Understanding the characteristics of Introductory notes in the presence and absence of HVC

A thesis submitted by

Prasanth P

For the study conducted under the guidance of

DR. RAGHAV RAJAN

At the

Department of Biology,

Indian Institute of Science Education and Research, Pune

Certificate

This is to certify that this dissertation entitled "**Understanding the characteristics of Introductory notes in the presence and absence of HVC**" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **PRASANTH P**, at **IISER, Pune** under the supervision of **Dr. Raghav Rajan, Department of Biology, IISER, Pune** during the academic year **2016-17**.

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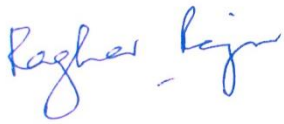

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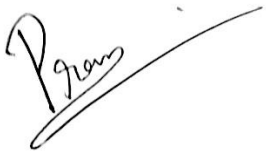
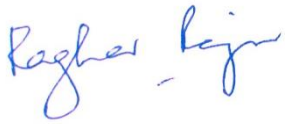
Declaration

I hereby declare that the matter embodied in the report entitled "**Understanding the characteristics of Introductory notes in the presence and absence of HVC**" are the results of the work carried out by me at the **Department of Biology, Indian Institute of Science Education and Research, Pune** under the supervision of **Dr. Raghav Rajan**, and the same has not been submitted elsewhere for any other degree.





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ABSTRACT

The Introductory notes (IN), a series of identical vocal elements which are produced at the beginning of a zebra Finch song, is considered important for the initiation of the song. However, the brain region responsible for IN production is not known. HVC, a song premotor nucleus responsible for song production of which INs are also part of, is known to show neural activity just before INs are produced. Hence to study the role of HVC in IN production, HVC lesions were done and the post-lesion song was studied. Before that, to identify the presence of IN in post-lesion songs and study the effects of HVC lesion on IN, the characteristics of IN in normal birds in which HVC is present were quantified. We found that in a normal bird song INs has a high probability to appear at the first 2-3 positions of a song. The INs were found to have a definite set of acoustic properties which in general are similar to short calls and no other song syllables. Also, the first few positions in a song are distinct from other song syllables in terms of its duration and spacing. In the absence of HVC, the INs are no longer acoustically identical to normal INs, while the distinction of beginning few positions from other song syllables in terms of it duration and spacing remains the same as before. Thus, acoustic properties of INs is controlled by HVC, while the duration, initiation, and spacing of INs are independent of HVC.

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Acknowledgments

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INTRODUCTION

Zebra finches are one of the most commonly studied organisms for understanding human vocalizations and their production, through the bird's song production and song learning abilities.

Bird Song

All kinds of sounds produced by zebra finches are referred to as vocalizations. A Zebra Finch's entire vocal repertoire is highly complex and consists many types of vocalizations. The entire vocalizations can be broadly divided two types: calls and songs. Calls can be further divided into short calls and long calls based on their duration.[1] A call can appear in isolation with no other syllables produced near it, or as a series of calls (set of calls within 500ms) or as a part of the song. The song is a stereotyped repeated sets of syllables sang in a specific sequence. Songs consist of many motifs which are the most repeated segment of a syllable. Thus, an entire song can also consist of multiple motifs. Songs also have a series of short duration identical notes at the beginning of the first motif which is called Introductory Notes (IN). Thus, the song consists of motif syllables (syllables which are part of the motif), INs and some calls. A set of continuous syllables with 500ms of silence before the first and after the last syllable were considered as a bout (Aronov et al., 2008).

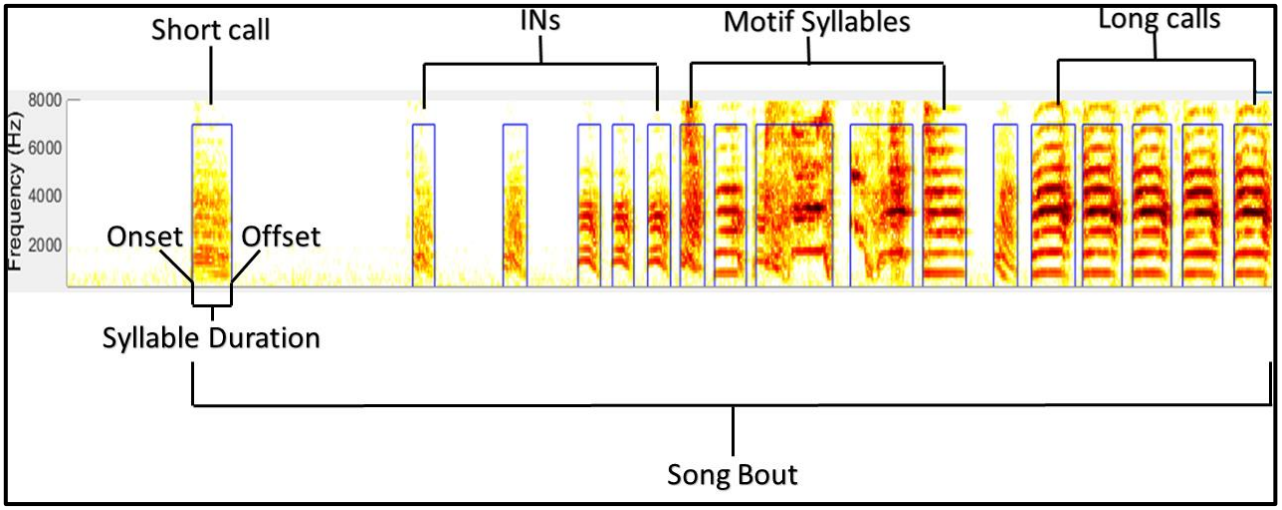

Figure 1: A sample spectrogram of a zebra finch song; different syllable categories, syllable duration and song bout has been represented in the picture

[1] A duration of a syllable is the time difference between the onset and offset of the syllable (see figure 1)

An important feature of Zebra Finch song is the production of a series of introductory notes (IN) preceding every song. INs can be defined as an Identical or stereotypical short vocalization produced repetitively at the beginning of the song based on the commonly observed patterns of the INs. But, the acoustics properties and its similarity or difference with other song syllables have never been quantified. The variability in the number of introductory notes and its features helps the bird attain the 'ready' state to initiate its song.(Rajan and Doupe, 2013)However, it is still not known which brain pathway is responsible for IN production and whether it is similar to the normally learned song production.

Dual Pathway

Juvenile birds reared in isolation, produced songs (untutored songs) which were different from that of the normal bird song (Williams, 1993) but still produced INs which were distinct from the rest of the song syllable(Price, 1979). This indicates that the INs are not learned and are primarily an innate vocalization. An interesting study has shown that the learned components of calls and songs have a separate brain pathway, which involves primarily pre-motor nuclei-syrinx pathway or a motor pathway other than that of the unlearned female vocalizations which do not have songs.(Simpson, 1990). Female vocalizations consist of only calls and hence calls, especially short calls, are mostly unlearned vocalizations. Also, when the dorsomedial nucleus of the intercollicular complex (DM) was stimulated, a long call like elements was produced and when DM was lesioned no long calls (distance calls) were produced(Fukushima and Aoki, 2000). All these together indicates a fact that there indicates that there exists two separate pathways: one involving HVC, a premotor nucleus, for song production and another involving DM for a call or unlearned vocalizations. (Figure 2) Even though INs are considered as a part of the song, it is also an innate or unlearned vocalization. This brings the questions where do INs come from? Are they produced by the song pathway or otherwise by the call pathway. In that case, if a separate pathway exists for learned and unlearned vocalizations then the brain region important for IN production, which is an unlearned vocalization, could be part of the call pathway or it could just be produced along with the songs from the song or learned vocalization motor pathway.

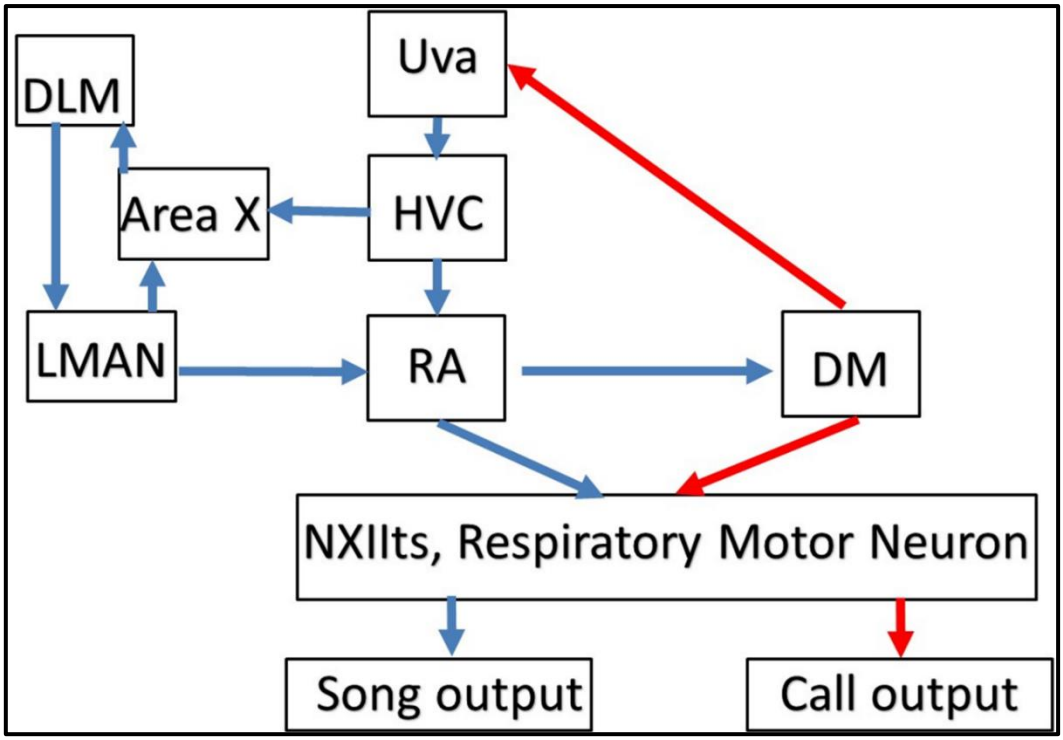

Figure 2 : A visualization of the dual pathway for song and call production; the blue arrows correspond to the Song or learned pathway and the red ones are for the unlearned ones. The learned pathway results in song output while the unlearned one is responsible for some calls and other innate vocalizations.

Effects of HVC lesion on bird song

HVC is considered as a central region of a song pathway and if we need to identify whether INs are produced by the song pathway or not, HVC could be the primary candidate for the lesion. HVC lesions in adults are known to produce noisy or "crappy" songs which differ from normal adult songs and do not have a definite structure (Aronov et al., 2008). (Figure 3) Neuron activity was seen during IN vocalizations in song premotor nucleus HVC (High vocal Center) which ended just before the song(Rajan and Doupe, 2013).

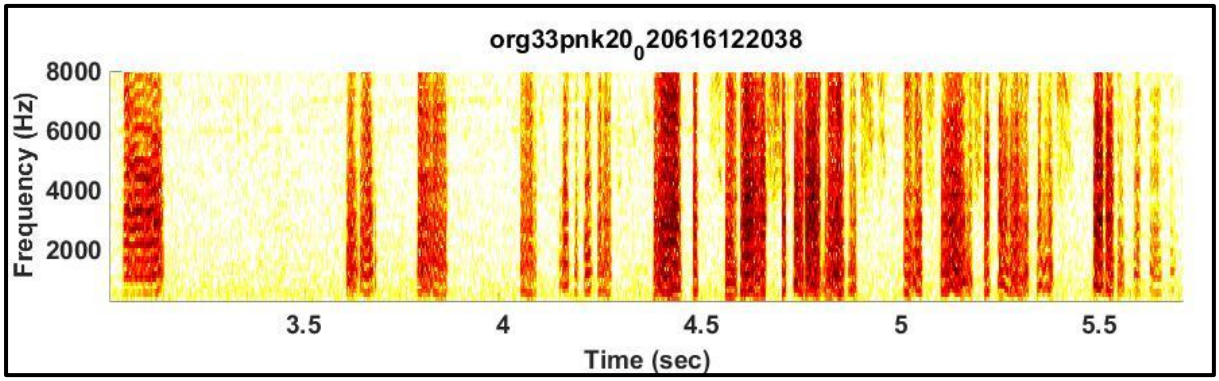

Figure 3: An example of a post lesion song.Notice that the syllables cannot be recognized from each other and the frequency patterns are homogeneous; hence noisy.

The songs produced after lesion are noisy[2] and these syllables are unidentifiable. Even if INs are still there after HVC lesion we will not be able to identify them just based on the spectrogram patterns. We need to quantify the features and properties of IN in Normal birds which we can rely on and can be used to identify the presence of IN in lesioned bird songs.

Aim and Hypothesis

Our aim of this project can primarily be split into two:

1. To quantify the properties of IN in normal birds with respect to other syllables, position in the bout and acoustic properties.
2. To study the role HVC in IN production by studying HVC lesioned birds.

We would be doing HVC lesion to study the role of song pathway in IN production. This could possibly have these three effects on song initiation:

1. The “Introductory Notes (IN) will not be produced at all and only variable unstructured vocalizations will be produced as described by *Aronov et al*.
2. The INs will be produced and will be noisy (similar to motifs), thus not differentiable from the motif. (not differentiable from motif).
3. The INs will be produced but will be different from the rest of the unstructured vocalizations. (differentiable IN and Song).

Methodology

All procedures and experiments were performed according to the guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi and with prior approval of the Institutional Animal Ethical Committee (IAEC), IISER Pune.

[2] Noisy means the harmonic stacks in a spectrogram become less clear and duration of the syllables in a bout varies a lot.

Song Recordings

A zebra finch is isolated from its colony 2-3 days before the recordings and kept in a sound box[3] for it to get adjusted to the new environment. The bird's vocalization is recorded over different days while keeping the bird isolated within the sound box. Two sets of birds[4] were used for recording:

i) Normal birds without any surgery or Lesion (sample size, n =16 birds); where 14 birds recorded at our lab while 2 birds were recorded by Raghav @ UCSF.

ii) HVC lesioned birds (sample size, n=8 birds); where 3 birds are recorded in our lab and the recordings for the rest of the 5 HVC lesioned birds has been obtained from C*hen et al* which they used to study "the role of basal ganglia and thalamus in vocal structure, tempo, and initiation". Their methods of recording are similar to ours and the detailed procedure has been given in their paper.(Chen et al., 2014).In post lesion recording, the bird is recorded continuously for an entire day from the first day after surgery till it starts singing good amount of songs per day which mostly after a 2-3 week. Then recorded on occasional days until we get enough data for analysis.

The recording was done using a microphone attached to the top of the bird's cage kept inside the sound box. All the recordings are undirected in nature which means the songs recorded are those which birds produced when it is alone and they are not directed towards a female. Even though directed recordings have been done, they have not been used for current analysis.

Surgical Procedure

Prior to surgery, a bird is given dosages of certain analgesics and anesthetics based on their body weight. Initially, an analgesics, Meloxicam (dosage = 0.25mg/kg) is given orally to the bird and later, after about 40 minutes, the bird is injected with three anesthetics: Ketamine (30mg/kg), Xylazine (3mg/kg) and Diazepam (7mg/kg)

[3] Acoustically isolated boxes used for recording bird songs to obtain least noisy recordings.

[4] For the current set of analysis, there is no overlap of birds between these two sets of birds. Normal bird song will help us to categorize and analyze INs in general irrespective of individual differences between birds and Lesion birds will help us to understand the role of HVC in IN production.

respectively. The bird is then placed in a stereotaxic apparatus in which its head is fixed to beak angle 50 degrees along the vertical axis. Prior to removing the skin over its head off and other layers of the skull, a local anesthetic, Lignocaine (100-200µl of Lox2%) is injected subcutaneously. The posterior divergence of the central sinus is considered as the origin. Now the center HVC is located using a Tungsten Microelectrode as 2.4mm from the origin along both the medial-lateral (ML) axis and no change in the anterior-posterior (AP) coordinates. Around this center, we will make a 4 X 3 grid with a spacing of 0.4mm among the points and at a depth of 0.4mm below the surface of the brain. At each of these coordinates, a constant current of 10µA is passed through the inserted electrode for 100 seconds. After completing the above process on both hemispheres, the bird's exposed skull is fully covered by applying dental-cement (dpi rr self-cure). The bird is kept under a heat lamp and constantly monitored until it completely recovers from the surgery. Then it is transferred back to the sound box for recordings.

