457 which is required for song production and contributes to syllable initiation and timing. Another

is through thalamic nucleus DMP, which projects bilaterally through mMAN to HVC (Schmidt, Ashmore and Vu, 2004; Seki and Okanoya, 2008). mMAN might therefore be well situated to coordinate syllable sequencing in the two hemispheres. Future studies will need to determine whether the effects we observed are the results of removing neuronal processing within mMAN itself, or whether mMAN is conveying signals to HVC from other brain areas, including the contralateral hemisphere, which contribute to the control of song syntax.

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Author contributions

T.L.W., J.C., S.S. and L.V. gathered the data. A.K. and L.V. analyzed the data, A.K., L.V. and M.S.B. wrote the paper.

Competing interests

The authors declare no competing interests.

References

Aldridge, J. W. and Berridge, K. C. (2003) 'Basal Ganglia Neural Coding of Natural Action Sequences', in *The Basal Ganglia VI. Advances in Behavioral Biology*, pp. 279–287. doi: 10.1007/978-1-4615-0179-4_28.

Ali, F. *et al.* (2013) 'The Basal Ganglia Is Necessary for Learning Spectral, but Not Temporal, Features of Birdsong', *Neuron*, 80(2), pp. 494–506. doi: 10.1016/j.neuron.2013.07.049.

Andalman, A. S. and Fee, M. S. (2009) 'A basal ganglia-forebrain circuit in the songbird biases motor output to avoid vocal errors', *Proceedings of the National Academy of Sciences*, 106(30), pp. 12518–12523. doi: 10.1073/pnas.0903214106.

Aronov, D. *et al.* (2011) 'Two Distinct Modes of Forebrain Circuit Dynamics Underlie Temporal Patterning in the Vocalizations of Young Songbirds', *Journal of Neuroscience*, 31(45), pp. 16353–16368. doi: 10.1523/JNEUROSCI.3009-11.2011.

Berwick, R. C. *et al.* (2011) 'Songs to syntax: the linguistics of birdsong.', *Trends in Cognitive Sciences*, 15(3), pp. 113–21. doi: 10.1016/j.tics.2011.01.002.

Cardin, J. A., Raksin, J. N. and Schmidt, M. F. (2005) 'Sensorimotor Nucleus NIf Is Necessary for Auditory Processing But Not Vocal Motor Output in the Avian Song System', *Journal of Neurophysiology*, 93(4), pp. 2157–2166. doi: 10.1152/jn.01001.2004.

Chatfield, C. and Lemon, R. E. (1970) 'Analysing sequences of behavioural events', *Journal of Theoretical Biology*, 29(3), pp. 427–445. doi: 10.1016/0022-5193(70)90107-4.

Cohen, Y. *et al.* (2020) 'Hidden neural states underlie canary song syntax', *Nature*, 582(7813), pp. 539–544. doi: 10.1038/s41586-020-2397-3.

Cohen, Y. *et al.* (2022) 'Automated annotation of birdsong with a neural network that segments spectrograms', *eLife*, 11:e63853. doi: 10.7554/eLife.63853.

Coleman, M. J. and Vu, E. T. (2005) 'Recovery of impaired songs following unilateral but not bilateral lesions of nucleus uvaeformis of adult zebra finches', *Journal of Neurobiology*, 63(1), pp. 70–89. doi: 10.1002/neu.20122.

Costalunga, G. *et al.* (2023) 'Wild nightingales flexibly match whistle pitch in real time', *Current Biology*, pp. 1–10. doi: 10.1016/j.cub.2023.06.044.

Dhawale, A. K., Smith, M. A. and Ölveczky, B. P. (2017) 'The Role of Variability in Motor Learning', *Annual Review of Neuroscience*, 40(1), pp. 479–498. doi: 10.1146/annurev-neuro-072116-031548.

Foster, E. F. and Bottjer, S. W. (2001) 'Lesions of a telencephalic nucleus in male zebra finches: Influences on vocal behavior in juveniles and adults', *Journal of Neurobiology*, 46(2), pp. 142–165. doi: 10.1002/1097-4695(20010205)46:2<142::AID-NEU60>3.0.CO;2-R.

