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Neural Synchrony in the Zebra Finch Brain

Sydney Pia Goings
Scripps College

Neural Synchrony in the Zebra Finch Brain

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Sydney Pia Goings

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ABSTRACT

I am interested in discovering the role of field potential oscillations in producing synchrony within the song system of the male zebra finch brain. An important function attributed to neural synchrony is sensorimotor integration. In the production of birdsong, sensorimotor integration is crucial, as auditory feedback is necessary for the maintenance of the song. A cortical-thalamic-cortical feedback loop is thought to play a role in the integration of auditory and motor information for the purpose of producing song. Synchronous activity has been observed between at least two nuclei in this feedback loop, MMAN and HVC. Since low frequency field potential oscillations have been shown to play a role in the synchronization of nuclei within the brain of other model animals, I hypothesized that this may be the case in the zebra finch song system. In order to investigate whether oscillatory activity is a mechanism behind the synchronous activity observed between HVC and MMAN, I performed dual extracellular recordings of neural activity within the zebra finch song system. Results suggest that oscillations are likely not involved in the synchrony observed in these nuclei. Future study may reveal that the structure of the feedback loop is necessary, and possibly even sufficient, for the synchronous activity in the zebra finch song system.

ACKNOWLEDGMENTS

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INTRODUCTION

This thesis grew from previous work in Professor Melissa Coleman's lab by Shayna Williams in which she noticed synchronous activity between two identified nuclei (MMAN and HVC) in the zebra finch (*Taeniopygia guttata*) song system. I became interested in the mechanism behind the synchrony she observed and decided to try to determine whether low frequency field potential oscillations were driving the synchrony between HVC and MMAN. In this report, I will begin by defining neural synchrony and introducing its proposed functions and mechanisms, which come from work in model systems such as cats and monkeys as well as work in humans. Then, I will discuss disorders which are related to abnormal neural synchrony, both when there is too little synchrony and too much of it. Since my research was conducted in the zebra finch as a model system, I will next provide background information on the song circuitry in the zebra finch brain. To test the hypothesis that oscillations drive the synchrony observed in this system, I performed dual extracellular recordings from three pairs of nuclei in the zebra finch brain: HVCleft/HVCright, HVC/MMAN, and HVC/NIf. I compared three types of activity in each of these pairs: individual action potentials, bursts of action potentials, and low frequency field potential oscillations. My results suggest that the low field potential oscillations do not play a role in synchronizing the activity of brain nuclei in this system.

Neural Synchrony: An Introduction

Neural synchrony has recently become a popular topic of study in the field of neuroscience, as it is thought to be involved in many different functions of the brain

(Uhlhaas et al., 2009). Interest in the topic began in the 1980's with the discovery that neurons in the visual system exhibit context-dependent synchronization of their oscillatory responses (Gray et al., 1989). Since the 1980's, research on neural synchrony has extended its known function in the brain to coordinating distributed neural activity for crucial tasks such as attention and consciousness (Uhlhaas et al., 2009). Over the years, many studies on neural synchrony have been conducted on animal models as well as humans (Uhlhaas et al., 2009; Kreiter and Singer,1996; Neuenschwander et al., 1996; Engel et al., 1991; Neuenschwander and Singer, 1996).

The term neural synchrony has been used in many ways in the literature (Rubin, 2007). Here, the term "correlated activity" is used to mean that the brain nuclei of interest exhibit some consistent temporal relationship in their activity, be it single action potentials, bursts of action potentials, or oscillations of membrane potentials. Synchrony is slightly more strict in that it requires that the activity is correlated with zero phase offset, or is simultaneous. The functions of neural synchrony are still not well known, although several theories have grown from many different studies. For example, researchers believe that neural synchrony is involved in important global functions like consciousness (Varela et al., 2001), attention (Roy et al., 2007; Uhlhaas et al., 2009), and sensorimotor integration (Womelsdorf and Fries, 2007; Uhlhaas et al., 2009). There are many ways in which neural synchrony is thought to arise, but only one of these mechanisms – low frequency field potential oscillations – will be a focus of this study.

Information about a stimulus may be encoded by neural synchrony in many ways, including strength of synchrony and oscillation phase at which neurons are correlated (Uhlhaas et al., 2009). The strength of neural synchrony is thought to provide

information regarding the relatedness of information arriving at a nucleus. The stronger the synchrony, the more related the information. For example, in the visual cortex, neurons are more likely to synchronize their responses when their orientation preferences are similar and their receptive fields are close to each other (Betsch et al., 2004). In this example, neural synchrony relays information about the similarity of visual features that activate those neurons (Singer, 1999). The phase offsets of correlated neural activity may also provide important information about a stimulus, but less is known about what information they convey (Uhlhaas et al., 2009). The information expressed by neural synchrony could be extensive. Therefore, when neural synchrony is somehow abnormal, information does not flow correctly within the brain, resulting in some kind of disorder. It is thought that epilepsy, schizophrenia, and autism may be related to abnormal neural synchrony (Uhlhaas and Singer, 2006; Uhlhaas et al., 2009).

Functions

Generally, neural synchrony is thought to bring together distributed information from many brain areas. It is believed to perform this unifying function in many important global tasks such as consciousness, attention, and sensorimotor integration.

Consciousness requires the integration of huge amounts of information about any external or internal stimuli being experienced. However, there is not one hub, or "consciousness center," within the brain through which all of this information passes (Uhlhaas et al., 2009). How, then, does the unified experience of consciousness emerge out of distributed brain activity? Studies on visual binding have provided evidence supporting neural synchrony as a mechanism for the integration of information in the brain. Visual binding is the process by which the different attributes of an object such as

the edges, color, texture, motion, and depth, which are processed by different brain areas, are brought together to become a unified representation of the object. In these studies, it has been proposed that the different areas of the brain which are responsible for different aspects of vision fire synchronously, which allows for the integration of all of their pieces of information about the object. Since neural synchrony spans many temporal and spatial scales, it seems that a more global version of the process thought to be involved in visual binding could be a mechanism for consciousness (Varela, et al., 2001). This idea is also supported by the fact that there is a close relationship between arousal and neural synchrony (Uhlhaas et al., 2009).

Whereas consciousness requires the integration of huge amounts of information, attention is the selection of only a small portion of that information to process in detail. There is quite a bit of evidence suggesting that temporal properties of neural activity are important in attention. Much of this evidence comes from studies done on the visual system in cats and monkeys. Steinmetz et al. (2000) investigated the synchronous firing of pairs of neurons in the secondary somatosensory cortex of monkeys trained to switch attention between two different types of sensory tasks and found that neural synchrony is correlated with attentional state. The opposite direction is also true: the degree of neural synchrony indicates whether or not a stimulus is attended. This is because increasing the degree of synchrony of nuclei representing an object, for example, increases the probability that the information about that object gets passed on, which increases the amount of detail that is passed on. As a result, it has been suggested that the neural correlate of attention is neural synchrony (Roy, et al., 2007).

Other studies conclude that neural synchrony is involved in the integration of sensory and motor information (Womelsdorf et al., 2006). It was shown in monkeys that there is a link between enhanced gamma-band (20-80 Hz) synchronization and the speed at which the monkeys can detect, and indicate via a specific motor output, a change in a visual stimulus. This link suggests that neural synchrony in the monkeys' visual cortex directly impacts visually triggered behavior, or visuomotor integration (Fries et al., 2008). Also, Sehatpour et al. (2008), found strong synchrony between the hippocampal formation, occipitotemporal cortex, and lateral prefrontal cortex when participants were shown fragmented images. This synchrony only occurred when the images were not scrambled, and were therefore recognizable. These results provide evidence that synchronization is possible at long distances within the brain and that this long-range synchrony coordinates widely distributed functions like those required in sensorimotor integration (Uhlhaas et al., 2009).

Mechanisms

Many mechanisms for neural synchrony have been proposed. There are two basic circuit-based mechanisms which can explain synchronous activity in two nuclei (Figure 1). First, neural synchrony may occur when there are bidirectional connections between the nuclei involved. Second, two synchronously active nuclei may be receiving input from some third nucleus at the same time (Uhlhaas et al., 2009). Clearly, these physiologically-based mechanisms are dependent upon the model animal and the structure of the circuit of interest.