The surgical procedure of the HVC lesion birds obtained from *Chen et al* are mostly similar to the above procedure except they used a 3 X 5 grid around the center of HVC. All other details of their procedure can be found in their paper. (Chen et al., 2014)

Vocalization categorization, bouts, and labeling

Songs and other vocalizations are labeled by going through the spectrograms and labeling them into different syllable based on the appearance of their spectrogram and repetition. Thus, for every bird, there will be a set of syllable labels distinct from each other which are part of their vocalizations such as w, i,j, a,b,c,d,e and so on (see figure 4). Among some of them will be called, some will be INs and others will be part of the syllable. Thus, the labeling of the syllables is based on a prior knowledge of what these syllable types look like and an experience of seeing the same syllables repeated and produced across the bird’s entire day of vocalizations.

For analysis purposes the vocalizations of a bird are classified into four broad categories:

1) Short call
2) Long call

3) Motif syllables
4) Introductory Notes (IN)

Most of the analysis is focussed on primarily song syllables and we need to have a definite idea what are we considering as song bouts. In the post-lesion song, due to his crappy nature, it would be difficult to identify and recognize syllables and their repetition and songs could only be seen as a series of vocalizations. Thus, it is important to have a sense of what a song will be when we don't have identifiable syllables and well-defined sequences. For that, we kept a 500ms sec rule to identify a bout. Any series of vocalizations which has an Inter-syllable[5] Interval among the syllables is less than 500ms and has a silence of 500ms before and after the beginning and end of the bout can be considered as a bout. Now, such a series of vocalization can be composed of any of the above syllables and based on what kind of syllables there is in a bout they are recognized as song bout, long call bout, and short call bout.

In Normal birds, Long call bout and Short call bout are those which have exclusively long call and short call respectively. While, Song bouts are those which has motif syllables, INs other than Long calls and short calls. (Figure 4)

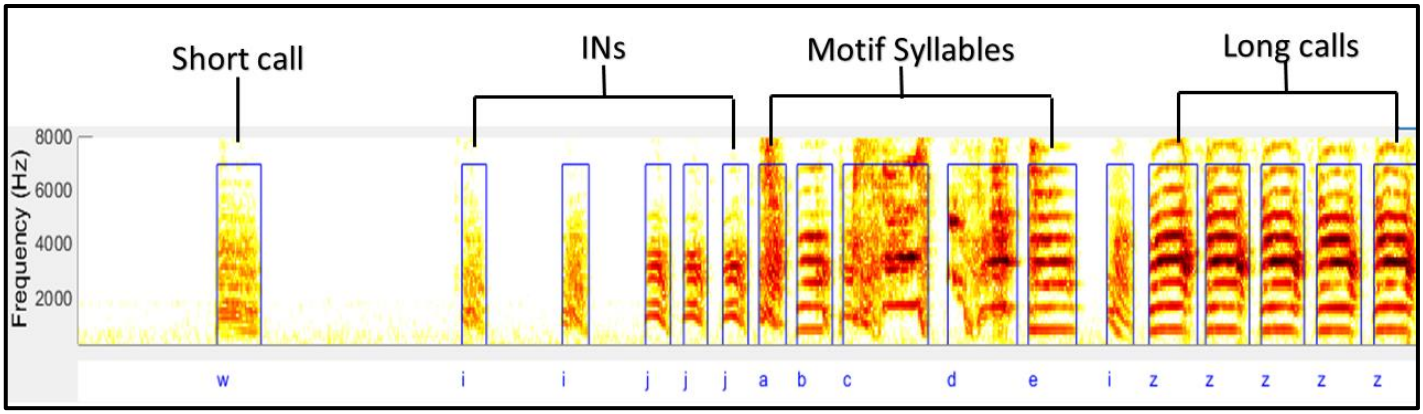

Figure 4: An example of how syllables are categorized within a song bout in a normal bird. i,j,a,b,c,d,e,z and w are labels for different syllables and syllable category they belong to indicated above.

In Lesioned bird, Long call bouts and short call bouts remain the same, while song bouts will be those which has many unidentifiable, noisy syllable along with the post-

[5] Inter-syllable interval is the duration of the gap (space of silence) between two syllables calculated as a difference between the onset of the second syllable and the offset of the first syllable of any two consecutive syllables.

lesion short calls and long calls. The short call and long calls identified as those which look similar to post-lesion short calls and long calls (Figure 5)

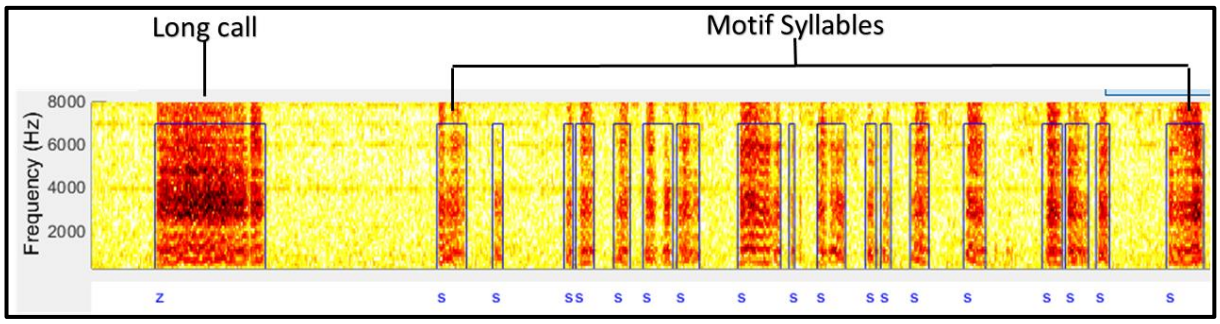

Figure 5 An example of how syllables are categorized within a song bout in a lesionedl bird. Notice that almost all the syllables which are part of the song bout is labelled as motif syllables as they are undistiniguishable from each other in the spectrogram.One syllable, though, is labelled long call based on its similarity to other long calls produced indepently by the bird after the lesion.

Criteria for Analysis

Based on the labeling done on the spectrograms, a set of 9 acoustic parameters were calculated which are used to do different types of analysis. The 9 bird parameters that were quantified for all the syllables are:

Duration, Log Amplitude – a measure of loudness, Entropy – accounts for the noisiness of the syllable, Mean Frequency, Amplitude Modulation, Pitch Goodness, Frequency Modulation, Entropy Variance and Mean Fundamental Frequency. (Tchernichovski et al., 2000)

(for more detailed definition go to URL: http://soundanalysispro.com/)

To minimize the error in labeling and noisy syllables, outliers were removed by removing

Any sample of a syllable having any feature value above its 75th percentile + 3* Interquartile range (IQR)

Any sample of a syllable having any feature value below 25th percentile – 3* Interquartile range (IQR)

Normal Bird Analysis

Position probability and syllable types

To analyze is the position of particular syllable category (Short call, Long call, IN and Motif syllables). Here, we will consider all bouts and calculate the probability of that

syllable category occurring at each of the positions within a bout. To test for significance, we will consider the same set of labels for each bout, but scramble them up within a bout and now calculate the probability of an IN at each position. We will do this 1000 time and then take the middle 95% percent as the confidence interval - anything outside this will be significant.

For example, 4 bouts of a bird with sequences as follows:

i i i i a b c d a b

i i i a b c d a i

i i i i a b c

i i a b c d a b i

All labels are pooled together, jumbled up and randomly assigned to any bout and any position irrespective of which bout or position it earlier belongs to. This is iterated for 1000 times and each time the new probability for each position is calculated and 95% confidence interval is calculated for all positions.

The probability significance for each position is categorized not significant if the probability is within the 95% confidence interval, “>chance” if it above 95% confidence interval coefficient value, and “<chance” if it is below 95% confidence interval.

Dimension reduction

i) Individual Feature PCA

We want to do a Principal component Analysis (PCA) as a dimension reduction analysis on all features of syllables and then compare the similarity of INs to all other categories of syllables - motif syllables, short calls and long calls. Consider only vocalizations that are part of song bouts - for this, we will include only birds that have enough short calls and long calls along with INs and motif syllables in their song bouts. We will not include only Fundamental Frequency (FF) in the list of features for PCA. Now go through and check if all syllables have at least a certain number of renditions (minimum trial) is kept as 20.Now for part 1 of analysis, all the syllables are normalized by its mean (z score). The dimension reduction is done on the Z score and the following values are calculated: PCA coefficient, PCA score and % of

variance explained per principal components are calculated. As the first four principal components (PC1, PC2, PC3 and PC4) explains the 75- 85% of the variance.

The Euclidean distance of INs from other syllables is calculated.

The shortest distance from an introductory note to a syllable in each of the three other syllable category is plotted and the minimum distance of INs to syllables of all the categories is plotted. In the minimum distance plots, to check the significance- Kruskal-Wallis test and posthoc Tukey-Kramer test ($p < 0.05$) is done. The minimum distance for each IN and see what type of syllable it is. (Excluding INs) .

i) All syllable PCA

This PCA analysis is done after combining syllables from all birds. We will take all valid bouts and take all syllables from these bouts. Put them all together across birds and normalize the whole thing and then do a PCA. Only 100 syllables of each type are kept since we have a lot of data.

Again the first 4 PCs and calculate distances within and between clusters.Now to check what is the minimum distance based on random assignment of syllables to the different categories.

The boundary of the IN's and all other syllable category's ellipse are calculated which would be later used to identify INs in post-lesion PCA analysis.

In the normal bird data, I have calculated distances in two ways.

1) I take the centroid of the cluster of all INs. Then I calculate the mean distance of all points of a particular category from the centroid of the IN cluster - this is the first plot that shows up for distance

2) I take the centroid of the clusters of the different types of syllables and then measure the mean distance of all INs from these centroids - this is the second plot.

In both cases, the dotted lines are the 95% confidence limits calculated by randomly re-assigning points to different clusters and repeating the same distance calculations as above (10000 times).

Plot the all bird PCA with pre-determined PCA dimensions and add the ellipse of IN from normal bird analysis.

Syllable Duration v/s Position

To analyze the distribution of syllable duration at different positions within a bout. The frequency distribution of syllable duration is plotted. Also, we want to correlate this distribution with the distribution over all positions. The mean syllable duration was also calculated for each position. For testing significance, we randomly changed durations within each bout and then repeat the procedure over 10000 times and we can use a p-value of 0.05. Correlations between the histogram at each position and the histogram for overall syllable durations across all positions are calculated and plotted. For testing significance, the same procedure to what we did with syllable duration was repeated. The group data for both syllable duration and correlation value was also plotted for both normal and lesion birds.

Inter-syllable duration and syllable duration

Plotting mean syllable duration vs. mean inter-syllable duration for the different categories in all birds.

Here, we pull out all the valid bouts[6] and then pick syllables of the same type and calculate the mean intervals between these syllables - they have to be within a single bout. Again mean and 1 standard deviation is used as the centroid and radius for plotting the ellipses.

Results

In pre-lesion songs, the song had some specific set of stereotyped syllables which frequently repeats within a song and also across each song renditions. The pre-lesion song is structured and has a specific sequence of syllables to it.(Figure 6) For example, if “i” is an IN and “a”, “b” and “c” are its motif syllables. Then the bird could have a sequence iiiiabc which repeats across all the renditions of a song.

[6] All bouts which are there in having 500ms silence before and after the bout.

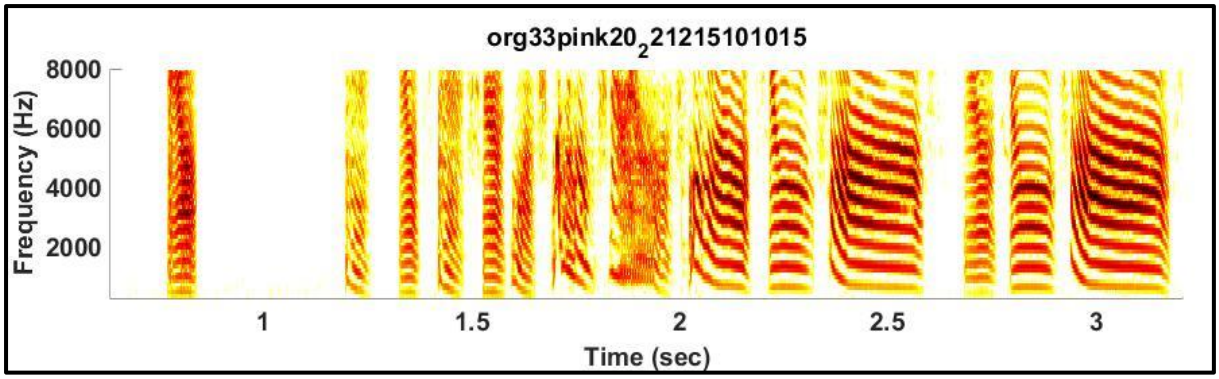

Figure 6: An example of a pre-lesion song

However, in post-lesion songs the frequency pattern in the spectrogram has become homogeneous for all the syllables, leaving each syllable unidentifiable by the virtue of observing spectrograms. In terms of syllable duration also, there is no repeating identical syllable with stereotype syllable duration values.(Figure 7) However, the syllable duration of first 2-3 positions in the bout seems to remain same across different bouts.

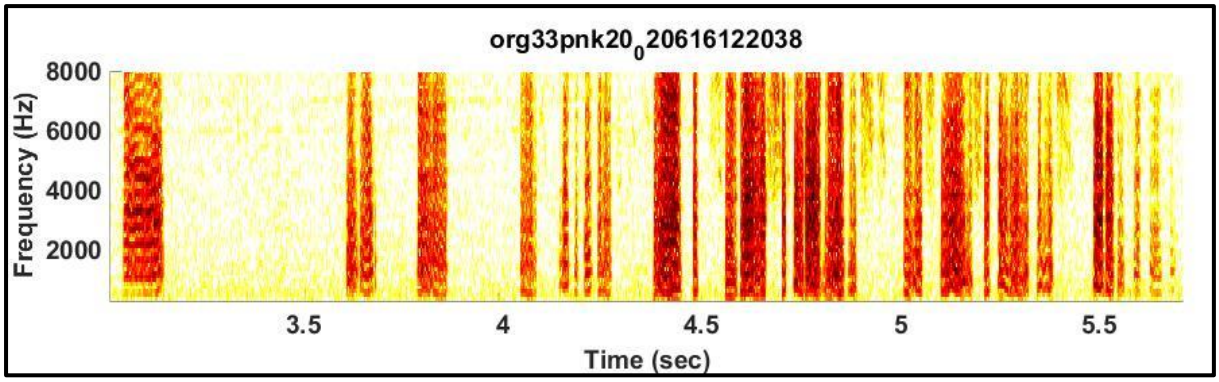

Figure 7: An example of a post-lesion song

However, we do not have enough birds with both pre- and post-lesion data (the 5 HVC lesion bird data which we borrowed from the Chen and Stepnick lab do not have pre-lesion data). Hence we decided to first characterize INs, in general, by the studying its properties in normal bird songs, where HVC is present, and using insights from those analysis to identify the presence of IN in post-lesion songs.

The following three defining characteristics were considered for the further analysis of INs in both presence and absence of the song:

1) Position of INs in a bout
2) Acoustic properties of INs

3) Spacing and Pacing: How INs are rendered within a song? Syllable duration and Inter-syllable Gap.

CHAPTER 1

How are INs arranged in a song bout?

i) INs are more likely to be found in the first 3-4 positions of a song bout

INs are always defined as syllables appearing at the beginning of a song but it does appear later position between different motifs. But, is it only IN which appears at the beginning of the song or any other syllable type appears at the beginning? In post-lesioned bird song since INs are not identifiable, if we could identify positions in a bout which are more likely to have IN in it then we could do further analysis on them to identify INs. Short calls do also frequently appear at the beginning of the bout. We need to know which position in a bout has a significantly high probability of finding a particular syllable type.