Fujimoto, H., Hasegawa, T. and Watanabe, D. (2011) 'Neural coding of syntactic structure in learned vocalizations in the songbird', *Journal of Neuroscience*, 31(27), pp. 10023–10033. doi: 10.1523/JNEUROSCI.1606-11.2011.

Hahnloser, R. H. R., Kozhevnikov, A. A. and Fee, M. S. (2002) 'An ultra-sparse code underlies the generation of neural sequences in a songbird', *Nature*, 419(6902), pp. 65–70. doi: 10.1038/nature00974.

Hamaguchi, K., Tanaka, M. and Mooney, R. (2016) 'A Distributed Recurrent Network Contributes to Temporally Precise Vocalizations', *Neuron*, 91(3), pp. 680–693. doi: 10.1016/j.neuron.2016.06.019.

Hampton, C. M., Sakata, J. T. and Brainard, M. S. (2009) 'An avian basal ganglia-forebrain circuit contributes differentially to syllable versus sequence variability of adult Bengalese finch song', *Journal of Neurophysiology*, 101(6), pp. 3235–3245. doi: 10.1152/jn.91089.2008.

Honda, E. and Okanoya, K. (1999) 'Acoustical and Syntactical Comparisons between Songs of the White-backed Munia (Lonchura striata) and Its Domesticated Strain, the Bengalese Finch (Lonchura striata var. domestica)', *Zoological Science*, 16(2), pp. 319–326. doi: 10.2108/zsj.16.319.

Horita, H., Wada, K. and Jarvis, E. D. (2008) 'Early onset of deafening-induced song deterioration and differential requirements of the pallial-basal ganglia vocal pathway', *European Journal of Neuroscience*, 28(12), pp. 2519–2532. doi: 10.1111/j.1460-9568.2008.06535.x.

Hosino, T. and Okanoya, K. (2000) 'Lesion of a higher-order song nucleus disrupts phrase level complexity in Bengalese finches.', *NeuroReport*, 11(10), pp. 2091–5. doi: 10.1097/00001756-200007140-00007.

Hultsch, H. *et al.* (2004) 'Long-term persistence of song performance rules in nightingales (Luscinia megarhynchos): a longitudinal field study on repertoire size and composition', *Behaviour*, 141(3), pp. 371–390. doi: 10.1163/156853904322981914.

Isola, G. R., Vochin, A. and Sakata, J. T. (2020) 'Manipulations of inhibition in cortical circuitry differentially affect spectral and temporal features of Bengalese finch song', *Journal of Neurophysiology*, 123(2), pp. 815–830. doi: 10.1152/jn.00142.2019.

Ivanitskii, V. V. and Marova, I. M. (2022) 'The Syntactic Organization of Bird Song', *Biology Bulletin*, 49(8), pp. 1158–1170. doi: 10.1134/S1062359022080076.

Jaffe, P. I. and Brainard, M. S. (2020) 'Acetylcholine acts on songbird premotor circuitry to invigorate vocal output', *eLife*, 9, p. e53288. doi: 10.7554/eLife.53288.

Jin, D. Z. (2009) 'Generating variable birdsong syllable sequences with branching chain networks in avian premotor nucleus HVC', *Physical Review E*, 80(5), p. 051902. doi: 10.1103/PhysRevE.80.051902.

Jin, D. Z. and Kozhevnikov, A. A. (2011) 'A Compact Statistical Model of the Song Syntax in Bengalese Finch', *PLOS Computational Biology*, 7(3), p. e1001108. doi: 10.1371/journal.pcbi.1001108.

Kao, M. H. and Brainard, M. S. (2006) 'Lesions of an Avian Basal Ganglia Circuit Prevent Context-Dependent Changes to Song Variability', *Journal of Neurophysiology*, 96(3), pp. 1441–1455. doi: 10.1152/jn.01138.2005.

Kao, M. H., Doupe, A. J. and Brainard, M. S. (2005) 'Contribution of an avian basal ganglia-forebrain circuit to real-time modulation of song', *Nature*, 433(7026), pp. 638–643. doi: 10.1038/nature03127.