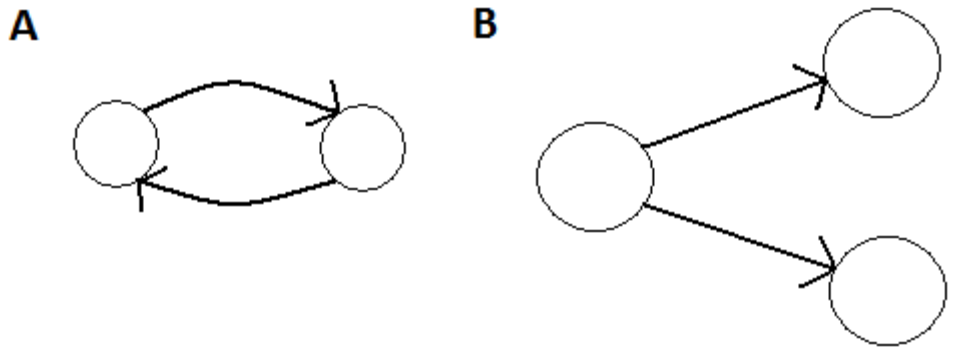

Figure 1. Two basic circuit-based mechanisms for neural synchrony. (A) Bidirectional connections between the nuclei. (B) A third nucleus providing simultaneous input to the nuclei of interest.

Other synchronizing mechanisms do not rely upon circuit structure. For example, oscillations in the beta (12-30 Hz) and gamma range (20-80 Hz) of frequencies have been shown to establish synchrony between distributed brain areas (Uhlhaas et al., 2009). There are several classifications of oscillation frequencies in the brain. From lowest frequency range to highest, they are delta waves (0.1-4 Hz), theta waves (4-7 Hz), alpha waves (8-12 Hz), mu waves (8-13 Hz), beta waves (12-30 Hz), and gamma waves (20-80 Hz). Each classification has been associated with different phenomena in the brain. For example, beta and gamma oscillations have been linked to attention, memory, and multisensory integration, which are proposed functions of neural synchrony (Uhlhaas et al., 2009). Gray et al. (1989) showed that action potentials in cortical cells occur at specific phases of the brain's oscillatory rhythm, synchronizing many cells in a very precise manner. In cats, evidence suggests that oscillatory activity is an efficient mechanism to adjust the precise timing of spikes and that these oscillations are involved in anticipation, suggesting that they could be a neural correlate of attention (Roelfsema et al., 1997).

Disorders

Further evidence that neural synchrony plays an important role in many functions of the brain lies in the fact that abnormal neural synchrony is thought to be a major mechanism responsible for disorders of the brain, including epilepsy, schizophrenia, and autism (Uhlhaas and Singer, 2006; Uhlhaas et al., 2009). As discussed above, neural synchrony plays an important role in the integration of information which results in consciousness (Uhlhaas and Singer, 2006). Many functions of the brain which are affected by schizophrenia are thought to involve the synchronization of beta- and gamma-band oscillations from multiple brain areas. Studies using EEG (Electroencephalography: the recording of electrical activity in the brain with electrodes on the scalp) and MEG (Magnetoencephalography: the recording of magnetic fields produced by electrical currents in the brain with arrays of superconducting quantum interference devices) have shown that responses to auditory and visual stimuli in the beta- and gamma-bands are greatly reduced in patients with schizophrenia. This reduction in oscillatory responses could cause a lack of neural synchrony, resulting in the cognitive dysfunctions seen in the disorder. Similarly, in Autism Spectrum Disorders (ASDs), recent work has attributed the disorders to deficits in the synchronization of distributed neural activity (Uhlhaas et al., 2009). Similar symptoms in both disorders, such as disorganized thoughts and language as well as abnormal perceptual integration during auditory and visual perception, suggest that schizophrenia and ASDs share similar mechanisms. Further studies on neural synchrony and the role of oscillations in synchrony could help in the search for better treatments for these disorders.

On the other hand, too much neural synchrony may also be pathological. For example, in epilepsy, seizures are a result of abnormal synchrony which is too long lasting and has too large of an amplitude (Uhlhaas and Singer, 2006; Penfield et al., 1954). High-frequency synchronous oscillatory activity is frequently observed in the brain before and during epileptic events (Allen et al., 1992; Fisher et al., 1992). Discovering ways to stop this over-synchronization and to predict seizures based on the presence of certain types of oscillations before seizures would increase the quality of life for sufferers of epilepsy worldwide.

The Zebra Finch (*Taeniopygia guttata*) as a Model System for Studying Neural Synchrony

Zebra finch (*Taeniopygia guttata*) were chosen as the model system in this study of neural synchrony. The zebra finch is commonly used as a model system to study vocal learning. Vocal learning is the ability of an animal to learn to produce sound by imitating what it hears. Most species produce innate sounds but do not exhibit vocal learning. For example, dogs will bark without having heard the sound produced by another animal. Many species exhibit auditory learning, which is the ability to recognize a word and respond behaviorally, rather than the ability to learn to speak the word itself. An example of auditory learning is a dog learning to sit when its owner says the word "sit." Very few species are known to exhibit vocal learning. Some examples are primates, bats, dolphins, parrots, hummingbirds, and a few songbirds (Jarvis, 2004). Songbirds are commonly used to study vocal learning, as many types of neurological studies are relatively easy to conduct in these animals (White, 2001).

There are many reasons why the zebra finch is one of the most common songbirds used as a model system for studying vocal learning. First, they are easily kept and bred in captivity, which allows for researchers to spend most of their energy doing experiments rather than trying to care for and breed the birds. Second, zebra finches sing year-round, permitting researchers to collect the maximum amount of data in a year. Third, they are sexually dimorphic in plumage, brain circuitry, and ability to produce song. The different brain circuitry means that only males can produce song, but female brains are specialized for differentiating between different males' songs and choosing a mate based on song characteristics. Also, the song circuitry in male zebra finches is relatively well-understood compared to the analogous systems in other animals, including humans. Fourth, the brain circuitry involved in song production, known as the song system, is unlike other vertebrate motor systems in that it is a physically discrete system specializing in one behavior -- producing song (Sutter and Margoliash, 1994). This allows researchers to be quite certain that activity observed in the song system is actually related to song. Fifth, a male finch only learns one song in his lifetime, making it possible to conduct long term studies on how songs change in response to certain experimental manipulations. Finally, there are many similarities between the processes by which humans learn to speak and zebra finches learn to sing. Both species experience critical periods for vocal learning, during which they must hear and practice their vocalizations or they will never be able to do so. If deafened or isolated from adult tutors during critical periods, vocalizations cannot be learned.

Vocal learning occurs in stages that both zebra finches and humans experience. The first stage is a sensory period of listening and learning the auditory pattern of the

appropriate vocalizations. No vocalizations other than innate sounds are made during this stage. In the next stage, the young birds or humans exhibit plastic vocalizations known as subsong or babbling, respectively, as they learn how to create the new sounds. Subsong is highly variable and generally quiet, as it is not meant for communication and is often produced as the bird appears to be on the verge of falling asleep (Nottebohm, 2005). Finally, after a change in hormone level, song or speech becomes stereotyped and changes very little over the rest of the animal's lifetime (Doupe and Kuhl, 1999).

Zebra finches were used in this study for the properties known about the nuclei and connecting pathways involved in song production, known as the song circuitry, in their brains (Figure 2). One reason the song circuitry is of interest is that vocal learning requires the ability to match one's own sound to a learned pattern. In other words, vocal learning requires sensorimotor integration. The integration of auditory and motor information is thought by many researchers to occur in a specialized nucleus within the song circuitry called HVC (used as a proper noun), since it receives both auditory and motor inputs. Another possibility is that the motor feedback loop including the nuclei known as RA (robust nucleus of the arcopallium), DMP (dorsomedial nucleus of the posterior thalamus), MMAN (medial magnocellular nucleus of the nidopallium), and HVC is involved in comparing the bird's motor output to its auditory output during song – another form of sensorimotor integration (Williams, 2009; Roberts et al., 2008). As previously mentioned, sensorimotor integration may be a function of neural synchrony, making this loop a good place to begin studying neural synchrony within the zebra finch model system. In fact, possible synchronous activity has been observed between at least two of these nuclei, HVC and MMAN (Williams, 2009). Also, both spontaneous and

auditory-evoked activity in HVC and MMAN are tightly correlated. The timing of auditory responses in MMAN has been shown to have a complex temporal relationship with those in HVC. Studies have shown that sometimes MMAN activity precedes HVC activity, other times HVC activity precedes MMAN activity, and even other times HVC and MMAN activity were exactly synchronous (Vates et al., 1997; Seki and Okanoya, 2008; Williams, 2009). This timing is odd because the nuclei are 5 mm apart, which should correspond to a specific nonzero synaptic delay time, however the existence of the loop structure could potentially influence the timing.