For this, the probability of a syllable being any one of the syllable types of Long call, Short call, IN and Motif syllables in a different position within the bout was calculated. The representation of these calculations as the probability of a syllable being a specific syllable type v/s syllable position within the bout showed the likeness of finding a particular syllable type at a particular position in the bout. For every bird and each syllable type, the above graphs were plotted and checked for significance. In most birds, INs had a high probability value (above 0.6) at the initial positions of the bout which decreased considerably after 4^{th} or 5^{th} position and occasionally increasing back to higher probability values at some of the latter positions but sufficiently remaining low than the values at the initial position. (Figure 8C) In contrary, the probability of finding a motif syllable at the beginning of the song is very unlikely but the probability values for motif syllables increases considerably after 5^{th} and 6^{th} positions. (Figure 8A) Similarly, short calls have high probability values at the very beginning of the song (the first 2-3 positions) and have very less probability at all other positions. (Figure 8B) The long calls have no fixed position where the

probability values are always high and most of its higher probability values in the later positions in a bout. (Figure 8D)

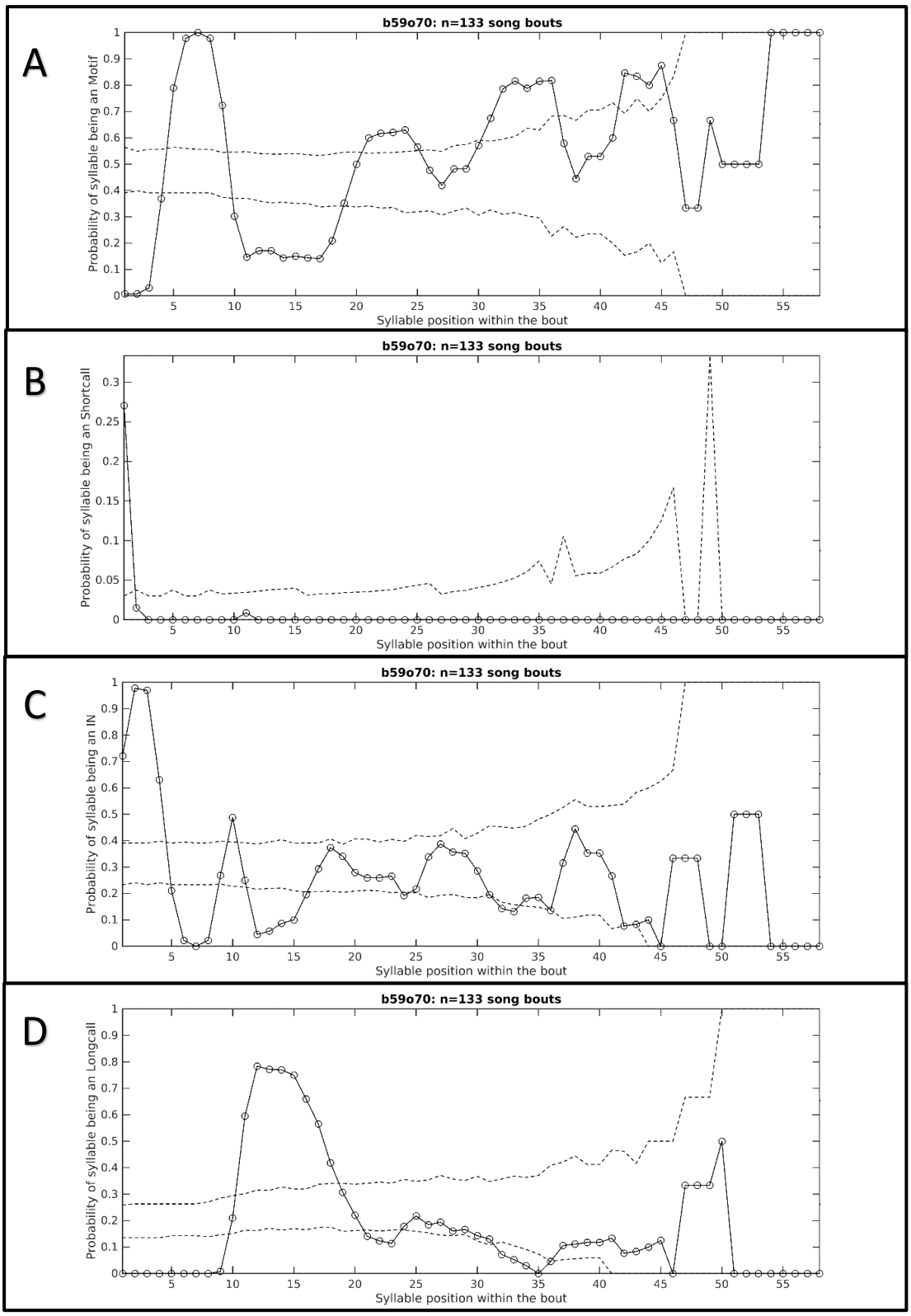

Figure 8: An example of a probability of a syllable being (A) a motif syllable (B) a short Call (C) an IN and (D) a long call v/s the syllable position within the bout plot. The dotted line indicates the interval for the value to be equal to chance (95% confidence interval of random iterations). Each point represents the probability values for a syllable in that position to be a syllable from the corresponding category

Even if we got probability values and trend across different position for each syllable type, it is important to find out which positions have significantly high probability. A 95% confidence interval can be calculated from the randomization of data.(see methods) Any probability value above this interval can be considered as "> chance", in the sense, the higher probability is significantly higher than the 95% of the confidence interval of the random distribution for it to happen merely by chance. Similarly, any probability value below this interval can be considered as "< chance", in the sense, the probability is significantly lower for it to happen merely by chance and '=chance' for the rest of it.

The heat map of the position v/s syllable-type significance of its probability is plotted for all birds together to understand the position specific probability on an individual basis and its differences from bird to bird. It can be seen that for IN about 13 out of 16 birds the INs are ">chance" in the first position, 14 out 0f 16 in the second position, 13 out of 16 in the third position and 9/16 in the fourth position. Thus, for the first 4 position, the INs has significantly high probability in the majority of the birds. (Figure 9A) Similarly, about 12 out of 16 birds the short calls are ">chance" in the first position and only 4 out of 16 in the second position and lesser fraction for subsequent positions. Thus short call has a significantly high probability of occurring at the first position in a bout. The motif syllables have "< chance "in the first two positions for all the birds meaning that there is a very low probability of finding a motif syllable in the first two positions of a bout. Only by 6^{th} position, the majority of birds has a ">chance" significance for motif syllable probability (11 out of 16) and this significant high probability is found the majority of birds till 15 positions. (Figure 9B) The higher position for all syllable types will have lesser significance values as total number positions in a bout from bout to bout and bird to bird so positions more than 20-30 will have lesser and variable sample size.

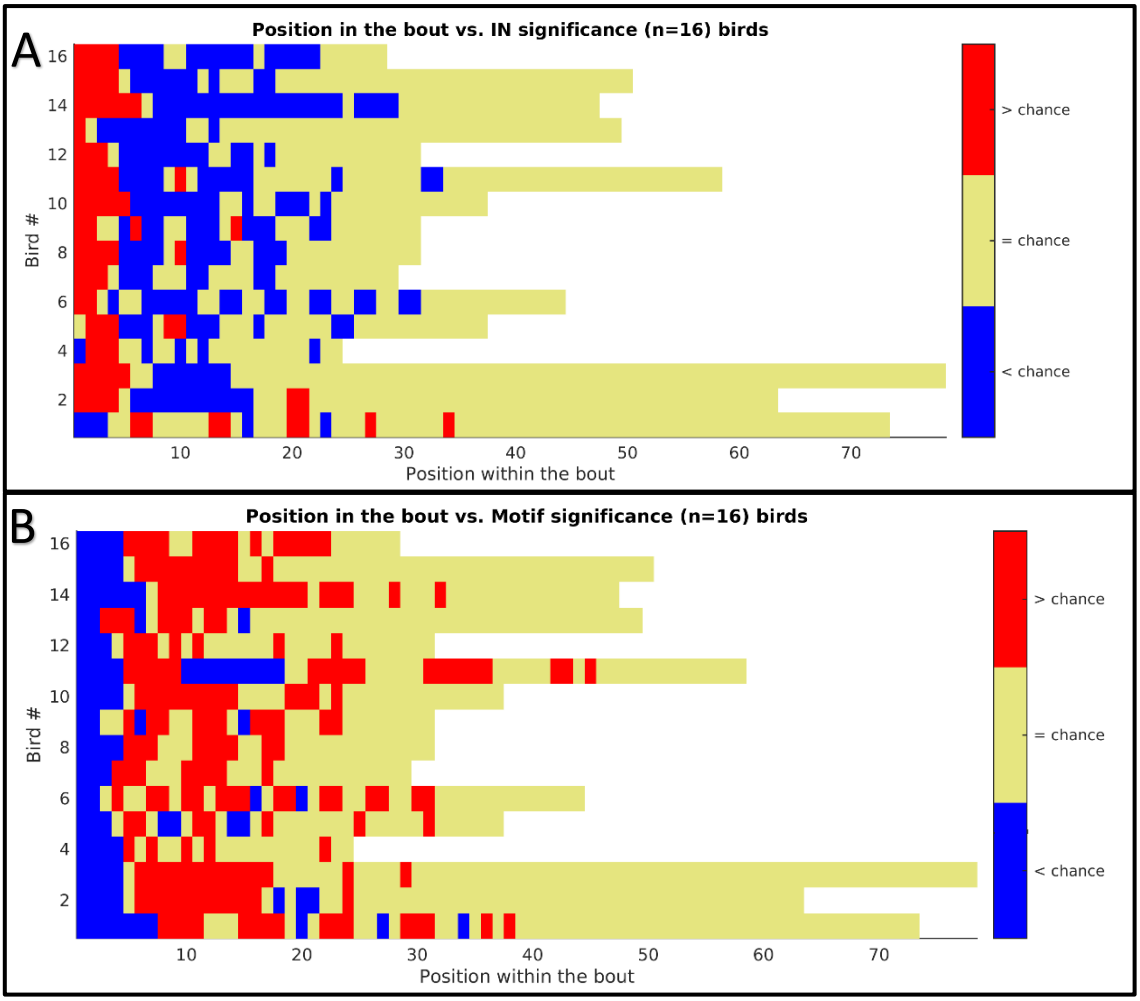

Figure 9: (A) Position in the bout v/s IN significance and (B) v/s Motif syllable significance. In the above figure, each row is a bird and each column represents the position in a bout and color coding represents the significance of probability.

To summarize these trends of probability for each syllable types across different birds (n= 16 birds), the probability v/s syllable positions plots for each syllable types and birds were averaged across all the birds and plotted together. In general, across all birds, INs has high probability values (above 0.6) at the initial five positions of the song and then suddenly decreases for subsequent positions (below 0.3) and remains low for the rest of the position values. In contrary to that, motif syllables initially has very low probability values (less than 0.2) but the probability values increase over positions and by 7^{th} position the values are as high as 0.6. The short calls have their highest probability at the very first position of the bout which decreases considerably for subsequently positions and remains very low for the rest

of the bout. The probability of finding a long call at any position within the bout remains low for all the positions in a bout. (Figure 12)

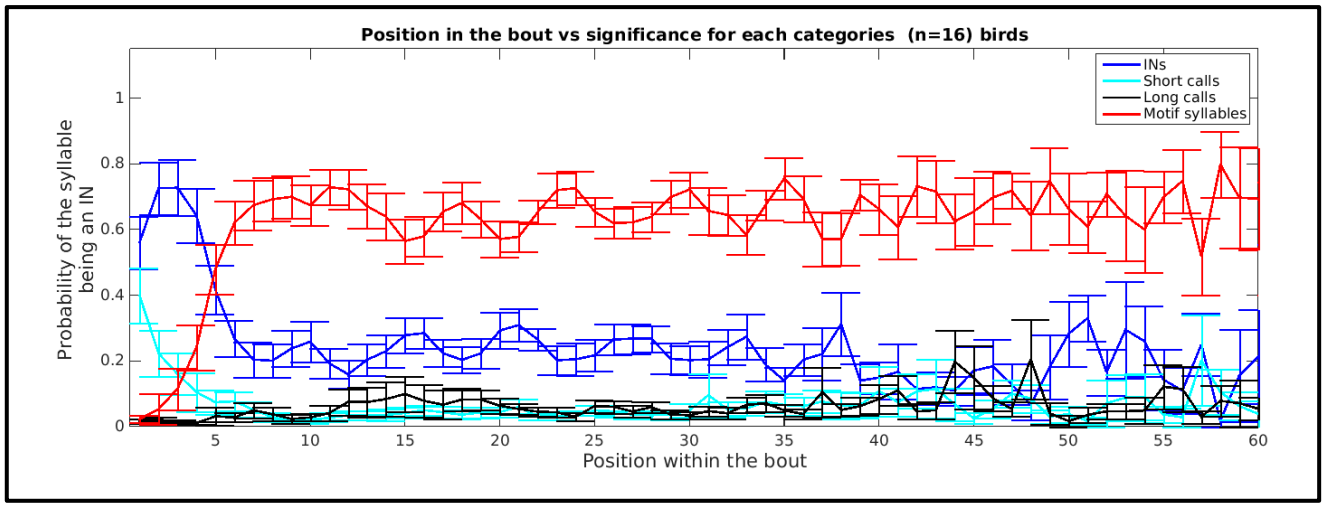

Figure 10: Probabilty of the syllable being a particular syllable for different syllable categories v/s position within the bout.

Thus, INs are more likely to be found in the first 3-4 positions in a song bout, while motif syllable is more likely to appear only after the 4-5th position in a song bout. The first two positions may also contain short calls, but their probabilities are less.

CHAPTER 2

Understanding the acoustic properties of INs in the presence and absence of HVC

i) In individual bird level, INs are similar, in terms of its acoustic properties to one of the Motif syllable or a Short call

Syllables can be identified and categorized by the virtue of its acoustics properties. As mentioned earlier in the methods, a set of 9 acoustics features can be obtained from the sound recording data. Each syllable type will have its own set of specificity in each acoustic property. For example, a long call will always have high syllable duration values and high log amplitude value. In order to account for all these acoustic features and find how much distinct or similar INs are from another syllable, a dimension reduction analysis was performed to account for all the variability from different features together. Initially, the dimension reduction will be done on an individual bird level to recognize the INs

closeness to other syllables which are part of the song regardless of any categorization.

8 acoustics features out of 9 (excluding Fundamental Frequency (FF) as FF calculation for syllables is difficult and needs much precision) obtained from the spectrogram is converted into 8 principal components doing Principal Component Analysis (PCA). The PCA helps to visualize the variance spread of a data cluster involving a set of variable. The new 8 principal components will represent the influence of all the 8 features in the distribution and help us to distinguish between different syllable labels. Each syllable labels is separated out as distinct clusters in a PCA. In PCA analysis for individual birds, cluster plotting and distance calculation has been done considering syllable labels and no syllable categories or types were employed. However, we do keep a track of syllable categories to which each syllable label belongs to. The PCA on an individual bird level and syllable label level will help us to understand how much INs are distinct from all other syllables irrespective of which category they belong to. The Variance explained (%) is also calculated to find how much variance is explained by each principal components. In most of the birds, the first four principal components explains nearly 80-85% percentage.(Figure 11)

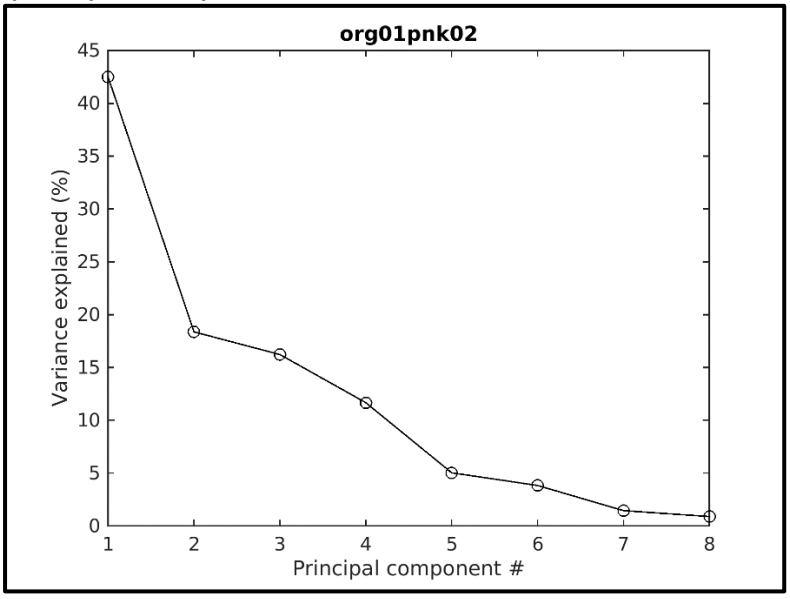

Figure 11: An example on how much variance are explained by different principal components in an individual bird PCA.