Katahira, K. *et al.* (2011) 'Complex Sequencing Rules of Birdsong Can be Explained by Simple Hidden Markov Processes', *PLOS ONE*, 6(9), p. e24516. doi: 10.1371/journal.pone.0024516.

Katahira, K. *et al.* (2013) 'A simple explanation for the evolution of complex song syntax in Bengalese finches', *Biology Letters*, 9(6), pp. 3–7. doi: 10.1098/rsbl.2013.0842.

Kobayashi, K., Uno, H. and Okanoya, K. (2001) 'Partial lesions in the anterior forebrain pathway affect song production in adult Bengalese finches', *NeuroReport*, 12(2), pp. 353–358. doi: 10.1097/00001756-200102120-00034.

Kojima, S. *et al.* (2018) 'The Avian Basal Ganglia Are a Source of Rapid Behavioral Variation That Enables Vocal Motor Exploration', *Journal of Neuroscience*, 38(45), pp. 9635–9647. doi: 10.1523/JNEUROSCI.2915-17.2018.

Kubikova, L. *et al.* (2014) 'Basal ganglia function, stuttering, sequencing, and repair in adult songbirds', *Scientific Reports*, 4. doi: 10.1038/srep06590.

Kubikova, L., Turner, E. A. and Jarvis, E. D. (2007) 'The pallial basal ganglia pathway modulates the behaviorally driven gene expression of the motor pathway', *European Journal of Neuroscience*, 25(7), pp. 2145–2160. doi: 10.1111/j.1460-9568.2007.05368.x.

Lashley, K. S. (1951) 'The problem of serial order in behavior.', in *Cerebral Mechanisms in Behavior*. Oxford: Bobbs-Merrill, pp. 112–146.

Lipkind, D. *et al.* (2013) 'Stepwise acquisition of vocal combinatorial capacity in songbirds and human infants', *Nature*, 498(7452), pp. 104–108. doi: 10.1038/nature12173.

Long, M. A. and Fee, M. S. (2008) 'Using temperature to analyse temporal dynamics in the songbird motor pathway', *Nature*, 456(7219), pp. 189–194. doi: 10.1038/nature07448.

Lynch, G. F. *et al.* (2016) 'Rhythmic Continuous-Time Coding in the Songbird Analog of Vocal Motor Cortex', *Neuron*, 90(4), pp. 877–892. doi: 10.1016/j.neuron.2016.04.021.

McCasland, J. (1987) 'Neuronal control of bird song production', *Journal of Neuroscience*, 7(1), pp. 23–39. doi: 10.1523/JNEUROSCI.07-01-00023.1987.

McInnes, L. *et al.* (2018) 'UMAP: Uniform Manifold Approximation and Projection', *Journal of Open Source Software*, 3(29), p. 861. doi: 10.21105/joss.00861.

Miller, M. N., Cheung, C. Y. J. and Brainard, M. S. (2017) 'Vocal learning promotes patterned inhibitory connectivity', *Nature Communications*, 8(1). doi: 10.1038/s41467-017-01914-5.

Moll, F. W. *et al.* (2023) 'Thalamus drives vocal onsets in the zebra finch courtship song', *Nature*, 616(June 2022). doi: 10.1038/s41586-023-05818-x.

Morita, T. *et al.* (2021) 'Measuring context dependency in birdsong using artificial neural networks', *PLOS Computational Biology*, 17(12), p. e1009707. doi: 10.1371/journal.pcbi.1009707.

Nottebohm, F., Stokes, T. M. and Leonard, C. M. (1976) 'Central control of song in the canary, Serinus canarius', *Journal of Comparative Neurology*, 165(4), pp. 457–486. doi: 10.1002/cne.901650405.

Okanoya, K. and Yamaguchi, A. (1997) 'Adult bengalese finches (Lonchura striata var.domestica) require real-time auditory feedback to produce normal song syntax', *Journal of Neurobiology*, 33(4), pp. 343–356. doi: 10.1002/(SICI)1097-4695(199710)33:4<343::AID-NEU1>3.0.CO;2-A.