The zebra finch song system consists mainly of two pathways: a vocal motor pathway and an anterior forebrain pathway. The anterior forebrain pathway is involved in the learning and modification of song, while the vocal motor pathway is involved in the actual production of the song. Within the song system resides several feedback loops that have been implicated in the integration of auditory and motor information. The feedback loops consist of song nuclei found both in the anterior forebrain pathway and the vocal motor pathway, as well as song nuclei which are included in neither of the two major pathways (Figure 2). HVC is a part of both the vocal motor and anterior forebrain pathways, and is implicated in sensorimotor integration and song pattern generation (Solis and Perkel, 2005). Some HVC cells project to RA, which is a member of the vocal motor pathway. RA is a premotor nucleus which is connected eventually to the bird's syrinx, the vocal organ, and is also involved in respiration control (Farries, 2006). Some neurons in RA then project to DMP, which is possibly involved in interhemispheric communication and coordination, as it connects bilaterally to both left and right MMAN (Vates et al., 1997). MMAN then completes the loop by sending projections back to

HVC. The function of MMAN is not well understood. One study suggested that MMAN is involved in song learning, since lesioning the nucleus bilaterally in juveniles resulted in more significant deficits than in adults (Foster and Bottjer, 2001). However, a different study suggested that MMAN could be involved in determining whether a stimulus is a zebra finch song or in bilaterally coordinating the song system (Williams, 2009). Due to the bilateral projections from DMP to MMAN, it has been proposed that this motor feedback loop may aid in coordinating bilateral HVC activity, which has been shown to be tightly coordinated during singing (Vates et al., 1997; Coleman and Vu, 2005; Vu et al., 1994).

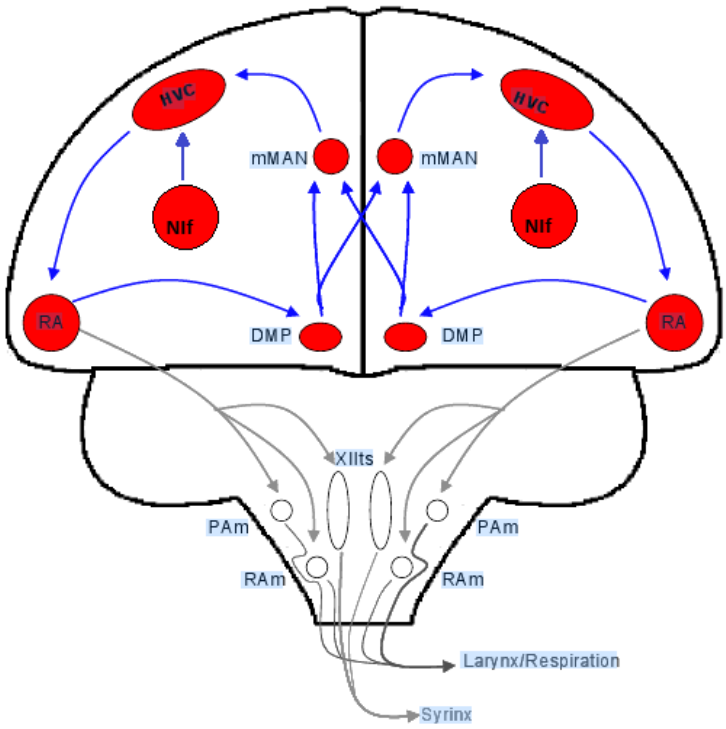

Figure 2. A bilateral feedback loop containing identified nuclei from the zebra finch song system in a cartoon of a coronal section of the zebra finch brain. HVC (used as a proper noun), RA (robust nucleus of the arcopallium), DMP (dorsomedial nucleus of the posterior thalamus), MMAN (medial magnocellular nucleus of the nidopallium), and NIf (interfacial nucleus of the nidopallium).

Experiment Summary and Goal

I am interested in discovering a mechanism behind the synchronous activity observed in this model system. Since there aren't bilateral connections between MMAN and HVC nor is there a third nucleus connected directly to both MMAN and HVC, the circuit architecture does not fit the parameters necessary for the circuit-based mechanisms described above. Therefore, I hypothesized that field potential oscillations may be a mechanism for neural synchrony in zebra finch system. It is also possible that the structure of the feedback loop, though much more complicated than the circuitry in the circuit-based mechanisms described above, is necessary and possibly even sufficient to cause the synchronous behavior.

In order to investigate whether oscillatory activity is a mechanism behind the synchronous activity observed in the zebra finch song system, I performed dual extracellular recordings of neural activity within the zebra finch song system. I recorded simultaneously from HVC and MMAN and compared their outputs. I also recorded simultaneously from NIf (interfacial nucleus of the nidopallium) and HVC because much of the auditory-evoked and spontaneous activity in HVC seems to arise from NIf (Coleman et al., 2004; Cardin et al., 2004) and NIf lies outside of the feedback loop outlined above, allowing me to compare data from the feedback loop to NIf data in an attempt to determine whether the loop structure is necessary for neural synchrony in the zebra finch song system. Finally, I recorded from HVC on both hemispheres of the brain simultaneously because their activity has been shown to be tightly coordinated during singing (Vates et al., 1997; Coleman and Vu, 2005; Vu et al., 1994). I hypothesized that I would find simultaneous activity between HVCleft and HVCright and between HVC and

MMAN. I also expected that NIf activity would be strongly correlated with HVC activity, but NIf would lead HVC activity rather than the two nuclei having simultaneous activity. As mentioned above, I expected to find evidence that low-frequency field potential oscillations played a part in creating or maintaining this synchronicity.

MATERIALS AND METHODS

Animal Care

A total of 6 birds, all more than 90 days post-hatch, were used in this experiment. All birds were housed in the W.M. Keck Science Department of Scripps, Claremont McKenna, and Pitzer Colleges and were provided with birdseed and water *ad libidum*. They were also regularly given minerals, grit, sand, water baths, eggs, spinach, and toys. The colony was kept on a 12:12 day:night (on at 7am, off at 7pm) light cycle. All procedures were approved by the Institutional Animal Care and Use Committee at the Keck Science Department.

Song Recording

To record a bird's song, a male bird was placed in a cage within an isolation box (Eckel Industries, Cambridge, MA) with a female for about 24 hours with unlimited food and water. Sounds were captured using a microphone attached to a sound digitizer (Firebox, PreSonus Electronics, Baton Rouge, LA) and a computer running Sound Analysis Pro (Tchernichovski et al., 2000). Approximately two seconds of the song containing two to three motifs were trimmed from the recording using Goldwave (Goldwave Inc., St. John's, Newfoundland, CAN). The resulting trimmed song was used during the experiment as the bird's own song (BOS). The reverse of BOS, (REV), was then created using Goldwave's built in Reverse function. Finally, for a conspecific song, (CON), a song file from a different bird in the colony was used. All three songs were normalized to ~70dB using a sound pressure meter (RadioShack).