Thus, Plotting just the PC1 and PC2 will itself give you an idea different syllable clusters. In, all the birds, PC1 v/s PC2 cluster plots shows clear distinctions between all the syllables. All the syllable has a distinct cluster from the rest and can be identified as separate clusters. The IN also form a separate cluster mostly

at the left middle extreme of the PC1 v/s PC2 plots. They in the majority of the cases can be seen closer to one of the motif syllable and short call.(Figure 12)

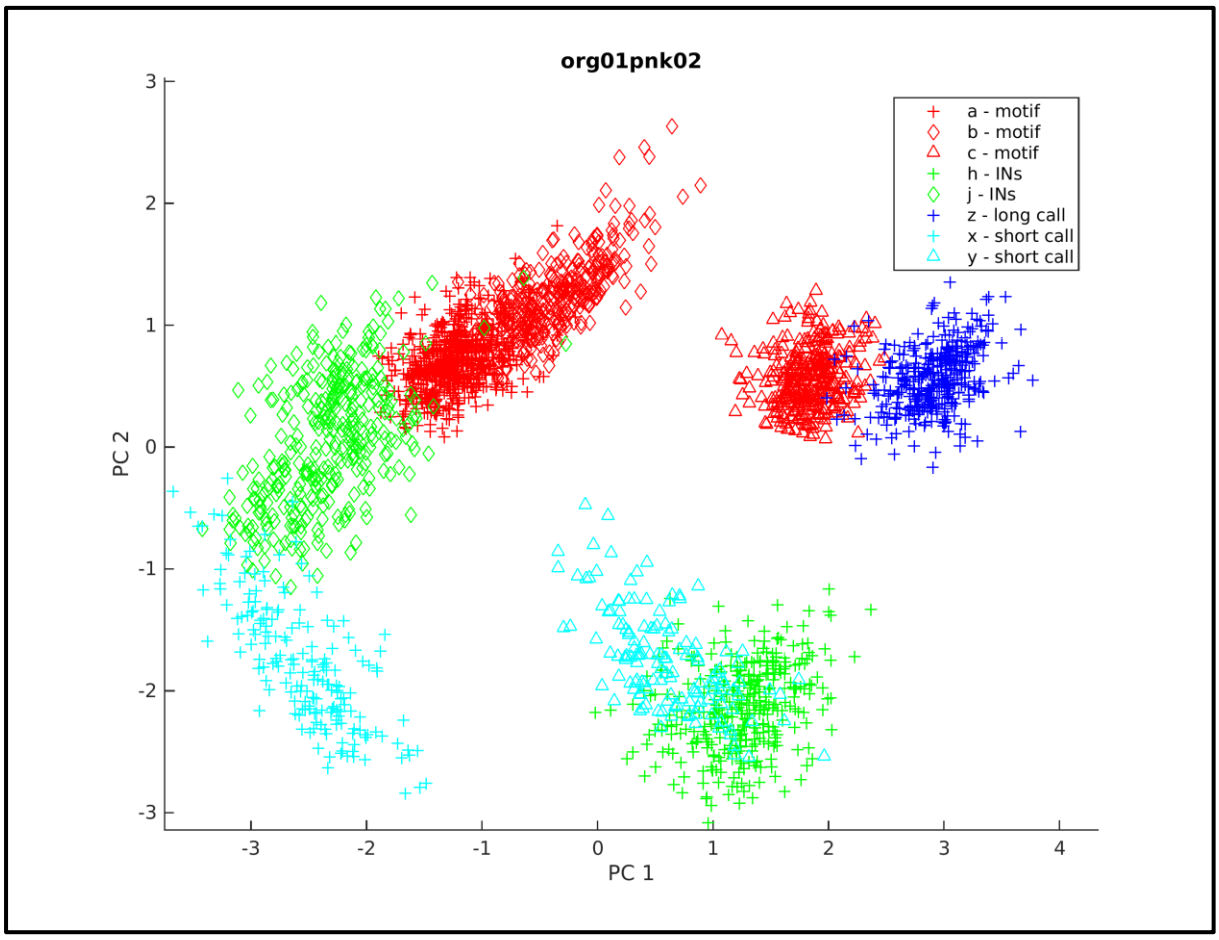

Figure 12: An example of the cluster plot of PC1 v/s PC2 for individual bird PCA. Each symbol and color combination represent each syllable. The red, blue, green or cyan colors represent which category the syllable belongs to motif syllable, long call, IN or short call respectively. Notice that each syllable, despite some outliers, forms a well-defined cluster of its own which seldom overlaps with other clusters. Here, the cluster of "j" (an IN) is closest to that of "a" (a motif syllable), while the cluster of "j" (another IN) is closest to that of "y" which is a short call.

Now to check for how much distinct or similar are INs from other syllable types, we need to find the distance of points belonging to one syllable in principal components axes to another set of points, closest the distance more similar will be the syllables from each other. The distance between each IN (n=18 INs from 9 birds) and syllables of other labels was calculated for each bird from first 4 PCs[7]. Only 9 out of 16 birds were considered for analysis which had all types of syllable categories as a part of its song. Then the syllable labels with minimum distance for

[7] The distance calculations are done on the points in the 4 -dimension distribution of PC1, PC2, PC3 AND PC4

each category were which also includes other INs. The mean of the minimum distance across all the birds in each category and across INs is calculated. The mean minimum distance plot shows that mean minimum distance of IN with itself has the lowest value among all other categories. While motif syllables, other INs, and short calls have comparable mean distances, long calls have the largest mean minimum distance from INs.(Figure 13)

In the minimum distance plots, all categories are significantly different from "Same IN (self)" - Kruskal-Wallis and posthoc Tukey-Kramer ($p < 0.05$).Motif syllables and other INs are not different from each other, but they are different from long calls. Short calls are not different from motif syllables or other INs or long calls.

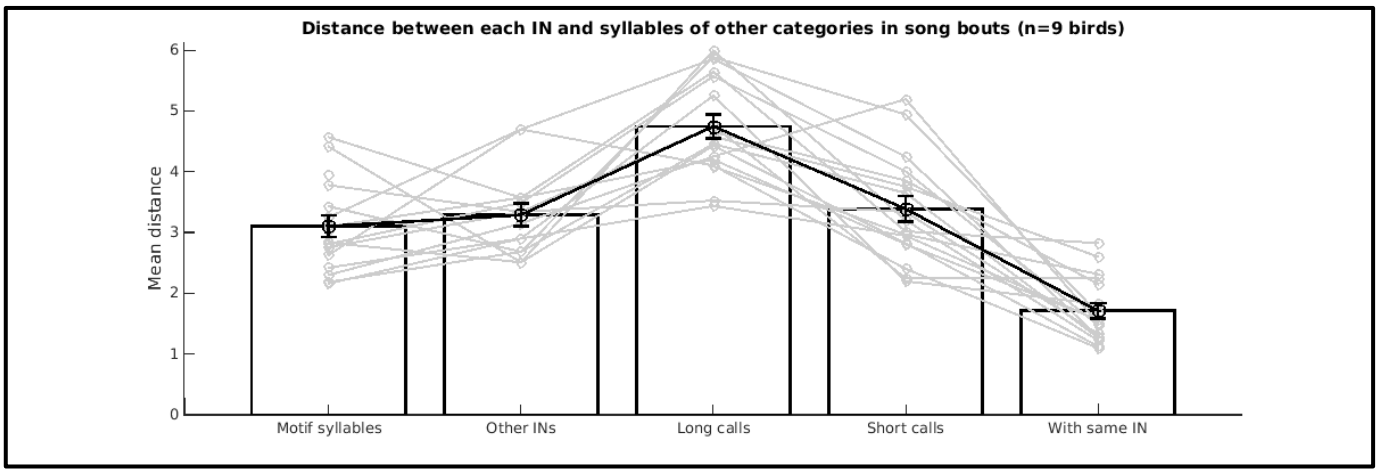

Figure 13: Mean distance, across all INs , of the minimum distance of each IN with other syllables for each syllable category. Each histogram represents the average minimum distance of all INs (across birds) from other syllables of different syllable categories, while the each line with dots represent minimum mean distance of INs from other syllables in different syllable categories for each bird.

From the mean distance calculations, it is evident that INs has closer to a motif syllables and other INs but short calls are close enough even if not significant. However, it is not clear from the above analysis that in an individual IN basis which syllable type has the shortest distance and how many INs are closer to, say a Motif syllable. The nearest neighbor for each INs is the syllable (label) which has the least minimum distances amongst all other syllables in a bird song. The nearest neighbor for each IN (excluding other INs[8]) is noted down and checked for which syllable category it belongs to. In 45% of IN (out of 18 INs) the nearest

[8] As it is intuitive that other INs will be much similar IN.

neighbor is a short call, 40% times it's a motif syllable and 15% it is a long call. (Figure 14)

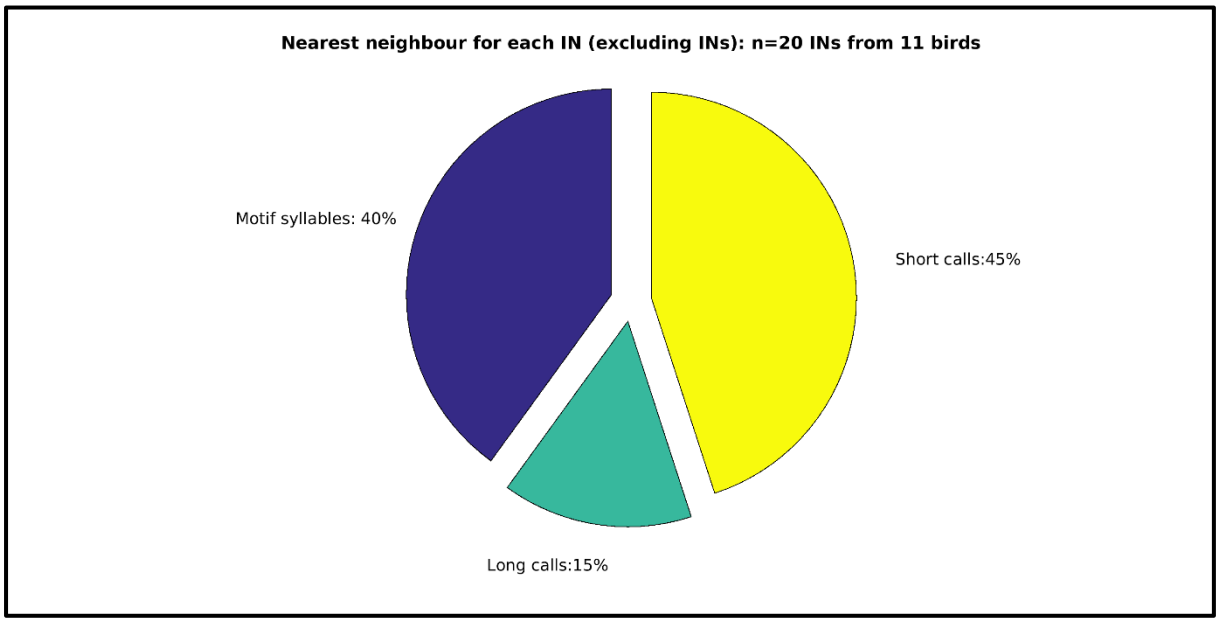

Figure 14: A pie-diagram representation of the share of syllables from different syllable categories which is the nearest neighbor for each IN.

Thus, each INs can be acoustically similar to either one of the motif syllables or a short call on an individual level. However, INs do also separate out as a single cluster in principal components indicating they have distinct acoustic properties of their own.

ii) A definitive boundary for IN can be identified using dimension reduction.

In general, how can we categorize INs? How can we define the properties of IN? So that no matter what the individual differences are there among INs we could have a universal categorization for IN. For this, all syllables belonging to each syllable categories (short calls, long calls, INs and motif syllables) are pooled together from all birds and a PCA is done on its acoustic features (normalized across all bird). This will help us to identify which motif type, in general, IN resembles the most. We could also define the definitive properties of IN which can always be used to identify the presence of IN in any sample. Now rather than, considering PCA for individual birds, the PCA is done on the all the syllables from all birds together and separating them

only as four syllable categories as before. About 70% of the variance is explained by the first 4 principal components[9].

The coefficient was noted down will be kept same for the post-lesion analysis. The PC1 v/s PC2 and PC1 v/s PC3 is plotted to visualize the clusters of different syllable types. The clusters are also superimposed with ellipses with the cluster's mean as its centroid and cluster's 2 standard deviations as its radius. There much more overlap among the clusters than we found in the individual PCA analysis but again in this PCA all syllables is pooled together to different syllable types and not to individual labels.(Figure 16) The coefficient values from the plot can also be used to understand which acoustic feature is contributing the distribution and in what way. (Figure 15) For example, PC1 has high positive coefficients for the duration and mean Fundamental Frequency (FF) and PC2 has a highly negative coefficient for the duration and again positive coefficient for FF which should explain the position of on INs and short calls are in the left extreme of PC1 and at the middle of the PC3 axis.

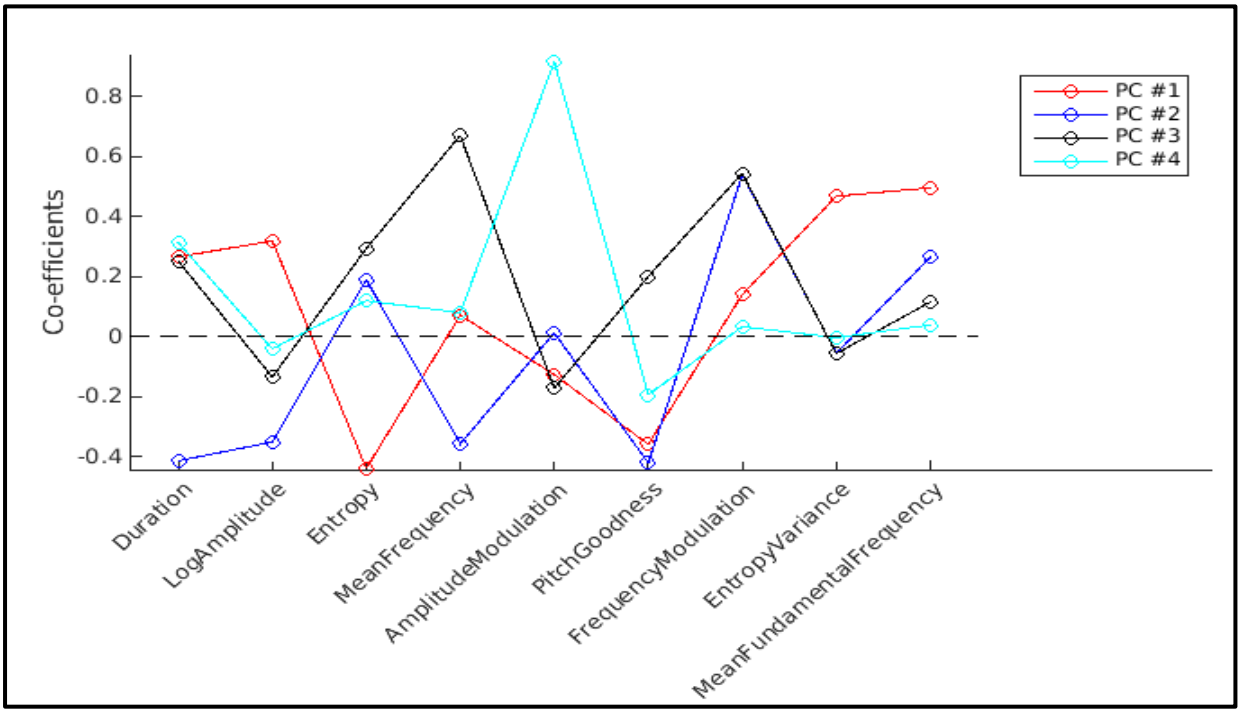

Figure 15: The principal coefficients of all bird PCA for each principal components across all 9 acoustic features.

[9] Note that for all bird we are using all the 9 acoustic features including mean Fundamental Frequency, hence it has a total of 9 principal components.