Ölveczky, B. P. *et al.* (2011) 'Changes in the neural control of a complex motor sequence

during learning', *Journal of Neurophysiology*, 106(1), pp. 386–397. doi: 10.1152/jn.00018.2011.

Otchy, T. M. *et al.* (2015) 'Acute off-target effects of neural circuit manipulations', *Nature*, 528(7582), pp. 358–363. doi: 10.1038/nature16442.

Picardo, M. A. *et al.* (2016) 'Population-Level Representation of a Temporal Sequence Underlying Song Production in the Zebra Finch', *Neuron*, 90(4), pp. 866–876. doi: 10.1016/j.neuron.2016.02.016.

Rajan, R. and Doupe, A. J. (2013) 'Behavioral and Neural Signatures of Readiness to Initiate a Learned Motor Sequence', *Current Biology*, 23(1), pp. 87–93. doi: 10.1016/j.cub.2012.11.040.

Sainburg, T., Thielk, M. and Gentner, T. Q. (2020) 'Finding, visualizing, and quantifying latent structure across diverse animal vocal repertoires', *PLOS Computational Biology*, 16(10), pp. 1–48. doi: 10.1371/journal.pcbi.1008228.

Sakata, J. T. and Brainard, M. S. (2006) 'Real-time contributions of auditory feedback to avian vocal motor control', *Journal of Neuroscience*, 26(38), pp. 9619–9628. doi: 10.1523/JNEUROSCI.2027-06.2006.

Sakata, J. T. and Brainard, M. S. (2008) 'Online contributions of auditory feedback to neural activity in avian song control circuitry', *Journal of Neuroscience*, 28(44), pp. 11378–11390. doi: 10.1523/JNEUROSCI.3254-08.2008.

Scharff, C. and Nottebohm, F. (1991) 'A comparative study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning.', *Journal of Neuroscience*, 11(9), pp. 2896–913. doi: 10.1523/JNEUROSCI.11-09-02896.1991.

Schmidt, M. F., Ashmore, R. C. and Vu, E. T. (2004) 'Bilateral control and interhemispheric coordination in the avian song motor system', in *Annals of the New York Academy of Sciences*. New York Academy of Sciences, pp. 171–186. doi: 10.1196/annals.1298.014.

Seki, Y. and Okanoya, K. (2008) 'Functional evidence for internal feedback in the songbird brain nucleus HVC', *NeuroReport*, 19(6), pp. 679–682. doi: 10.1097/WNR.0b013e3282fb7dae.

Simpson, H. and Vicario, D. (1990) 'Brain pathways for learned and unlearned vocalizations differ in zebra finches', *Journal of Neuroscience*, 10(5), pp. 1541–1556. doi: 10.1523/JNEUROSCI.10-05-01541.1990.

Sober, S. J. and Brainard, M. S. (2012) 'Vocal learning is constrained by the statistics of sensorimotor experience', *Proceedings of the National Academy of Sciences*, 109(51), pp. 21099–21103. doi: 10.1073/pnas.1213622109.

Sober, S. J., Wohlgemuth, M. J. and Brainard, M. S. (2008) 'Central contributions to acoustic variation in birdsong', *Journal of Neuroscience*, 28(41), pp. 10370–10379. doi: 10.1523/JNEUROSCI.2448-08.2008.

Stepanek, L. and Doupe, A. J. (2010) 'Activity in a Cortical-Basal Ganglia Circuit for Song Is Required for Social Context-Dependent Vocal Variability', *Journal of Neurophysiology*, 104(5), pp. 2474–2486. doi: 10.1152/jn.00977.2009.

Suge, R. and Okanoya, K. (2010) 'Perceptual chunking in the self-produced songs of

Bengalese finches (Lonchura striata var. domestica)', *Animal Cognition*, 13(3), pp. 515–523. doi: 10.1007/s10071-009-0302-4.

Tanaka, M. *et al.* (2016) 'Focal expression of mutant huntingtin in the songbird basal ganglia disrupts cortico-basal ganglia networks and vocal sequences', *Proceedings of the National Academy of Sciences*, 113(12), pp. E1720–E1727. doi: 10.1073/pnas.1523754113.