Anesthesia and Surgery Preparation

Before each experiment, the birds were anesthetized with 3 injections of 30 to 40 μL of 20% urethane in the pectoral muscle for a total of 90 to 100 μL. The injections were spread over an hour, with half an hour between injections. One and a half to two hours after the final injection of urethane, the bird was placed in a small blanket and then placed in a stereotaxic apparatus (custom made by Herb Adams), which held its head in place with ear bars and a beak holder. A small amount of 2% lidocaine (Hospira Inc., Lake Forrest, IL) was injected under the bird's scalp, which was then opened at the midline on the top of the head. The upper skull was removed over the bifurcation of the midsagittal sinus. The bifurcation was used to measure and mark the approximate location of MMAN and NIf (MMAN: 5.2 mm anterior and 0.5 mm lateral of the bifurcation of the midsagittal sinus, NIf: 2 to 2.5 mm anterior and 1.7 mm lateral of the bifurcation of the midsagittal sinus). Finally, a stainless steel headpost was mounted to the skull just behind the beak with dental cement (Coltene/Whaledent Inc., Cuyahoga Falls, OH) and cyanoacrylate (Krazy Glue, Elmer's, Columbus, OH).

Surgery Procedures

The bird was moved to another stereotaxic apparatus on an air table with a faraday cage (manufacturer) lined with sound foam. The bird's head was held in place at approximately 40° from horizontal. The bird's body, still wrapped in the blanket, was supported by a heating pad (FHC, Bowdoin, ME) at about 37°C. A speaker, used to play BOS, REV, and CON, was placed approximately 30 to 40 cm from the bird within the recording rig, facing the bird. Following the previously made score-marks over the

relevant nuclei, small craniotomies were made in the skull for access to HVC, NIf, and MMAN with the recording electrodes.

To perform the dual extracellular recordings, carbon fiber electrodes (Carbostar-1, Kation Scientific, Minneapolis, MN) were positioned by micromanipulators (Siskiyou, Grants Pass, OR) over the craniotomies and were lowered into the brain using actuators (Siskiyou) controlled by motion controllers (Siskiyou or Newport, Irvine, CA). The recordings were amplified (A-M systems, Sequim, WA), digitized at 20000 Hz (Micro1401, CED, Cambridge, England), and collected using Spike 2 software (CED). The files were saved onto a PC and an external hard drive.

Electrodes for HVC were positioned at 2.4 mm lateral of the bifurcation of the midsagittal sinus and 200 to 500 m ventral to the dorsal surface of the brain. Electrodes for recording from MMAN were positioned 5.2 mm anterior and 0.5 mm lateral of the bifurcation of the midsagittal sinus and were 1.8 to 2.0 mm ventral to the dorsal surface of the brain. Electrodes recording from NIf were 2 to 2.5 mm anterior and 1.7 mm lateral of the bifurcation of the midsagittal sinus and were 1.8 to 2.4 mm ventral to the dorsal surface of the brain. All three nuclei were identified by their individual characteristic firing patterns (Coleman et al., 2004). HVC is known for its spontaneous bursting with increased firing when BOS is played. MMAN has bursts correlating with those in HVC and often responds to BOS, but it generally has more background activity than HVC (Williams, 2009).

At each recording site, four types of recordings were collected (Figure 3). Two were filtered at 300 Hz lowpass and 5000 Hz highpass (Figure 3, A and C) and two were left relatively unfiltered at 1 Hz lowpass and 20000 Hz highpass (Figure 3, B and D).

The filtered data were used to analyze action potentials and bursts of action potentials, while the unfiltered data were used to analyze the low frequency oscillations. At each filter level ("filtered" or "unfiltered"), one recording consisted of spontaneous activity (Figure 3, A and B) and another of song-evoked activity (Figure 3, C and D). Both song-evoked and spontaneous activity were collected in order to determine whether synchrony was enhanced or possibly only existed during song. For each recording of song-evoked activity, 20 repetitions each of BOS, REV, and CON were played in random order with 7±2 seconds inter-stimulus interval. Activity was recorded simultaneously from HVC and ipsilateral MMAN, HVC and ipsilateral NIf, or bilaterally in both HVCs. After recording from HVC and MMAN and before removing the MMAN electrode, the MMAN recording site was marked by an electrolytic lesion (+8 A for 10s, single pulse). The recording site was later identified by histological methods described below.

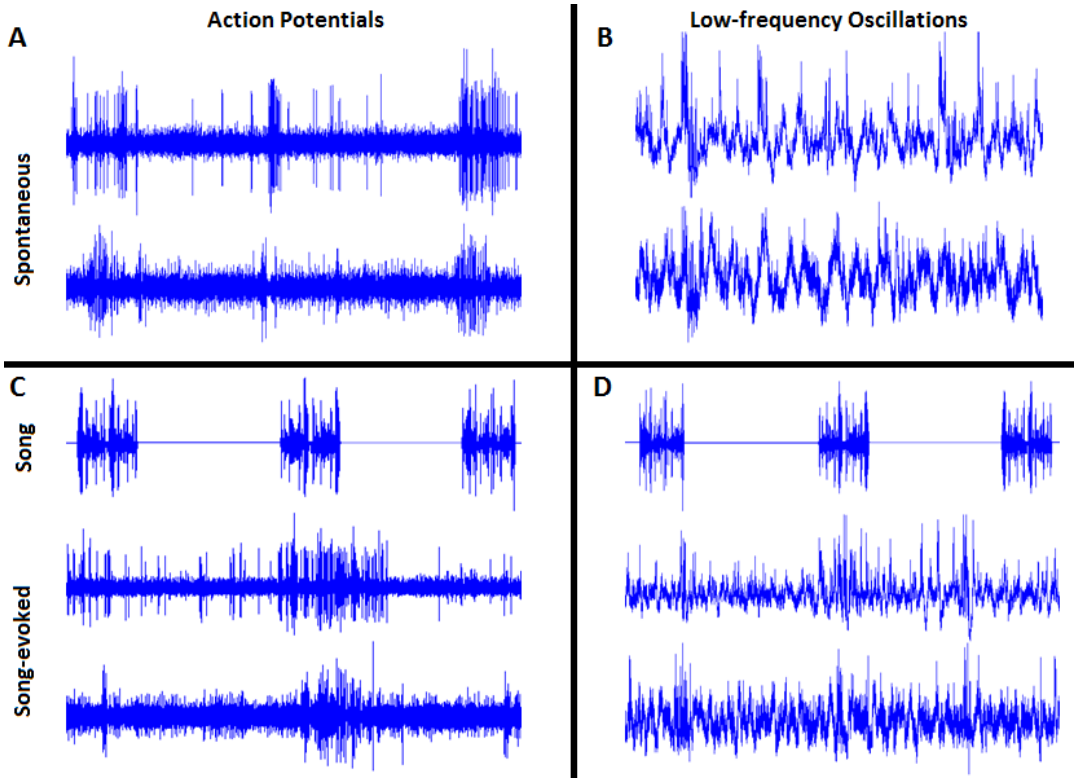

Figure 3. Example raw data traces from HVC (top data trace in each quadrant) and MMAN (bottom data trace in each quadrant). (A) filtered spontaneous, (B) unfiltered spontaneous, (C) filtered song-evoked, and (D) unfiltered song-evoked activity. Filtered data were used to analyze action potentials and bursts of action potentials, while unfiltered data were used to analyze low-frequency oscillations.

Histology

At the conclusion of each experiment, the bird was euthanized with about 0.15 mL of equithesin (7.1 mL distilled water, 0.42 g $MgSO_4$, 6.92 mL propylene glycol, 1.78 mL 100% Ethanol, 4.2 mL Pentobarbital (Nembutal 50 mg/mL), 0.85 g Chloral Hydrate). The bird was then perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. The brain was immediately removed from the skull and stored in 4% paraformaldehyde at 4°C until histological processing. Prior to histological processing, the brains were cryoprotected with 30% sucrose in 4% paraformaldehyde overnight. Brains were then sliced coronally into 100 μm sections using a freezing microtome (Thermo Fisher Scientific). Sections were then mounted on subbed slides and

subsequently stained with cresyl-violet and coverslipped with Krystalon (EMD Chemicals/Harleco, Darmstadt, Germany). To identify lesion sites, the sections were then viewed and photographed on a microscope.

Data Analysis

Four analyses were performed on the data collected in the experiments.