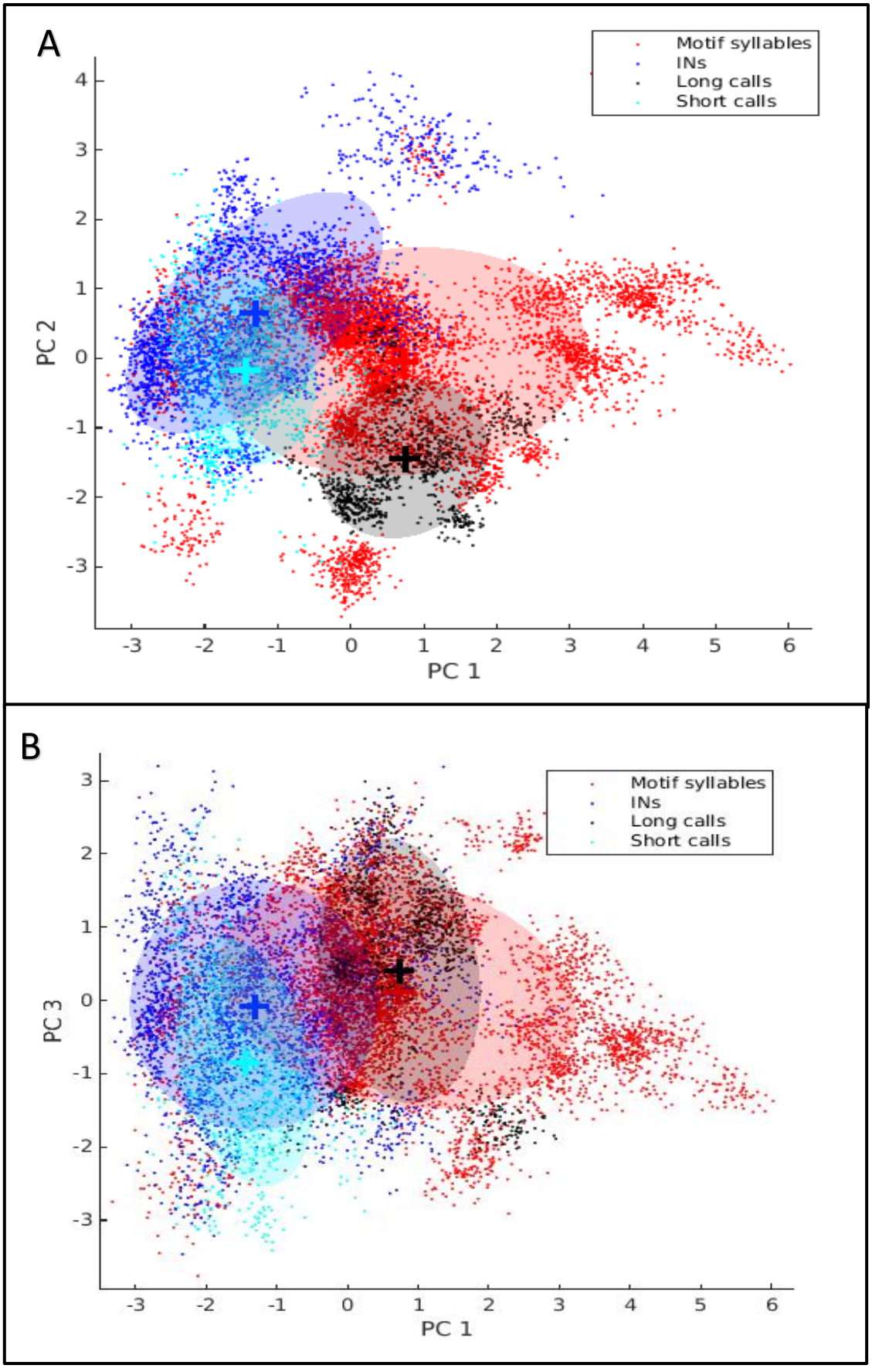

Figure 16: A) PC1 V/S PC2 and B) PC1 V/S PC3 plots of all bird PCA of normal bird representing the clusters of different syllable categories. Each coloured points represent each syllable category(Red: Motif syllables, Blue: INs, Black: Long calls

and Cyan: Short calls). The ellipses with corresponding lighter colours are the with a mean of the cluster as its center and its axes is ± 1 SD from the mean.

The cluster plot helped us to find the mean of IN cluster points from centroid of all other syllable category centroid. In the corresponding figure, the dotted lines are the 95% confidence limits calculated by randomization of the entire data set. (Figure 17) According to this plot, all the mean distance values of each category are above or below the confidence interval indicating all the distant values are significant and are not due to randomness or chance. While the IN and the short call has distance values is much "<Chance", Motif syllables and Long calls has mean distances ">Chance". Thus, INs are much closer to short calls in all bird PCA than motif syllables and long calls (which is the farthest).

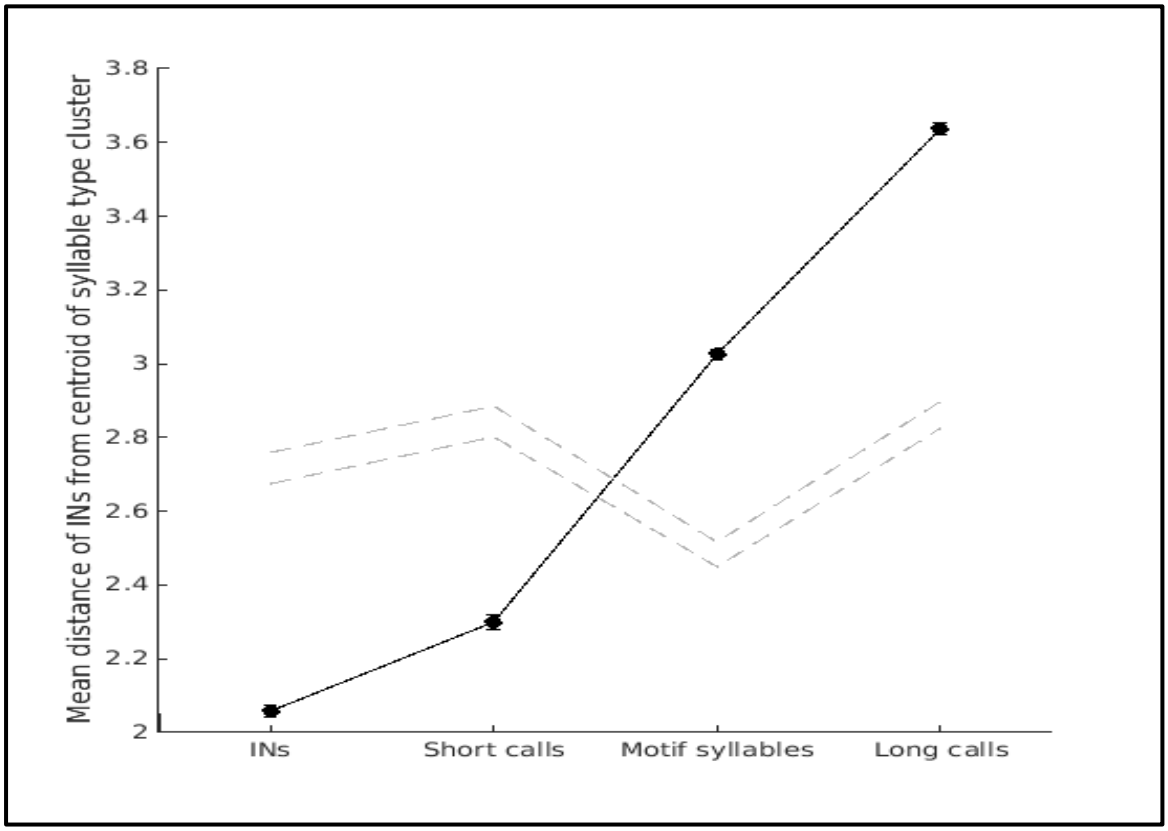

Figure 17: Mean distance from the centroid of IN cluster to other syllable categories over first four principal components in an all bird PCA analysis of normal birds. Mean distance of INs from the centroid of other syllable type clusters over first four principal components in an all bird PCA analysis of normal birds. the dotted line indicates the interval for the value to be equal to chance(95% confidence interval of random iterations).

Thus, from the normal bird principal component analysis, we obtained a definitive boundary for IN which can be used to identify INs in a post-lesion song and also INs, in general, are more similar to a short call than any other syllable type.

iii) All Syllables has lost its acoustic properties irrespective of position after HVC lesion and hence INs too.

From the normal bird dimension reduction analysis, we now have a set of acoustic boundaries in the principal component plots to identify INs. So, if we plot the principal component for the song syllable from post-lesion bird songs. We could be able to identify the presence of IN in songs after lesion given that the principal components values and the coefficients over different are kept the same as the normal bird plots (figure 15). By plotting the principal components, the principal components dimensions are kept the same from all bird analysis by keeping the same set of coefficients for different features in each principal components. The new syllables are superimposed on the normal bird PCA over the earlier boundary of ellipses for each syllable category.

In PC1 v/s PC2 plots and PC2 V/S PC3 plots, all the post-lesion motif syllables[10] are scattered over as a single cluster and spread across all the syllable boundaries (ellipses). (Figure 18) This was expected as the post-lesion songs are highly variable and noisy, they should be randomly distributed. We know that, from the probability v/s position analysis for the normal birds earlier, there is a high probability for INs to appear in the first 2-3 position in a bout. Thus, the first two positions are colored separately in the plot to check their position with respect to the syllable category boundaries and how they are spread across the graph. In both graphs, the position of first two position points are spread across the plots and are not localized over any of the syllable category boundaries.

This could indicate that irrespective of position, all post-lesion syllables have lost their acoustic features and become noisy in the absence of HVC. However, a quantitative analysis needs to be done to confirm this. So even if INs are still there in the post-lesion songs we will not be able to confirm that, as INs would also have lost their acoustic properties.

[10] Note that all the syllables in a post-lesion song are labelled the same and only short calls and long calls which can be identified were labelled separately.

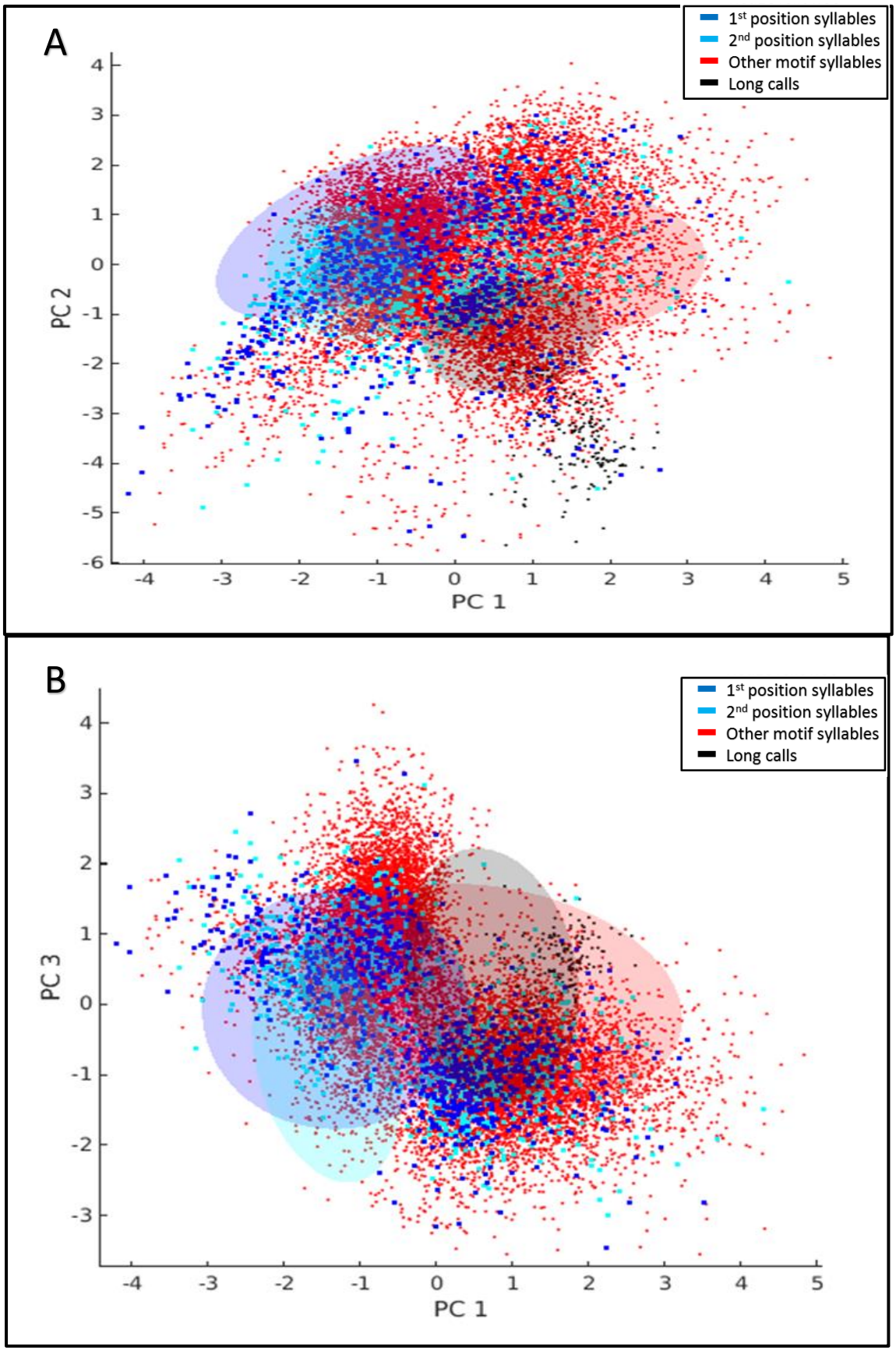

Figure 18: A) PC1 V/S PC2 and B) PC1 V/S PC3 plots of all bird PCA of lesion bird to identify syllable category of post lesion motif syllables, the violet and cyan points represent the syllables "at first position" and "second position respectively.

All the syllables seem to have lost its acoustic properties irrespective of their position in the absence of HVC. Even the first two position syllables do not have acoustic properties similar to any of the normal bird syllable categories.

CHAPTER 3

How are different syllables spaced and paced based on their position in the bout?

i) Syllable duration distribution varies with respect to the position in Normal Songbirds.

In terms of the acoustic properties, INs do not seem to be present in the Post-lesion song. But, since the post-lesion songs are noisy, with its frequency pattern in the spectrogram is homogenous even if the INs are there we will not be able to identify them by the acoustic features. However, the syllable duration seems to be a better measure to check for INs as they do not depend on the frequency patterns in the spectrogram entirely and is based on the beginning (onset) and end (offset) of a syllable. Then, how syllable duration varies in a normal bird with the position? The syllable duration distribution is a histogram or frequency distribution of syllable duration. In normal birds, the bird produces

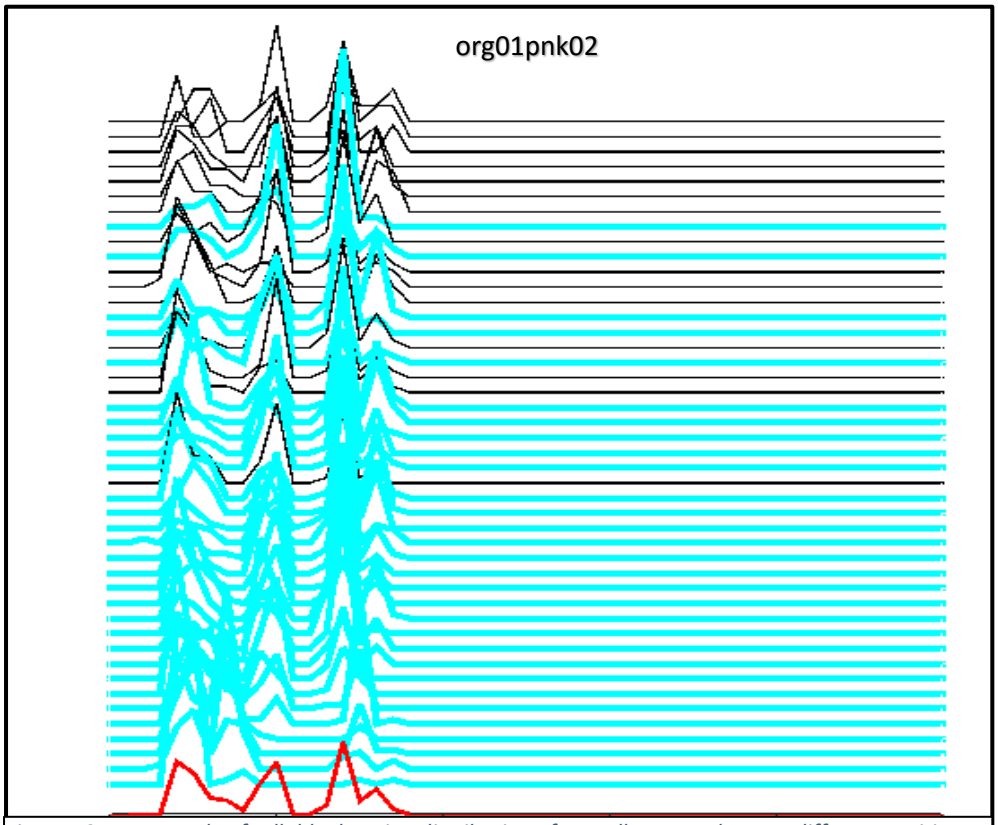

Figure 19: An example of syllable duration distribution of overall song and across different positions of a normal bird. The red line corresponds to the overall syllable duration distribution and all other lines represent each position starting from first position just above the overall syllable duration distribution to subsequent positions one above the other.The blue lines are those which are significantly correlated with the overall syllable duration histogram.

a set of repeating stereotypic song elements in a particular sequence. Thus, syllable duration distribution in a normal bird will have many high peaks at a certain range of syllable duration values as a similar type of syllable duration is repeated frequently. The syllable duration distribution for each position has single or double peaks and is different from the overall song distribution.(For example, see figure 19)