Tanji, J. (2001) 'Sequential Organization of Multiple Movements: Involvement of Cortical Motor Areas', *Annual Review of Neuroscience*, 24(1), pp. 631–651. doi: 10.1146/annurev.neuro.24.1.631.

Tian, L. Y. and Brainard, M. S. (2017) 'Discrete Circuits Support Generalized versus Context-Specific Vocal Learning in the Songbird', *Neuron*, 96(5), pp. 1168-1177.e5. doi: 10.1016/j.neuron.2017.10.019.

Tian, L. Y., Warren, T. L. and Brainard, M. S. (2022) 'Dynamic top-down biasing implements rapid adaptive changes to individual movements', *bioRxiv*, p. 2022.06.16.496455. doi: https://doi.org/10.1101/2022.06.16.496455.

Tumer, E. C. and Brainard, M. S. (2007) 'Performance variability enables adaptive plasticity of "crystallized" adult birdsong.', *Nature*, 450(7173), pp. 1240–4. doi: 10.1038/nature06390.

Vates, G. E., Vicario, D. S. and Nottebohm, F. (1997) 'Reafferent thalamo- "cortical" loops in the song system of oscine songbirds.', *Journal of Comparative Neurology*, 380(2), pp. 275–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9100137.

Veit, L. *et al.* (2021) 'Songbirds can learn flexible contextual control over syllable sequencing', *eLife*, 10, pp. 1–19. doi: 10.7554/eLife.61610.

Vu, E. T., Mazurek, M. E. and Kuo, Y. C. (1994) 'Identification of a forebrain motor programming network for the learned song of zebra finches', *Journal of Neuroscience*, 14(11 II), pp. 6924–6934. doi: 10.1523/jneurosci.14-11-06924.1994.

Vyssotski, A. L. *et al.* (2016) 'A Neural Code That Is Isometric to Vocal Output and Correlates with Its Sensory Consequences', *PLOS Biology*, 14(10), pp. 1–21. doi: 10.1371/journal.pbio.2000317.

Wang, C. Z. H. *et al.* (2008) 'Rapid interhemispheric switching during vocal production in a songbird', *PLOS Biology*, 6(10), pp. 2154–2162. doi: 10.1371/journal.pbio.0060250.

Wang, H. *et al.* (2019) 'Transcriptional regulatory divergence underpinning species-specific learned vocalization in songbirds', *PLOS Biology*, 17(11), p. e3000476. doi: 10.1371/journal.pbio.3000476.

Warren, T. L. *et al.* (2011) 'Mechanisms and time course of vocal learning and consolidation in the adult songbird', *Journal of Neurophysiology*, 106(4), pp. 1806–1821. doi: 10.1152/jn.00311.2011.

Warren, T. L. *et al.* (2012) 'Variable sequencing is actively maintained in a well learned motor skill', *Journal of Neuroscience*, 32(44), pp. 15414–15425. doi: 10.1523/JNEUROSCI.1254-12.2012.

Williams, S. M., Nast, A. and Coleman, M. J. (2012) 'Characterization of Synaptically Connected Nuclei in a Potential Sensorimotor Feedback Pathway in the Zebra Finch Song System', *PLOS ONE*, 7(2), p. e32178. doi: 10.1371/journal.pone.0032178.

Wiltschko, A. B. *et al.* (2015) 'Mapping Sub-Second Structure in Mouse Behavior', *Neuron*, 88(6), pp. 1121–1135. doi: 10.1016/J.NEURON.2015.11.031.

Wittenbach, J. D. *et al.* (2015) 'An Adapting Auditory-motor Feedback Loop Can Contribute to Generating Vocal Repetition', *PLOS Computational Biology*, 11(10), p. e1004471. doi: 10.1371/journal.pcbi.1004471.