1. In order to determine whether the nuclei exhibited related bursting, a correlation was performed on the bursts of action potentials between each of the pairs of nuclei (MMAN/HVC, HVCleft/HVCright, and NIf/HVC). Before performing the burst correlation analysis, the DC offset was removed and raw filtered data was rectified and smoothed over a time constant of 0.2 seconds (Figure 4). These waveforms were then compared using the Spike2 waveform correlation function. In MMAN/HVC and NIf/HVC pairs, HVC was used as the reference, while the other nucleus was used as the search channel. For data from the HVCleft/HVCright pair, HVCright was used as the reference and HVCleft as the search channel. The peak correlation value was used for comparisons. The offset of this peak correlation value was used to determine how synchronous the correlated bursts were.

2. For the second analysis, an oscillation correlation was performed on each pair of nuclei to determine whether the oscillations within the nuclei were related. Oscillation correlation analyses were performed on raw unfiltered data using the waveform correlation function in Spike2 after removing the DC offset (Figure 5). HVC or HVCright was used as the reference channels and the peak correlation values were used

for comparisons. The offset of this peak correlation value was used to determine the phase offset of the correlated oscillations.

3. Third, within each nucleus a correlation was performed to determine whether bursts of action potentials and low-frequency field potential oscillations within a single nucleus were related. These analyses used the raw, unfiltered data from a single nucleus which was high-pass filtered and then prepared as in the burst correlation analyses and low-pass filtered for oscillation analyses (Figure 6). These two preparations were then compared with the waveform correlation function in Spike2. As in both of the above analyses, HVC or HVCright was used as the reference channel and the peak correlation values were used for comparisons. The offset of this peak correlation value was used to determine whether the bursts of action potentials were synchronized with the oscillations.

4. Finally, an action potential correlation was performed on each pair of nuclei to determine whether the firing of action potentials occurred at similar times within the two nuclei. Action potentials were defined by a user-defined threshold (Figure 7). Correlations in time between action potentials in two nuclei were then compared with the event correlation function in Spike2, with HVC or HVCright as the reference channel as above. The value of the largest bin in the resulting histogram was used for comparisons. The offset of this largest bin was used to determine the level of synchronicity with which action potentials were fired between the two nuclei.

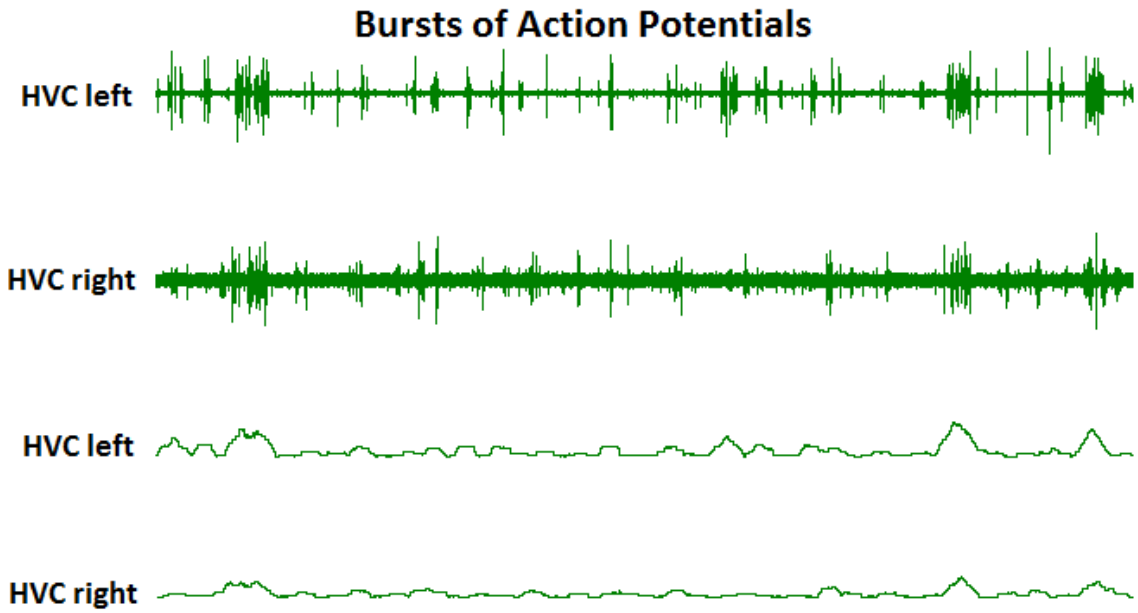

Figure 4. *Top two traces:* raw filtered HVCleft/HVCright data. *Bottom two traces:* data from the top two traces processed for burst correlation analysis.

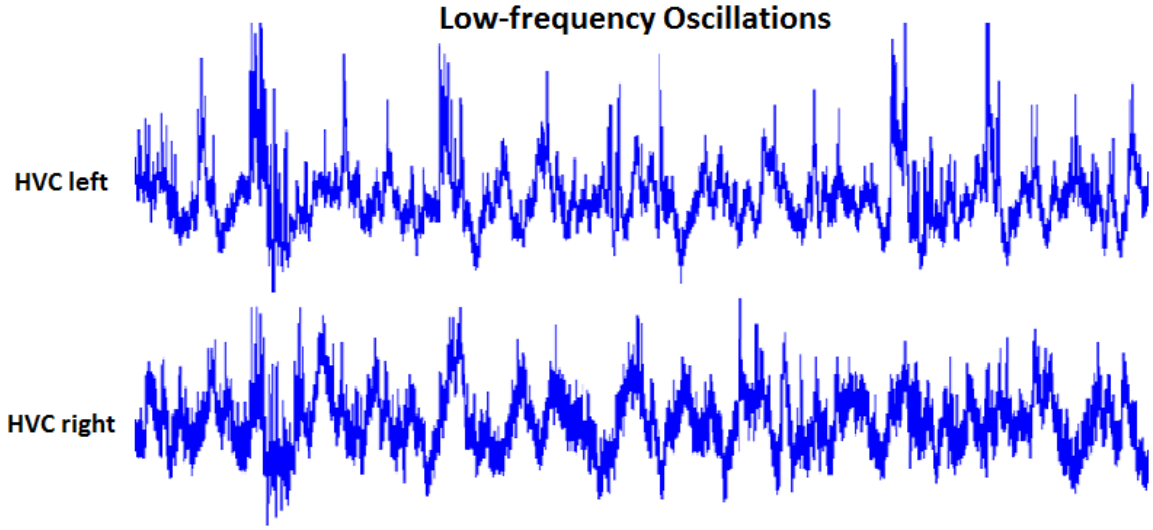

Figure 5. Example unfiltered HVCleft/HVCright data used for oscillation correlation analysis.

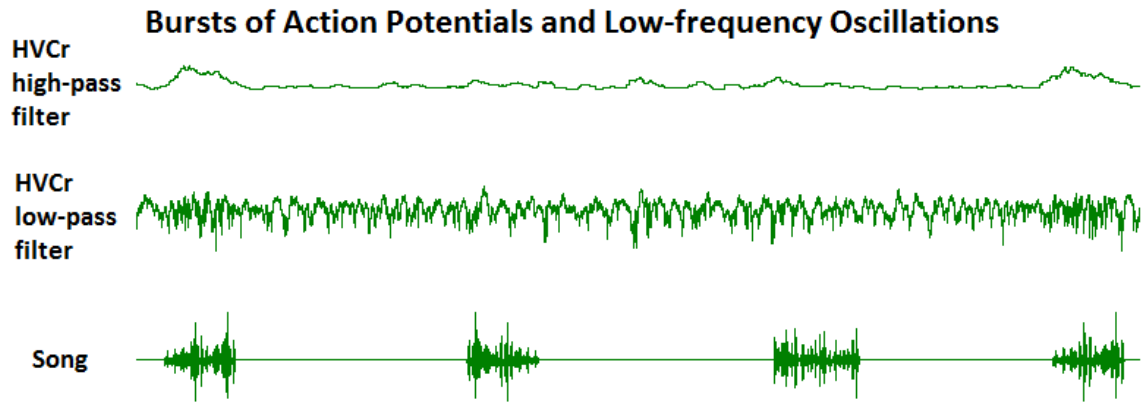

Figure 6. Example HVCright song-evoked data processed for correlation analysis of bursts of action potentials and low-frequency oscillations. *Top trace*: HVCright data that has been put through a high-pass filter, rectified, and then smoothed (time constant 0.2s). *Bottom trace*: The same HVCright data that has been low-pass filtered.