The correlation of each position with overall syllable duration histogram will tell us how distinct are the syllable distribution at different positions from the overall syllable distribution. The probability analysis for syllable types with the position for each shows that at initial positions there is a significantly high probability of having one type of syllable over other (say IN at second position), which becomes less significant for subsequent positions. If only one kind of syllable is appearing frequently at a particular position, then a syllable duration will be more or less the same value and hence the syllable duration has only one peak. Hence, that particular position will have low correlation with the overall syllable distribution which has multiple peaks. If more than one syllable is appearing frequently at a particular position, then it will have multiple peaks in the syllable distribution as many 2-3 sets of syllable duration values are repeated continuously. Thus the correlation should be less at the initial position and increases for subsequent positions. In the correlation with overall duration histogram for each bird, most of the bird has significantly lower correlation values at initial 6-10 positions which increase with position within the bout.(Figure 20)

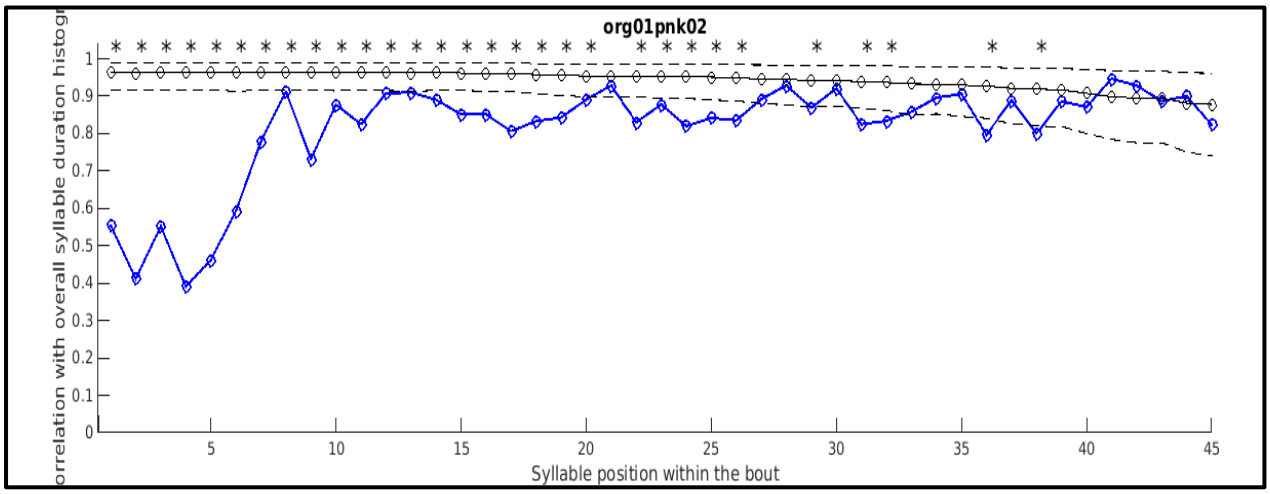

Figure 20: An example for the correlation with overall syllable duration histogram v/s syllable position within the bout for a normal bird; the dotted line indicate the interval for the value to be equal to chance(95% confidence interval of random iterations);

Similarly, the Mean syllable duration v/s each position shows that the mean syllable duration increases with the position. The positions after 6-10 positions, for most of the birds, the syllable duration distribution will start resembling the overall syllable duration distribution. (Figure 21)

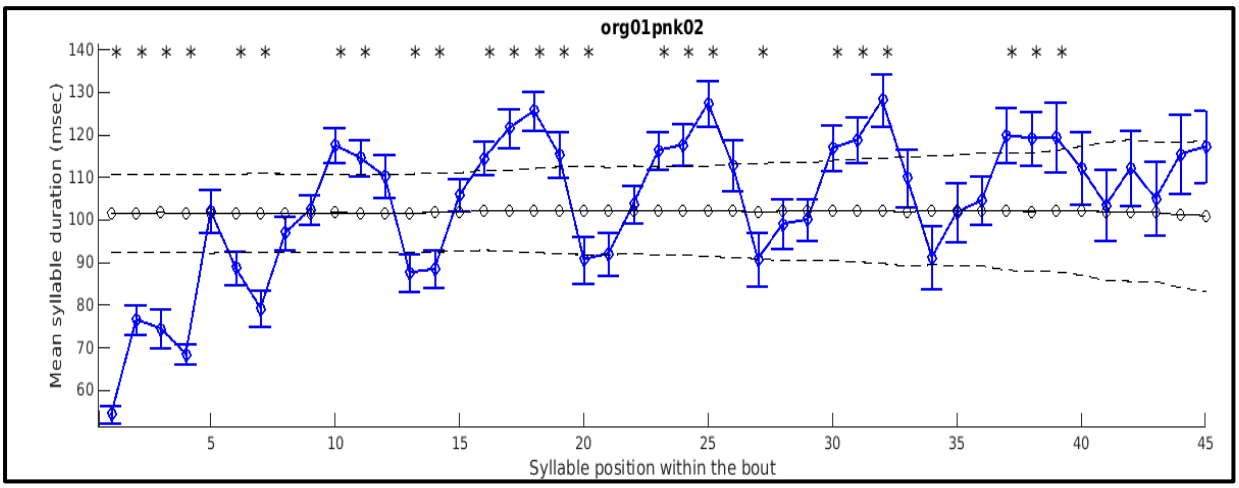

Figure 21: An example for the mean syllable duration v/s syllable position within the bout for a normal bird; the dotted line indicate the interval for the value to be equal to chance (95% confidence interval of random iterations).

Now, the mean syllable duration is averaged across birds for each position. The average mean syllable duration across all birds v/s position shows that the mean syllable duration increases in the initial positions and more or less remains same for the latter position.(Figure 22) Based on this differences in slope in two sets of positions and also based on the difference seen in the syllable distribution plots, the linear correlation was calculated separately for two sets of position: 1) First six positions and 2) Positions between 6^{th} and 15^{th} position (not all birds have position more than 15).

The linear correlation between first six positions and mean syllable duration: r = 0.69843; p=2.5629e^-15.

The linear correlation between 6^{th} and 15^{th} position and mean syllable duration: r = 0.031035; p=0.69685.

The first 6 positions have a high positive correlation with highly significant while the subsequent 6th to 15th positions has a low correlation which is not significant.

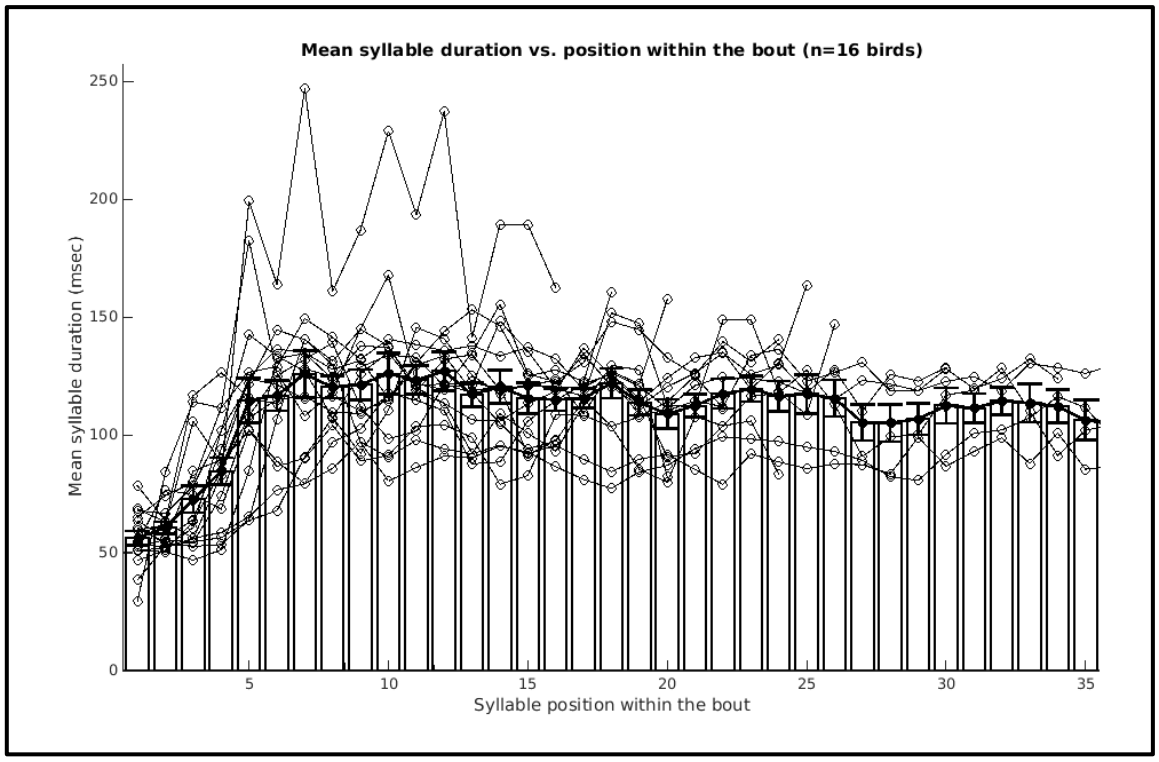

Figure 22: Mean syllable duration averaged across all birds v/s syllable position within the bout of normal birds. Each histogram represents the average mean syllable duration (across birds) at that position, while the each line with dots represent mean syllable duration values across each position for each bird.

Now, the correlation with overall syllable duration histogram is averaged across birds for each position. The average correlation with overall syllable duration histogram v/s position shows that the correlation with overall syllable duration histogram is lower for initial positions which increase with positions and in the same range for the rest of the position.(Figure 23)

The linear correlation between first six positions and mean syllable duration: r = 0.40035; p=5.3085e^-05.

The linear correlation between 6th and 15th position and mean syllable duration: r = 0.2165; p=0.005965.

The linear correlation between 1-6 positions and 6-15 is significant, but the r-value is smaller for 6-15 positions and not significant.

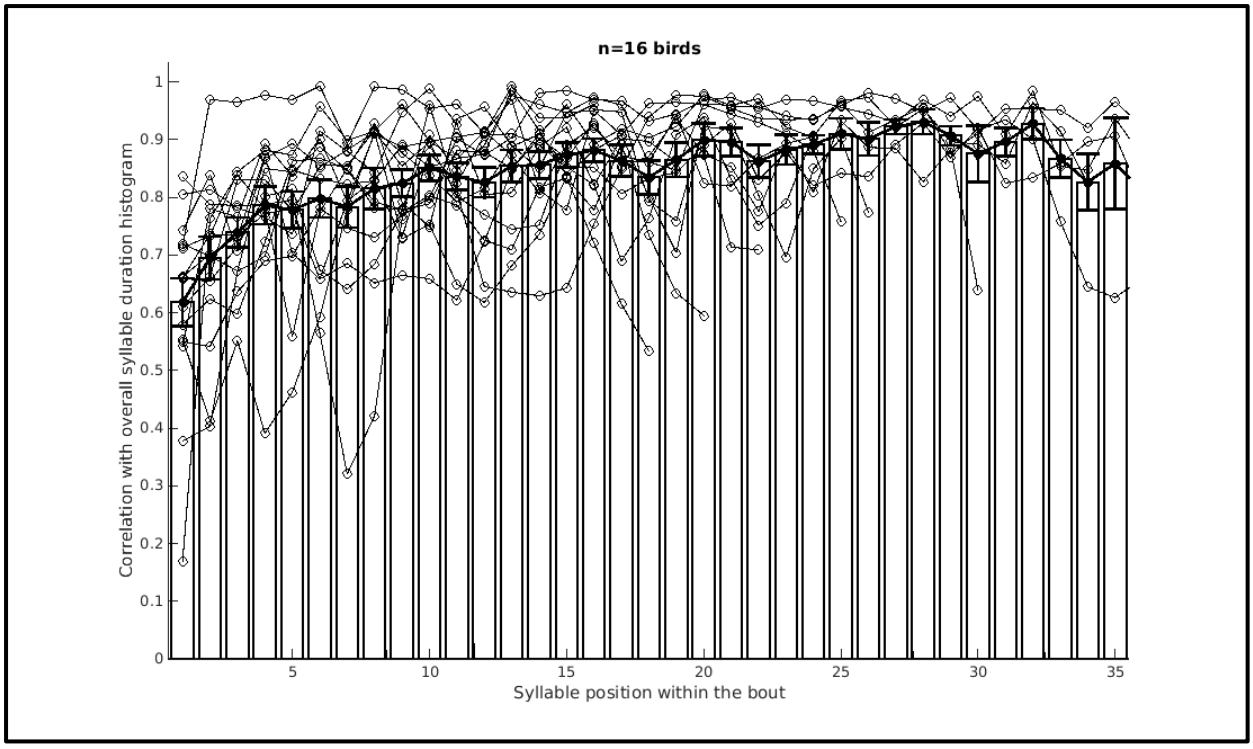

Figure 23: Mean correlation with overall syllable duration histogram averaged across all birds v/s syllable position within the bout of normal birds. Each histogram represents the average correlation with overall syllable duration histogram (across birds) at that position, while the each line with dots represent correlation values across a position for each.

Thus, there is a distinction in the syllable duration distribution between first few positions and the rest of the positions in a normal bird song.

ii) The distinction between the first few positions and the rest of the song due to Syllable duration distribution is still maintained even after HVC lesion.

The analysis of syllable distribution of a lesioned bird song will help us to check whether there is a distinction in the syllables between the first few positions and subsequent positions similar to what we found in the normal bird data. In post-lesion syllables are highly variable and except the first few positions, the syllable duration is also highly variable. Hence, the syllable duration distribution of the overall post-lesion song will be exponential due to, as mentioned in the *Aronov et. al*, the "absence of repeatable acoustic elements of a stereotyped length". But it does not seem to be the case entirely as the first 2-3 positions in each bird has a non-exponential distribution while most of the other positions follow the same exponential distribution. The number of syllables from just the first 2-3 positions is very small compared to a number of syllables from all other position together which is why the

stereotypy (repeated syllables with identical syllable durations) in the first 2-3 positions is not reflected in the overall syllable distribution and it remains exponential. (For example, see figure 24)

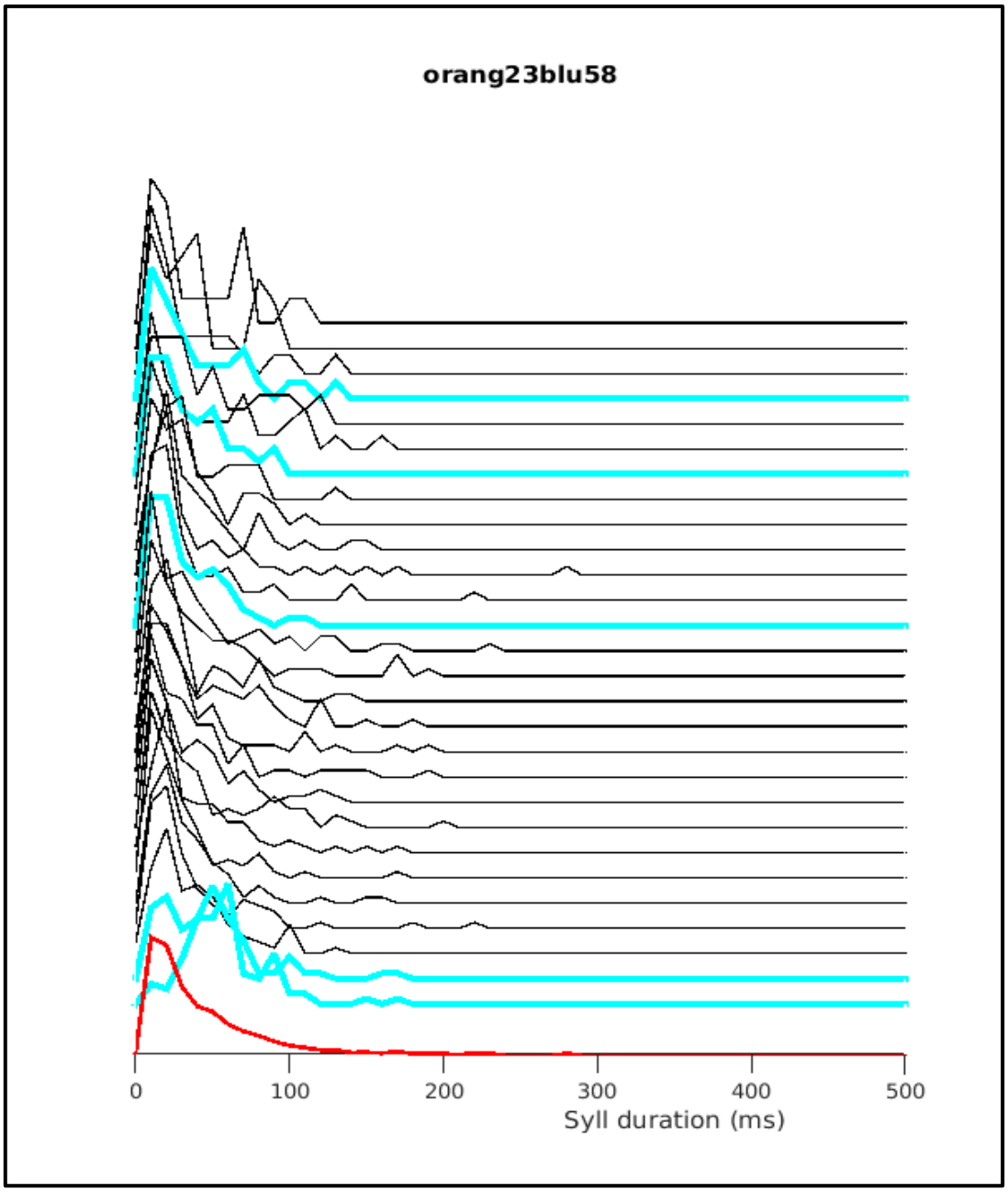

Figure 24: An example of syllable duration distribution of overall song and across different positions of a lesion bird. The red line corresponds to the overall syllable duration distribution and all other lines represent each position starting from the first position just above the overall syllable duration distribution to subsequent positions one above the other. The blue lines are those which are significantly correlated with the overall syllable duration histogram.