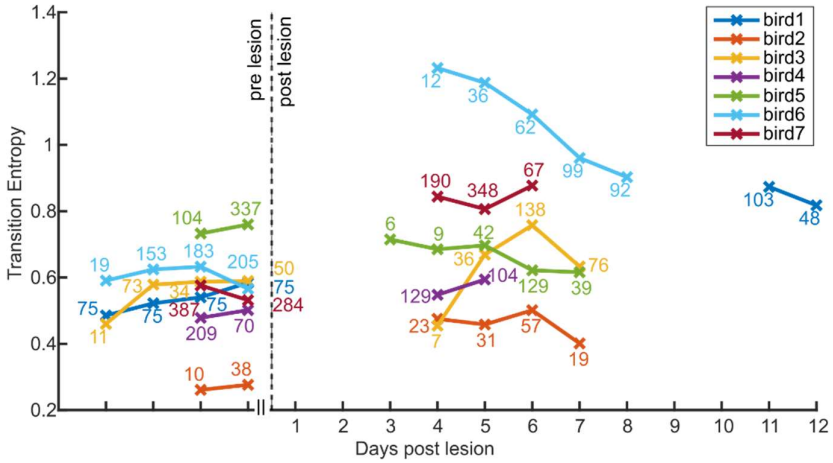
Wohlgemuth, M. J., Sober, S. J. and Brainard, M. S. (2010) 'Linked control of syllable sequence and phonology in birdsong', *Journal of Neuroscience*, 30(39), pp. 12936–12949. doi: 10.1523/JNEUROSCI.2690-10.2010.

Xiao, L. *et al.* (2021) 'Expression of FoxP2 in the basal ganglia regulates vocal motor sequences in the adult songbird', *Nature Communications*, 12(1). doi: 10.1038/s41467-021-22918-2.

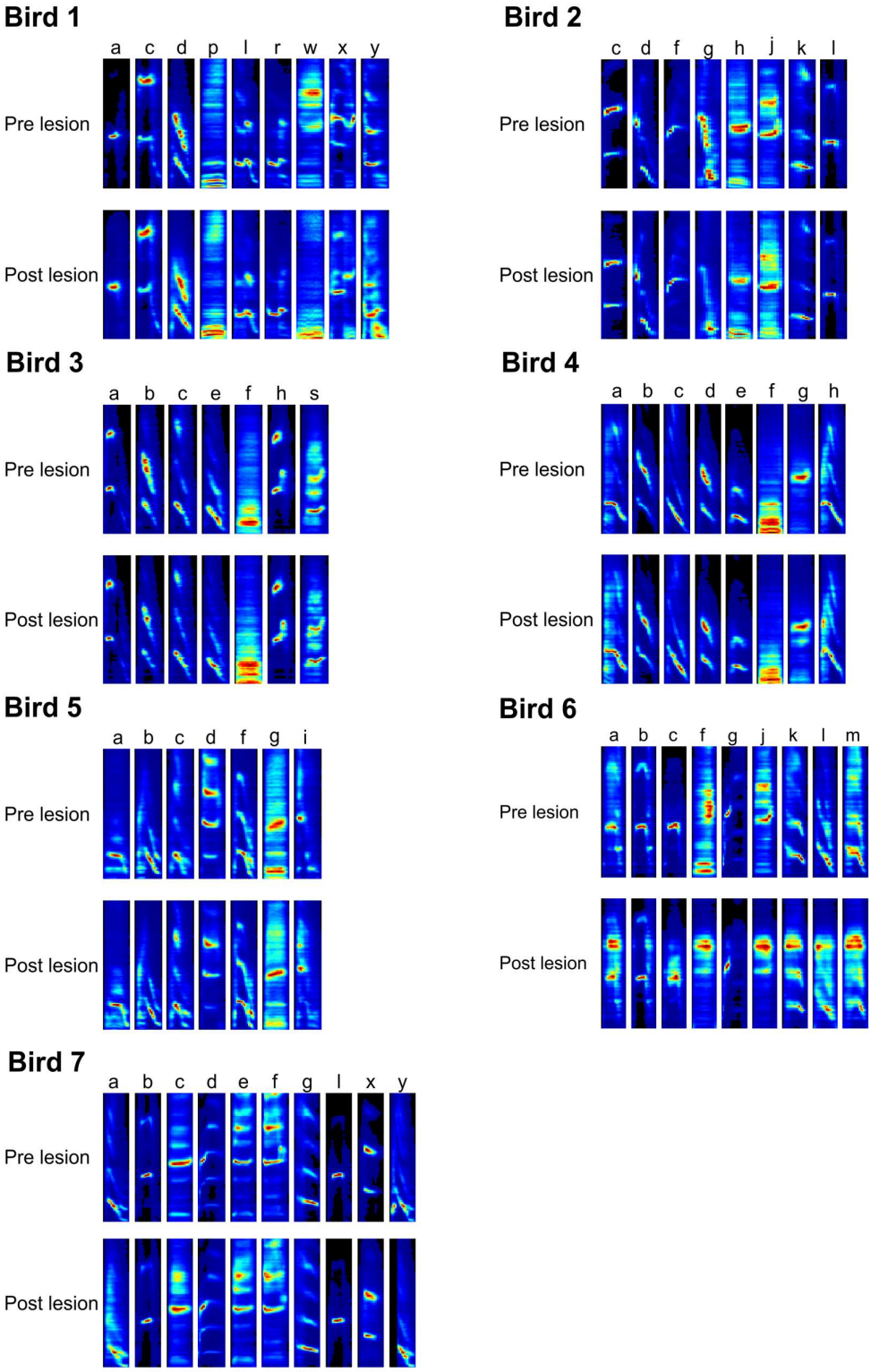
Zann, R. A. (1996) *The Zebra Finch: A Synthesis of Field and Laboratory Studies*, *Oxford ornithology series*. Oxford University Press. Available at: https://www.journals.uchicago.edu/doi/10.1086/420003.