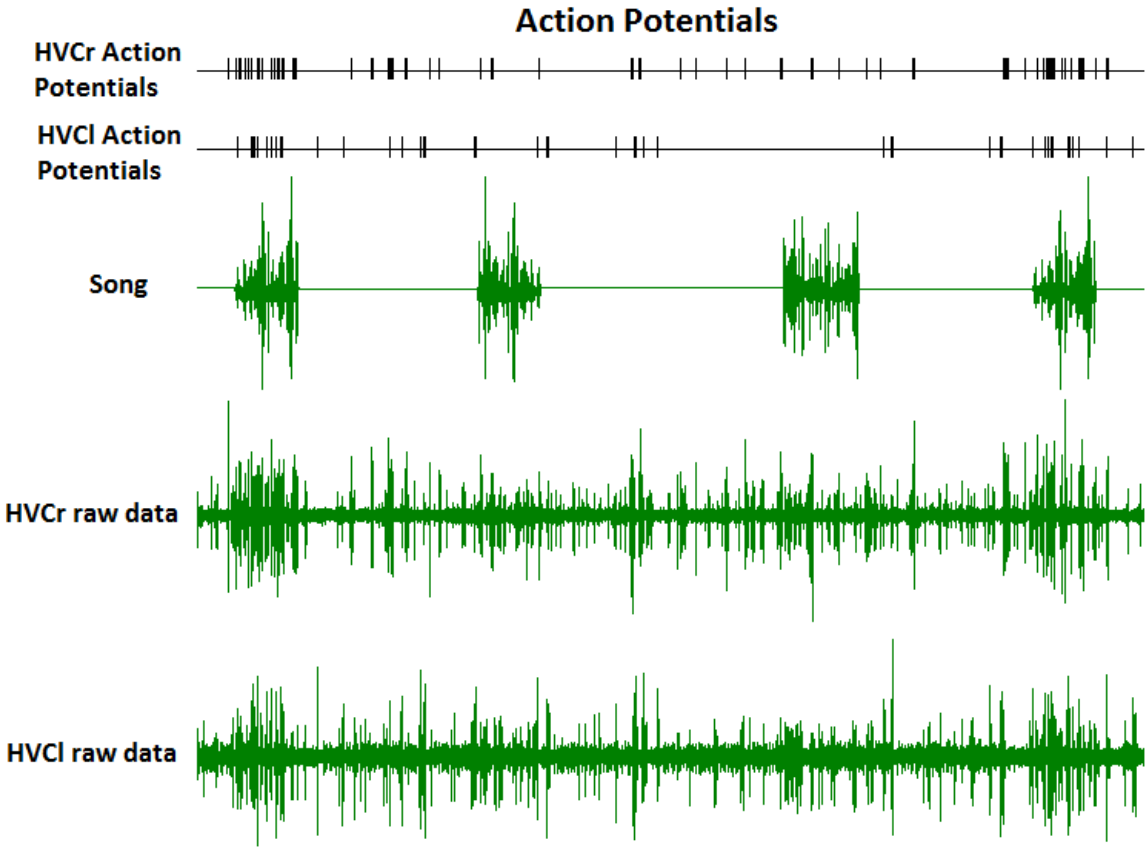

Figure 7. Example HVCleft/HVCright song-evoked data processed for action potential correlation analysis. *Top two*: action potentials above threshold for HVCright and HVCleft. *Bottom two*: raw filtered data for HVCright and HVCleft.

Each of these four analyses was performed in three different song contexts: spontaneous activity, auditory-evoked activity during song, and auditory-evoked activity

between songs. Spontaneous activity was recorded from a 5-minute trial where no song was played. For both auditory-evoked activity during song and between songs, activity was recorded from a trial in which song (BOS, REV, and CON) was played intermittently. For the song context, data were only analyzed from when BOS was playing. For the between song context, data were only analyzed from between songs, specifically just before BOS was played.

For each nucleus, data were also collected from just outside of the nucleus (not necessarily the same place each time) for comparison. These data were analyzed as described above and then compared to the data collected from within the nuclei.

Some data collected during experiments were removed before analysis for at least one of the following reasons. In some trials, breathing artifacts, movement artifacts, 60-cycle noise, or charge buildup were present throughout the trial. Also, in some trials, the gain was set too high, cutting off many of the spike peaks. If any of these problems affected the data to the point where the entire trial was compromised by occurring several times or overwhelming the pattern (especially in unfiltered trials), the trial was not included in the analysis.

RESULTS

Correlations of Bursts of Action Potentials

To determine whether the nuclei exhibited correlated bursting, waveform correlations were performed on the bursts of action potentials in each of the pairs of identified nuclei involved in song learning and production. Waveform correlation analysis of dual extracellular recordings from these pairs of nuclei showed that bursts of action potentials in NIf and HVC as well as MMAN and HVC are highly correlated in all song contexts, while HVCleft and HVCright did not exhibit highly correlated bursting in any context (Figure 8a; MMAN spontaneous activity n=5, MMAN song-evoked activity during song n =5, MMAN song-evoked activity between song n=5, NIf spontaneous activity n=4, NIf song-evoked activity during song n =4, NIf song-evoked activity between song n=4, HVC spontaneous activity n=6, HVC song-evoked activity during song n=5, HVC song-evoked activity between song n=5; single sample t-tests versus 0: MMAN/HVC spontaneous activity p=0.000, MMAN/HVC song-evoked activity during song p=0.001, MMAN/HVC song-evoked activity between song p=0.000, NIf/HVC spontaneous activity p=0.003, NIf/HVC song-evoked activity during song p=0.007, NIf/HVC song-evoked activity between song p=0.002, HVCleft/HVCright spontaneous activity $p<0.0001$, HVCleft/HVCright song-evoked activity during song p=0.000, HVCleft/HVCright song-evoked activity between song p=0.005; one-way ANOVAs: MMAN vs. NIf vs. HVC spontaneous activity p=0.001 (Tukey HSD: MMAN vs. NIf p = nonsignificant, NIf vs. HVC $p<0.01$, MMAN vs. HVC $p<0.01$), MMAN vs. NIf vs. HVC song-evoked activity during song p=0.546, MMAN vs. NIf vs. HVC song-evoked activity between song p=0.033 (Tukey HSD: MMAN vs. NIf p=nonsignificant, NIf vs.

MMAN p=nonsignificant, MMAN vs. HVC p=nonsignificant)). The same analysis on data from areas just outside of these nuclei showed that the bursts were not very correlated, if at all, within those pairs (Figure 8b; outside of MMAN spontaneous activity n=3, outside of MMAN song-evoked activity during song n =1, outside of MMAN song-evoked activity between song n=1, outside of NIf spontaneous activity n=4, outside of NIf song-evoked activity during song n =3, outside of NIf song-evoked activity between song n=3, outside of HVC spontaneous activity n=2, outside of HVC song-evoked activity during song n=2, outside of HVC song-evoked activity between song n=2; single sample t-tests versus 0: outside of MMAN/HVC spontaneous activity p=0.190, outside of MMAN/HVC song-evoked activity during song p=N/A, outside of MMAN/HVC song-evoked activity between song p=N/A, outside of NIf/HVC spontaneous activity p=0.021, outside of NIf/HVC song-evoked activity during song p=0.113, outside of NIf/HVC song-evoked activity between song p=0.079, outside of HVCleft/HVCright spontaneous activity p=0.102, outside of HVCleft/HVCright song-evoked activity during song p=0.024, outside of HVCleft/HVCright song-evoked activity between song p=0.094; one-way ANOVAs: outside of MMAN vs outside of NIf vs outside of HVC spontaneous activity p=0.779, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity during song p=0.780, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity between song p=0.612). To determine whether the activity observed in these pairs of nuclei were synchronous, the offset of the peak correlation values for each of the above analyses were plotted (Figure 9). The timing of the peak correlation values in each of these analyses were not significantly different from each other and most were not significantly different from zero, suggesting that the bursts were