Hence, the first 2-3 position in the bout will have significantly low correlation values compared to the rest of the positions and which, in indeed, seen in the mean correlation with overall syllable duration histogram against position graph for each bird. (Figure 25) The mean syllable durations values are either more than the rest of the positions or less and it is different in different birds (For example, see figure 26)

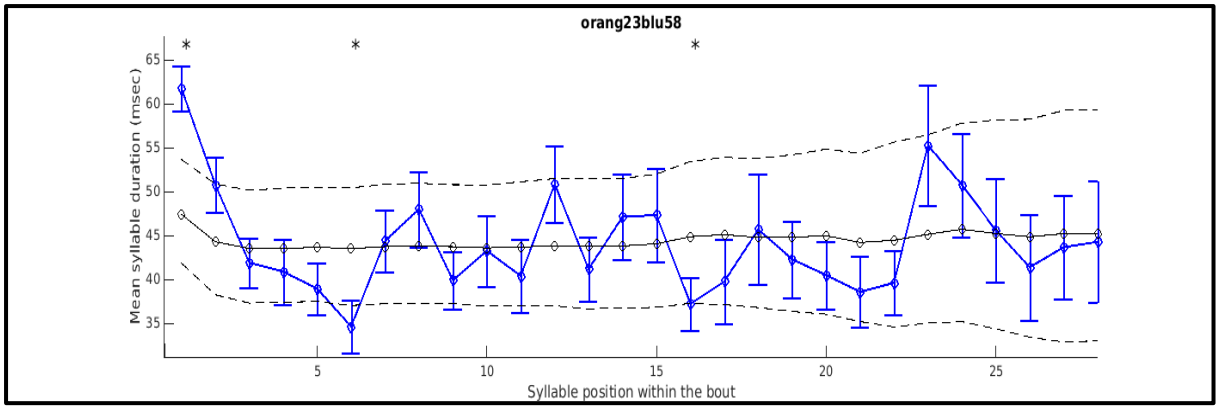

Figure 25: An example for the mean syllable duration v/s syllable position within the bout for a lesioned bird; the dotted line indicates the interval for the value to be equal to chance(95% confidence interval of random iterations).

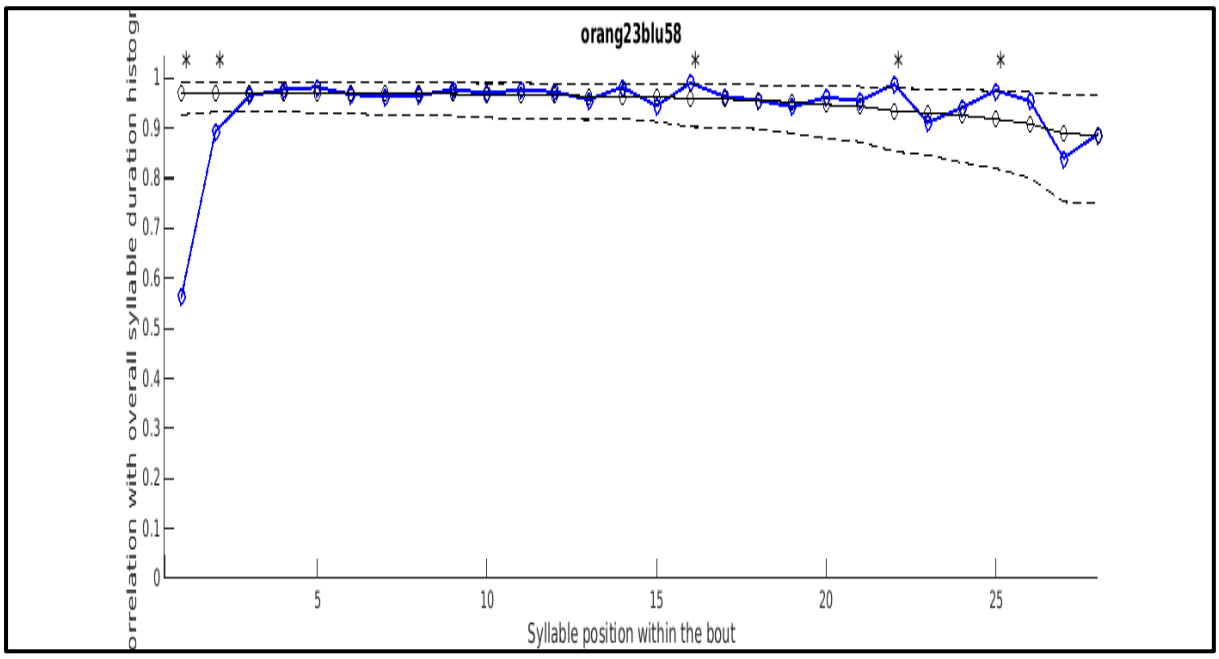

Figure 26: An example of the correlation with overall syllable duration histogram v/s syllable position within the bout for a lesion bird; the dotted line indicates the interval for the value to be equal to chance(95% confidence interval of random iterations).

The mean syllable duration is averaged across birds for each position. In post-lesion songs, the average mean syllable duration across all birds v/s position shows that the mean syllable duration increases in the initial positions and more or less remains same for the latter position. (Figure 27)

The linear correlation between first six positions and mean syllable duration: r = 0.23128; p=0.12198.

The linear correlation between 6th and 15th position and mean syllable duration: r = 0.02034; p=0.87123.

The first 6 positions have high positive correlation but are not significant and similarly, the subsequent 6th to the 15th position has a very low positive correlation with a high p-value. The difference in the first 6 positions is not strong correlated because the direction of change is different for different birds, for some the syllable duration is higher for first few positions while for some it is lower from the rest of the positions.

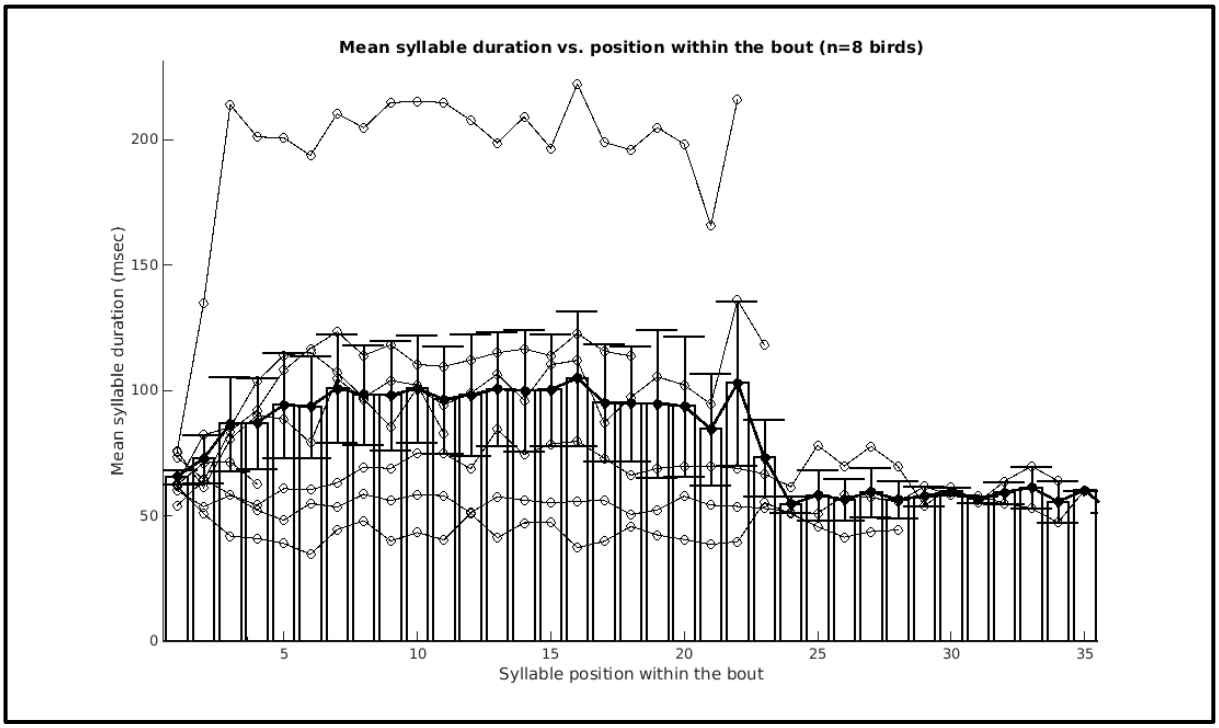

Figure 27: Mean syllable duration averaged across all birds v/s syllable position within the bout of lesion birds. Each histogram represents the average mean syllable duration (across birds) at that position, while the each line with dots represent mean syllable duration values across each position for each bird.

The average correlation with overall syllable duration histogram v/s position shows that similar to pre-lesion songs, correlation with overall syllable duration histogram is lower for initial positions which increase with positions and in the same range for the rest of the position. (Figure 28)

The linear correlation between first six positions and mean syllable duration: r = 0.59018; p=1.83e^-0.5.

The linear correlation between 6^{th} and 15^{th} position and mean syllable duration: r = 0.084971; p=0.49755.

The first 6 positions have a high positive correlation which is highly significant while the subsequent 6th to 15th positions has a very low positive correlation with the higher p-value (not significant).

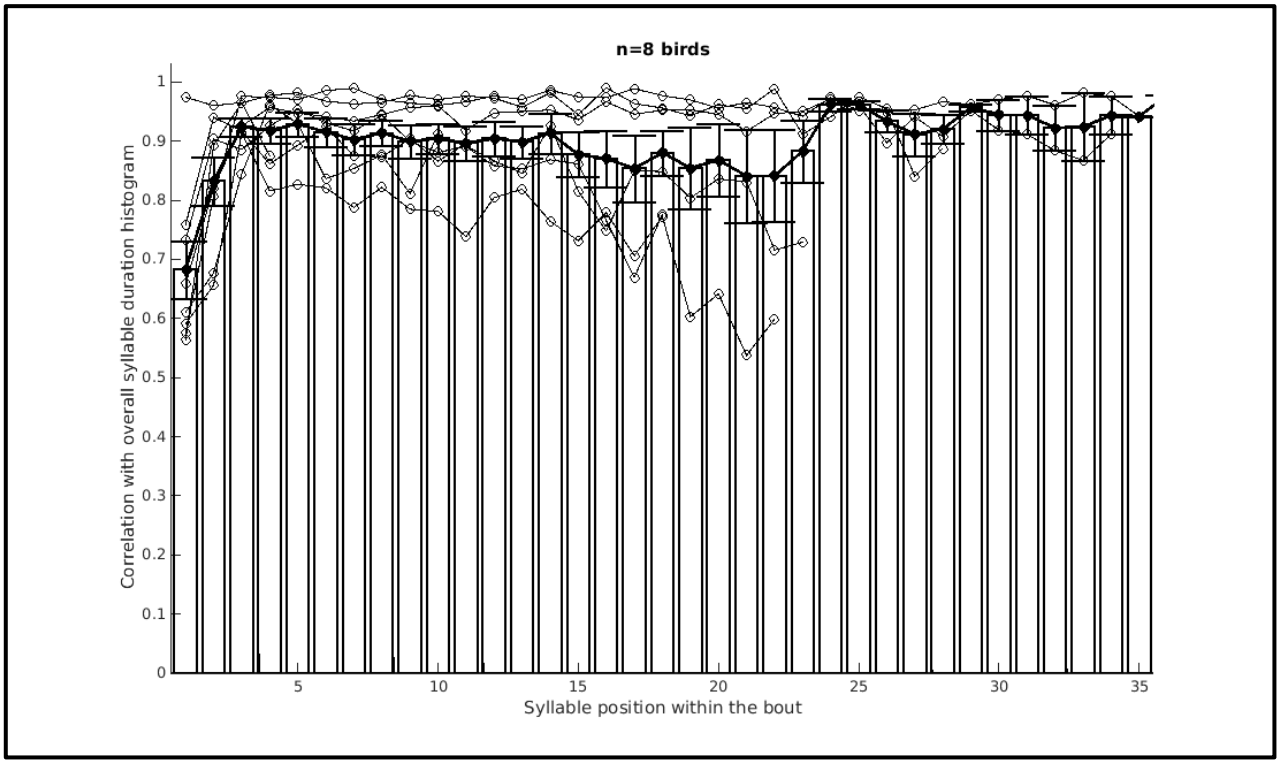

Figure 28: Correlation with overall syllable duration histogram averaged across all birds v/s syllable position within the bout of lesion birds. Each histogram represents the average correlation with overall syllable duration histogram (across birds) at that position, while the each line with dots represent correlation values across a position for each.

In post-lesion songs, the first few position still maintains a syllable duration distribution similar to that of the normal bird song while rest of the positions lost theirs. Hence, maintaining the distinction between initial positions and rest of the positions even after HVC lesion

iii) The "spacing and pacing" of syllables at the first few positions of a bout are maintained even after the HVC lesion while rest of the motif syllables lost theirs.