Zhang, Y. S. *et al.* (2017) 'Temperature manipulation in songbird brain implicates the premotor nucleus HVC in birdsong syntax', *Journal of Neuroscience*, 37(10), pp. 2600–2611. doi: 10.1523/JNEUROSCI.1827-16.2017.

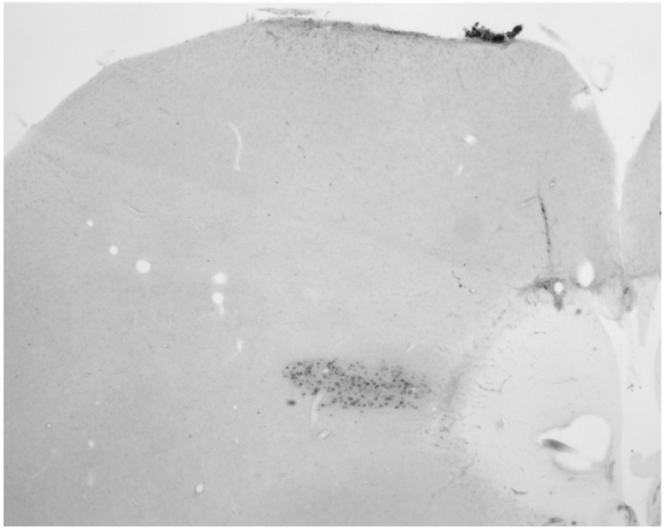
Supplementary Figs:



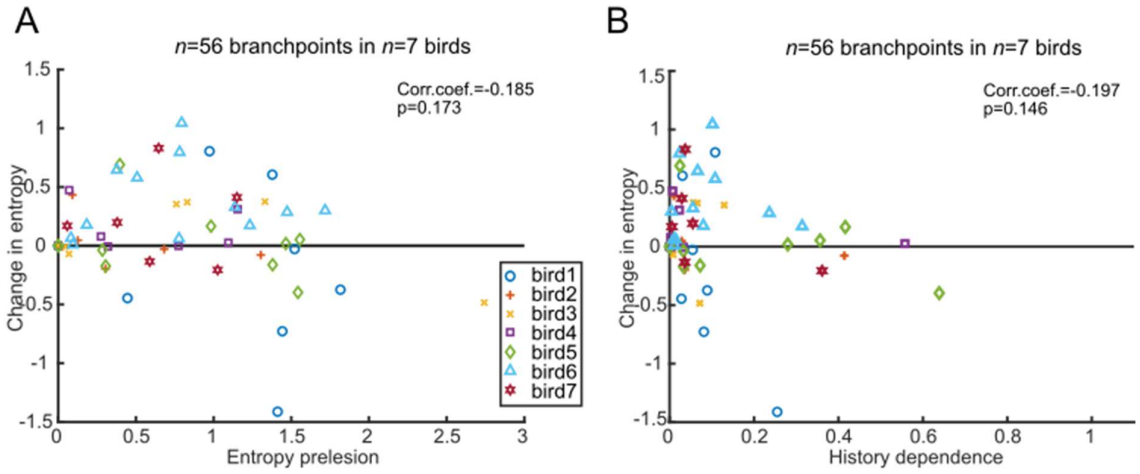
Sup. Figure 1: Increase in transition entropy after mMAN lesions remains consistent over several days. Transition entropy did not change in a consistent way after recovery from the lesion for the followed time period. Numbers near the datapoints indicate number of song bouts recorded and analyzed for that day.



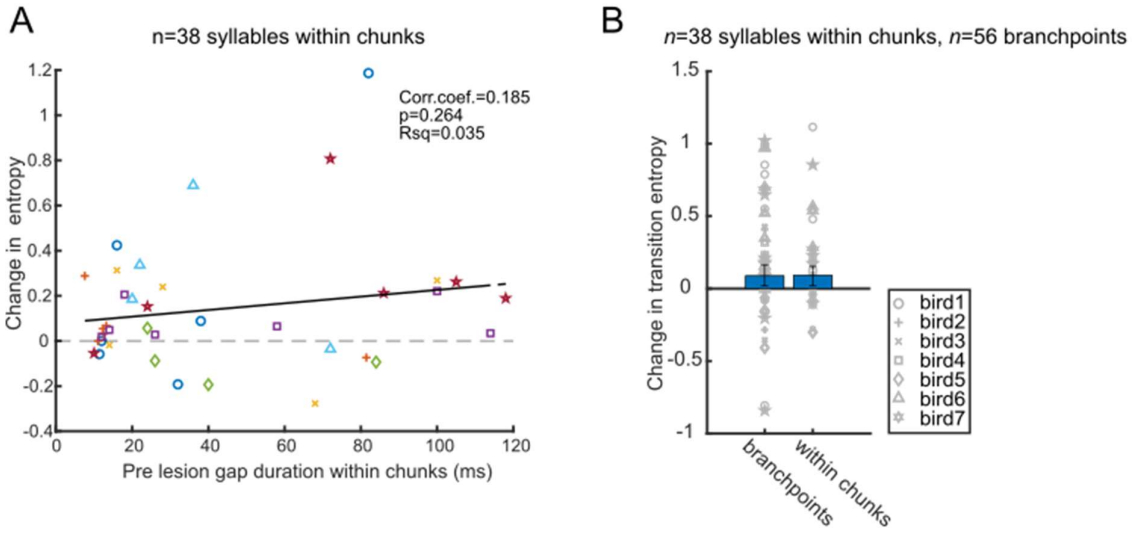
Sup. Figure 2: Syllable types are preserved after mMAN lesions. Average spectrograms of 200 instances of all syllable types for all birds before and after mMAN lesions. Letters over the spectrograms indicate syllable labels.



Sup. Figure 3: Example image of calcitonin gene-related peptide (CGRP)-stained frontal section of bird 5. CGRP labels cells in both lMAN (seen in black to the left of the lesion) and mMAN (completely destroyed).



Sup. Figure 4: Change in transition entropy for branchpoints (A) Transition entropy increased for branchpoints after mMAN lesions. (B) Branchpoints with low history dependence before mMAN lesions tended to show a higher change in transition entropy.



Sup. Figure 5: Change in transition entropy for transitions within chunks vs. branch points (A) Duration of gaps within chunks before mMAN lesions was weakly correlated with entropy change at the corresponding transition. (B) Change in transition entropy was approximately equal for transitions within chunks and at branchpoints.