fairly simultaneous between NIf and HVC and between MMAN and HVC (Figure 9; MMAN spontaneous activity n=5, MMAN song-evoked activity during song n =5, MMAN song-evoked activity between song n=5, NIf spontaneous activity n=4, NIf song-evoked activity during song n =4, NIf song-evoked activity between song n=4, HVC spontaneous activity n=6, HVC song-evoked activity during song n=5, HVC song-evoked activity between song n=5, outside of MMAN spontaneous activity n=3, outside of MMAN song-evoked activity during song n =1, outside of MMAN song-evoked activity between song n=1, outside of NIf spontaneous activity n=4, outside of NIf song-evoked activity during song n =3, outside of NIf song-evoked activity between song n=3, outside of HVC spontaneous activity n=2, outside of HVC song-evoked activity during song n=2, outside of HVC song-evoked activity between song n=2; single sample t-tests versus 0: MMAN/HVC spontaneous activity p=0.031, MMAN/HVC song-evoked activity during song p=0.001, MMAN/HVC song-evoked activity between song p=0.063, NIf/HVC spontaneous activity p=0.003, NIf/HVC song-evoked activity during song p=0.207, NIf/HVC song-evoked activity between song p=0.011, HVCleft/HVCright spontaneous activity p=0.374, HVCleft/HVCright song-evoked activity during song p=0.342, HVCleft/HVCright song-evoked activity between song p=0.139, outside of MMAN/HVC spontaneous activity p=0.413, outside of MMAN/HVC song-evoked activity during song p=N/A, outside of MMAN/HVC song-evoked activity between song p=N/A, outside of NIf/HVC spontaneous activity p=0.026, outside of NIf/HVC song-evoked activity during song p=0.056, outside of NIf/HVC song-evoked activity between song p=0.125, outside of HVCleft/HVCright spontaneous activity p=0.341, outside of HVCleft/HVCright song-evoked activity during song p=0.294, outside of

HVCleft/HVCright song-evoked activity between song p=0.245; one-way ANOVAs: MMAN vs NIf vs HVC spontaneous activity p=0.821, MMAN vs NIf vs HVC song-evoked activity during song p=0.053, MMAN vs NIf vs HVC song-evoked activity between song p=0.316, outside of MMAN vs outside of NIf vs outside of HVC spontaneous activity p=0.481, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity during song p=0.239, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity between song p=0.523).

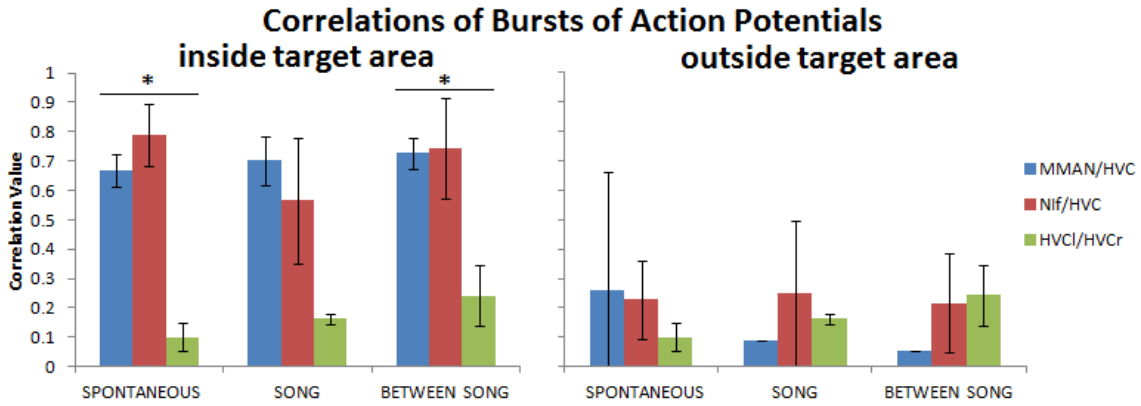

Figure 8. Peak correlation values for the burst correlation analysis. Bursts of action potentials in NIf and HVC as well as MMAN and HVC are highly correlated.

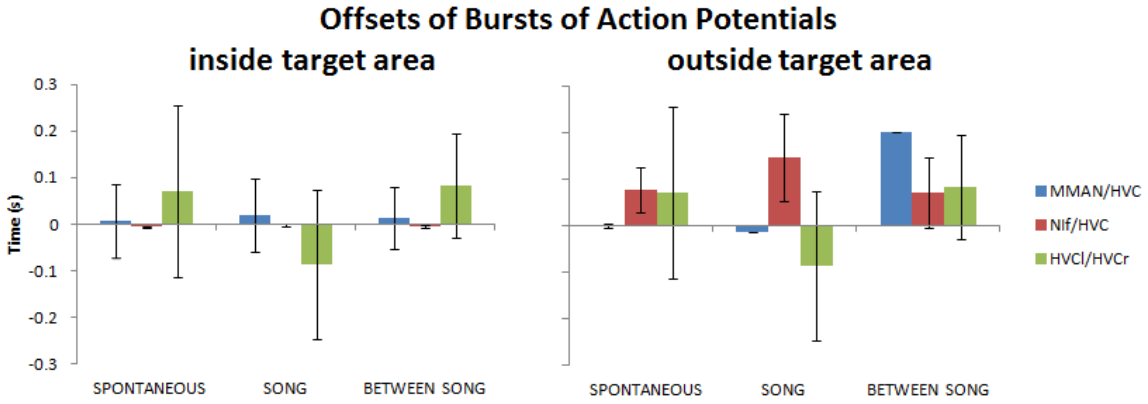

Figure 9. Offset times of peak burst correlation values.

Oscillation Correlations

In order to determine whether low-frequency oscillations within the pairs of

nuclei were related, waveform correlations were performed on the low-frequency oscillations in each of the pairs of nuclei. These waveform correlation analyses showed that oscillations in NIf and HVC are highly correlated, those in MMAN and HVC are mildly correlated, and those in HVCleft and HVCright are not very correlated at all (Figure 10a; MMAN spontaneous activity n=4, MMAN song-evoked activity during song n =4, MMAN song-evoked activity between song n=4, NIf spontaneous activity n=4, NIf song-evoked activity during song n =3, NIf song-evoked activity between song n=3, HVC spontaneous activity n=4, HVC song-evoked activity during song n=3, HVC song-evoked activity between song n=3; single sample t-tests versus 0: MMAN/HVC spontaneous activity p=0.004, MMAN/HVC song-evoked activity during song p=0.004, MMAN/HVC song-evoked activity between song p=0.001, NIf/HVC spontaneous activity p=0.000, NIf/HVC song-evoked activity during song p=0.008, NIf/HVC song-evoked activity between song p=0.004, HVCleft/HVCright spontaneous activity p=0.000, HVCleft/HVCright song-evoked activity during song p=0.023, HVCleft/HVCright song-evoked activity between song p=0.003; one-way ANOVAs: MMAN vs. NIf vs. HVC spontaneous activity $p<0.0001$ (Tukey HSD: MMAN vs. NIf $p<0.01$, NIf vs. HVC $p<0.05$, MMAN vs. HVC $p<0.01$), MMAN vs. NIf vs. HVC song-evoked activity during song p=0.007 (Tukey HSD: MMAN vs. NIf p=nonsignificant, NIf vs. HVC p=nonsignificant, MMAN vs. HVC $p<0.01$), MMAN vs. NIf vs. HVC song-evoked activity between song p=0.000 (Tukey HSD: MMAN vs. NIf $p<0.01$, NIf vs. MMAN $p<0.01$, MMAN vs. HVC $p<0.01$)). The corresponding data from areas just outside of these nuclei showed a similar pattern (Figure 10b; outside of MMAN spontaneous activity n=3, outside of MMAN song-evoked activity during song n =1, outside of