Inter-syllable duration of a syllable (the period of silence between two consecutive syllables) is calculated when two syllables of the same syllable category occur together within a bout. The Inter-syllable duration and syllable duration gives together gives an idea of the "pacing" and "spacing" of syllables when they are together. In Normal birds, the mean Inter-syllable duration among Short calls, Long calls, Motif syllables, INs and first three positions of a bout are plotted against corresponding mean syllable duration. All the syllable, categories are separating out as distinct clusters in this plot. For example, Short call occupies the upper left half of the plot. Interestingly, INs are separated out from the rest of the motif syllables. Also,

the first 3 bout syllables[11] which overlap between IN cluster and Short call cluster which was expected as we know the first 3 syllables of bout are highly likely to be an IN or a short call. (Figure 29)

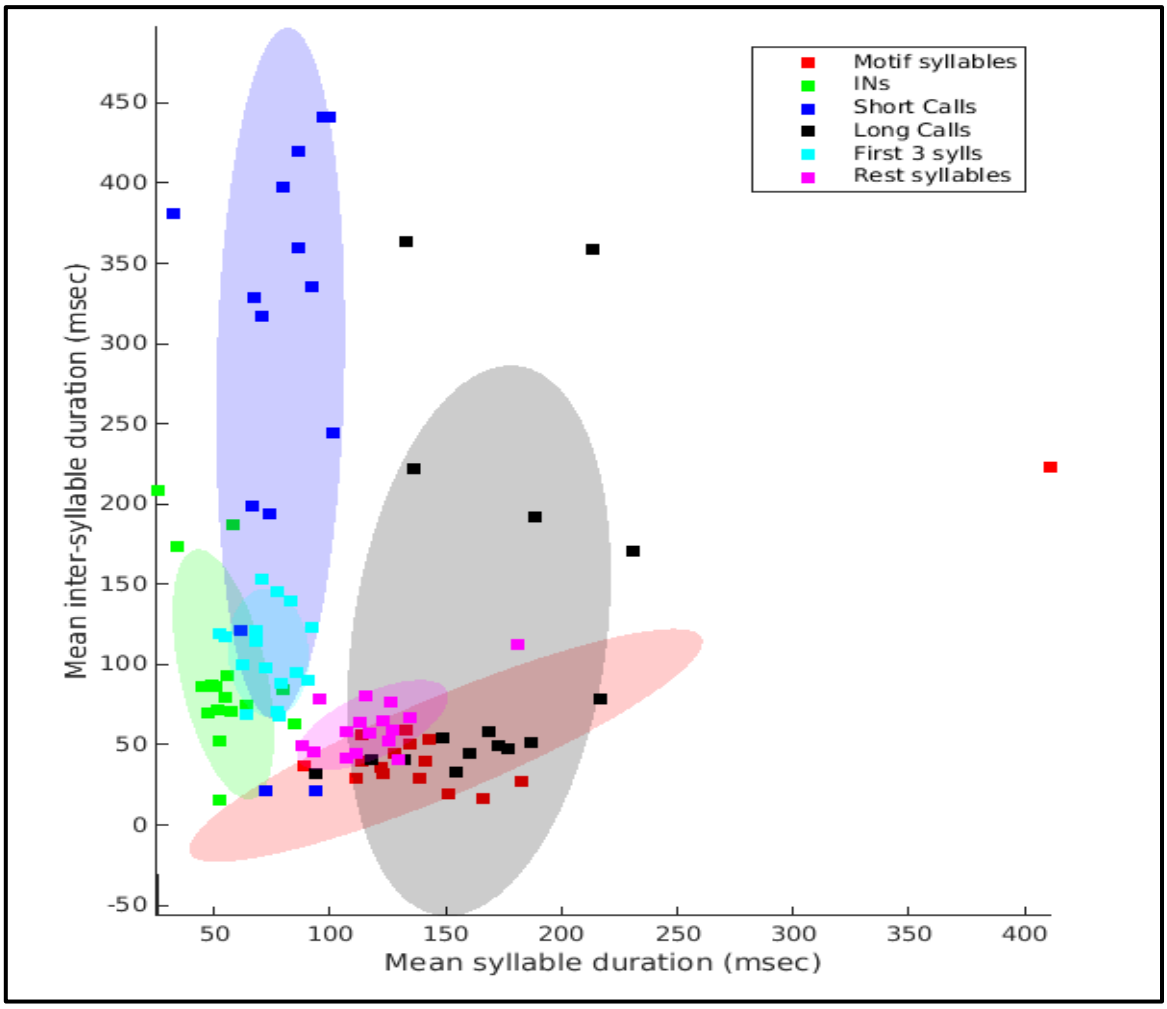

Figure 29: Mean inter-syllable duration v/s Mean syllable duration plot for different syllable categories of Normal birds. Each ellipse in different colors (each color represents the lighter version are the cluster boundaries plotted

Again, to check the how much of the distinction that we observed for the first 3 bout position with the rest of the motif syllable is maintained. We want to know whether the first few syllables are still repeated in the same fashion i.e., spaced differently from the rest of the syllables. The Mean Inter-syllable duration v/s Mean syllable duration are plotted for post-lesion syllables and first 3 syllables of the bout. The post-lesion syllables are superimposed over normal bird plot, keeping the scale same and maintaining the cluster outlines for normal bird syllable categories. From the graph, it is evident that none of the post-lesion motif syllables are similar to normal motif syllables. (Figure 30) However, first 3 bout syllables along with other

[11] syllables which are at the beginning of the song including all syllable categories

syllable types maintained their older position on the plot. The first three, even after HVC lesion, is part of the normal first 3 syllable cluster and hence also overlapping with long calls and short calls. Thus, the distinction between first few positions and the rest remains. However, we need to do a quantitative analysis to validate the same. For now, it can be seen from the plot itself that the 6 out of 8 birds lies inside or closer to the ellipse boundaries of first 3 position syllables from the normal bird analysis. However, no motif syllable (0 out of 8 birds) falls within the boundaries of normal bird motif syllable indicating the HVC lesion has affected the spacing and pacing of motif syllables but not the first three position syllables. It can also be noted that the HVC lesioned long calls have higher syllable duration values as compared to normal bird long calls which support earlier known fact that long call becomes stretched or longer in duration after HVC lesion.(Simpson, 1990)

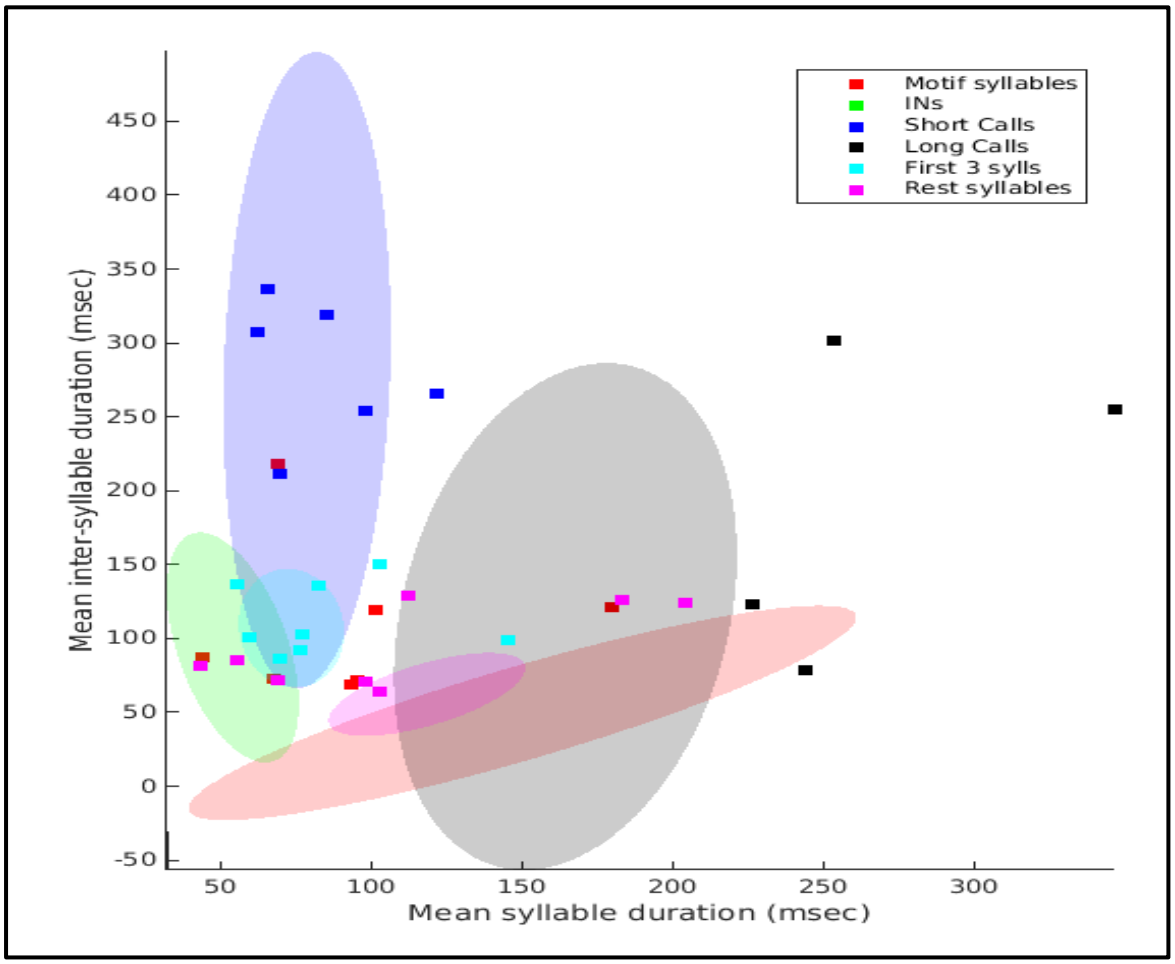

Figure 30: Mean inter-syllable duration v/s Mean syllable duration plot for different syllable categories of lesion birds. The ellipses represent the cluster of normal birds and each colored point represent different syllable categories; "First 3 sylls" are the syllables within the first three positions of a song bout. Cyan, light pink ellipse represents the cluster for "first 3 sylls" and motif syllables respectively in normal birds

In the absence of HVC, Most of the syllables, expect the first few positions lost their "Spacing and Pacing". Hence, the initial part of a post-lesion song is still rendered in the same way as in normal bird song.

DISCUSSION

The results so far are:

The INs has significant '>chance' probability for first 1-4 positions. The short calls also have a '>chance' probability to be present in the first position. However, as you progress along a bout, the probability of finding motif syllable is very high and low probabilities for INs and Short calls. This distinction in the position of a bout for IN, Motif syllable and the Short call will help to locate IN in a bout and will be helpful for cases such as Post-Lesion sub songs or Juvenile songs where syllables are not identifiable and position in the bout is the factor which we can rely upon.

In individual PCA for normal birds, we can see INs are distinct from other motifs syllable in terms of its acoustic features. This is evident from the fact the mean minimum distance of IN to itself is significantly different from all other syllable categories. While, motif syllables and INs has lower Also, the nearest neighbor of each IN is mostly a motif syllable and followed by Short calls, while it is rarely closest to a Long call. Thus, in normal birds on an individual bird basis the INs are acoustically distinct rest of the syllables and, mostly, is much similar to one of the motif syllables or a short call which is part of the song bout.

In general, for all bird PCA, the mean distance from the centroid of IN cluster from other syllable clusters is significantly lower from short calls and increases in the order of motif syllables and then long calls. The mean distance of IN from the centroid of syllable type cluster also shows the similar increase in mean distance in the order of Short calls, Motif syllables and Long calls. This indicates that in general INs are more similar in acoustic features to a short call and is different from Long calls and motif syllables. The PCA also helped us to identify a definite categorization of IN by maintaining the IN dimensions (of the ellipse) from these principal components. These principal components, its coefficient for a set of pre-determined 9 acoustic feature can be used to identify INs from any song bouts and will extremely

useful for those cases when you cannot manually identify them such as in lesion song and Juvenile Sub-songs.

Utilizing these definite dimensions of INs in a PCA, the post-lesion song syllables and other syllable types were mapped on the pre-determined PC dimensions. The motif syllables are plotted on the PCA. The motif syllable is scattered over the PCA and is scattered across all syllable category boundaries. Even the location of first two positions in the bout is scattered across all syllable category boundaries indicating that all syllables in the post-lesion song bouts have lost all its acoustic features irrespective of which position it belongs to in a song bout.

In normal birds, in Mean syllable duration v/s syllable position plot the mean syllable duration significantly increases with syllable position. Similarly, the correlation of syllable duration histogram with overall syllable duration histogram is lower at initial positions and increases over with an increase in position. The difference in linear correlation between first few position and rest of the song indicates that there is a distinction, in terms of syllable duration distribution, between the initial few positions and rest of the syllable. After the lesion also the first few syllables are less correlated with the overall syllable histogram, which is exponential now, compared to rest of the positions which are highly correlated with the overall distribution. The linear correlation for first few positions is again high and significant, and hence different from the rest of the positions all of which are equally well correlated with the overall distribution. This indicates that the syllables in the first few position are more stereotype in terms of syllable durations and has syllables with identical duration repeated frequently at the same position across bouts. While in the rest of the positions the syllables have highly variable syllable duration values and are not stereotyped. However, the mean syllable duration in lesion birds does not show significant linear correlation values even if there is a difference in the mean syllable duration of first few syllables and the rest. This could be because, in lesion birds, the first few positions have either higher mean syllable duration or lower mean syllable duration from the rest and this varies from bird to bird and this variability could account for the fact why the linear correlation is not significant. More sample size may help to improve the significance. Thus, even in the absence of HVC, the first few syllables are still stereotyped and distinct from the rest in terms of syllable duration distribution.

The inter-syllable interval v/s syllable duration shows us that all bout-types are distinct from each other and INs are also distinct from the song bouts. This means the spacing and pacing of song bout is distinct for each bout type. After lesion the song bout and other bout types were plotted on the above graph, it is found that post-lesion song bouts are no longer similar to normal bird song bouts. The lesion songs are different. But, the first few positions maintained the same “spacing” and “pacing” before and after the lesion. Thus, the unique way in which the first few syllables are rendered is independent of HVC and is also different from the rest of the song.

In normal birds, the INs, hence the first few positions, are distinct from rest of the song in terms of its acoustic properties and syllable duration distribution. While, after HVC lesion the bird, the distinction between first few position and rest of the song in terms of syllable duration distribution and Inter-syllable gap is still maintained but the acoustics properties of the initial part of the song is variable and is not distinguishable from rest of the song and no particular position seems to have acoustic properties similar to INs. This means that in absence of HVC, the acoustic properties of the initial few position is different, lost or is indistinguishable from the rest of the noisy lesion syllables while they still maintain the distinction of syllable duration and spacing from the rest of the positions.

This distinction of first 2-3 positions from the rest of the motif syllables in terms of its syllable duration and inter-syllable interval could be because of two possibilities:

One, the difference in syllable spacing and duration at the beginning of the song from the rest of the positions could be just due to the respiratory constraints or the syrinx, the sound box of the birds, inability to produce larger lengths of syllable durations and closer spacing of syllable at the beginning of the song. The syrinx of the bird may need some time after some period of silence (no activity of vocal production) to produce longer syllables with greater pace so it produces shorter syllables with lesser pace till it reaches a point when it can extend it respiratory constraints and produce long strings of motif syllables. Hence, the distinction between first few positions and rest of the motif syllables. Thus, indicating that INs could be an analogous behavior of the human behavior of “clearing the throat” before singing, speeches or long conversations. However, long call, which are syllables of

long duration, are produced in isolation, meaning preceding a long silence. Also, they are also produced in series which primarily starts with a long call only. To check for such possibility, we could try studying the series of long calls and how they are "spaced" and "paced" along with its position of series and do they resemble what we observed with the first few positions of the song.

When DM and HVC were lesioned together, the bird produced no song and only produced some vocalizations similar to INs instead of songs.(Chen et al., 2014) It means even if the main song pathway is cut-off. If they are actually INs then it could imply that the INs are produced either intrinsically by RA which is still intact or INs or the beginning of the song is controlled by entirely another region which is not part of the Song pathway. Given its similarity to short calls, there is also a possibility that INs are controlled by any other region which is part of the call pathway say DM. (see figure 31 for reference) Further lesions such as RA, DM or combination of lesions will help to map the origin of INs or the beginning of the song. However, In general, INs could just be a template of specifically spaced sounds associated with the initiation of a song which gets its acoustic properties when it gets associated with the song pathway (HVC) and is produced as a part of the song.

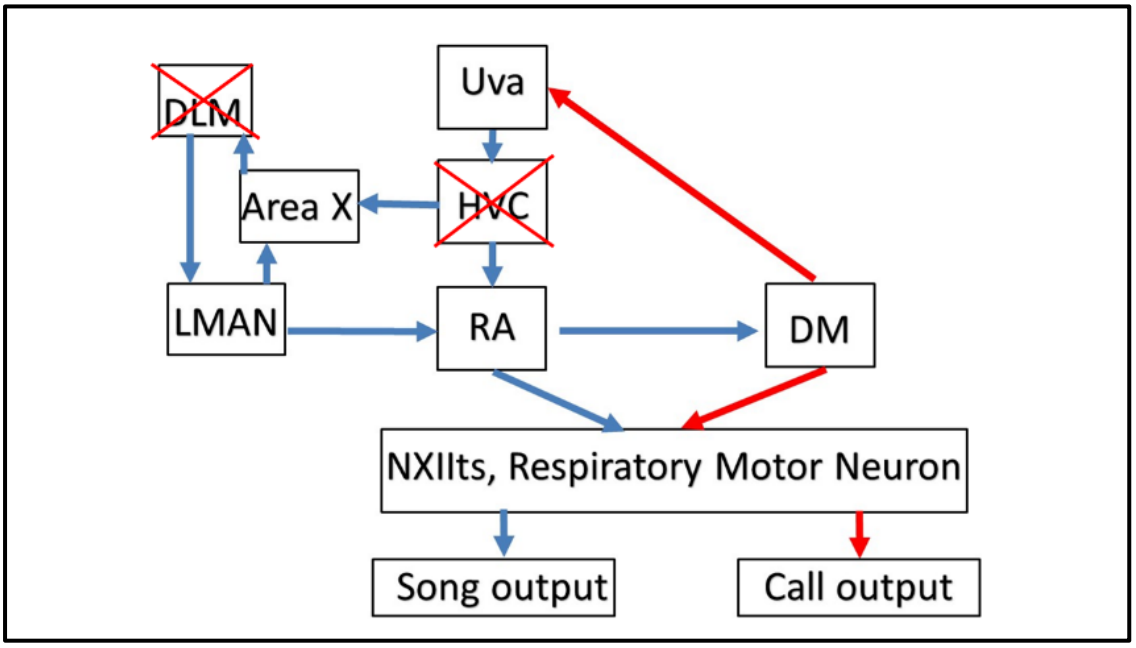

Figure 31: A representation of the dual pathway and regions which are left when both DLM and HVC are removed. Again, the blue and red lines represent the learned and innate pathway respectively.when DM and HVC are removed, only RA and DM is left to provide vocal inputs.

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