MMAN song-evoked activity between song n=1, outside of NIf spontaneous activity n=3, outside of NIf song-evoked activity during song n =3, outside of NIf song-evoked activity between song n=3, outside of HVC spontaneous activity n=2, outside of HVC song-evoked activity during song n=1, outside of HVC song-evoked activity between song n=1; single sample t-tests versus 0: outside of MMAN/HVC spontaneous activity $p<0.0001$, outside of MMAN/HVC song-evoked activity during song p=N/A, outside of MMAN/HVC song-evoked activity between song p=N/A, outside of NIf/HVC spontaneous activity $p=0.004$, outside of NIf/HVC song-evoked activity during song $p=0.014$, outside of NIf/HVC song-evoked activity between song $p=0.009$, outside of HVCleft/HVCright spontaneous activity $p=0.114$, outside of HVCleft/HVCright song-evoked activity during song p=N/A, outside of HVCleft/HVCright song-evoked activity between song p=N/A; one-way ANOVAs: outside of MMAN vs outside of NIf vs outside of HVC spontaneous activity $p=0.003$ (Tukey HSD: outside of MMAN vs. outside of NIf $p<0.05$, outside of NIf vs. outside of MMAN p=nonsignificant, outside of MMAN vs. outside of HVC $p<0.01$), outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity during song $p=0.109$, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity between song $p=0.162$). These data suggest that the field potential oscillations may be synchronous throughout each hemisphere of the brain. To determine the phase offsets of the oscillations within each pair of nuclei, the offset time of each peak correlation value was plotted (Figure 11). The timing of the peak correlation values in most of these analyses were not significantly different from zero, which suggests that the oscillations were nearly in phase between the pairs of nuclei, except with NIf and HVC (Figure 11; MMAN spontaneous activity n=4, MMAN song-

evoked activity during song n =4, MMAN song-evoked activity between song n=4, NIf spontaneous activity n=4, NIf song-evoked activity during song n =3, NIf song-evoked activity between song n=3, HVC spontaneous activity n=4, HVC song-evoked activity during song n=3, HVC song-evoked activity between song n=3, outside of MMAN spontaneous activity n=3, outside of MMAN song-evoked activity during song n =1, outside of MMAN song-evoked activity between song n=1, outside of NIf spontaneous activity n=3, outside of NIf song-evoked activity during song n =3, outside of NIf song-evoked activity between song n=3, outside of HVC spontaneous activity n=2, outside of HVC song-evoked activity during song n=1, outside of HVC song-evoked activity between song n=1; single sample t-tests versus 0: MMAN/HVC spontaneous activity p=0.185, MMAN/HVC song-evoked activity during song p<0.0001, MMAN/HVC song-evoked activity between song p=0.196, NIf/HVC spontaneous activity p=0.007, NIf/HVC song-evoked activity during song p=0.042, NIf/HVC song-evoked activity between song p=0.033, HVCleft/HVCright spontaneous activity p=0.201, HVCleft/HVCright song-evoked activity during song p=0.500, HVCleft/HVCright song-evoked activity between song p=0.211, outside of MMAN/HVC spontaneous activity p=0.217, outside of MMAN/HVC song-evoked activity during song p=N/A, outside of MMAN/HVC song-evoked activity between song p=N/A, outside of NIf/HVC spontaneous activity p=0.092, outside of NIf/HVC song-evoked activity during song p=0.500, outside of NIf/HVC song-evoked activity between song p=0.101, outside of HVCleft/HVCright spontaneous activity p=0.172, outside of HVCleft/HVCright song-evoked activity during song p=N/A, outside of HVCleft/HVCright song-evoked activity between song p=N/A; one-way ANOVAs: MMAN vs NIf vs HVC spontaneous activity p=0.915, MMAN vs NIf vs HVC

song-evoked activity during song p=0.008 (Tukey HSD: MMAN vs. NIf p=nonsignificant, NIf vs. HVC p=nonsignificant, MMAN vs. HVC p=nonsignificant), MMAN vs NIf vs HVC song-evoked activity between song p=0.003 (Tukey HSD: MMAN vs. NIf p<0.05, NIf vs. HVC p<0.05, MMAN vs. HVC p<0.05), outside of MMAN vs outside of NIf vs outside of HVC spontaneous activity p=0.579, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity during song p=0.763, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity between song p=0.297).

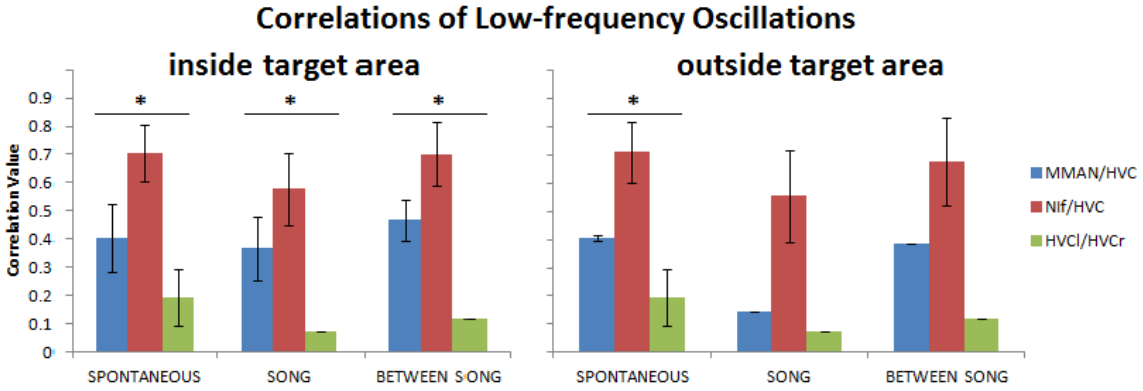

Figure 10. Peak correlation values for the oscillation correlation analysis. Oscillations are highly correlated between NIF and HVC, but not very correlated between HVCleft and HVCright, both when within the nuclei and when just outside of the nuclei.

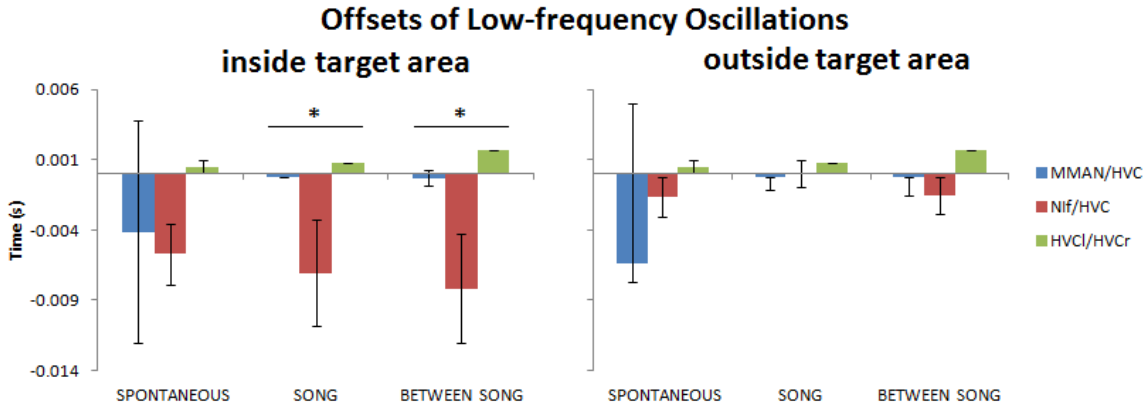

Figure 11. Offset times of peak oscillation correlation values.

Correlations of Bursts of Action Potentials and Low-frequency Oscillations

To determine whether low-frequency oscillations in a nucleus are correlated with the bursts of action potentials in that nucleus, waveform correlations were performed on bursts of action potentials and low-frequency oscillations from a single nucleus. These waveform correlation analyses showed that, for MMAN, NIf, and HVC the oscillations in a nucleus were not very correlated, if at all, with the bursts of action potentials within the same nucleus (Figure 12a; MMAN spontaneous activity n=4, MMAN song-evoked activity during song n =4, MMAN song-evoked activity between song n=4, NIf spontaneous activity n=4, NIf song-evoked activity during song n =3, NIf song-evoked activity between song n=3, HVC spontaneous activity n=8, HVC song-evoked activity during song n=6, HVC song-evoked activity between song n=6; single sample t-tests versus 0: MMAN/HVC spontaneous activity p=0.002, MMAN/HVC song-evoked activity during song p=0.004, MMAN/HVC song-evoked activity between song p=0.003, NIf/HVC spontaneous activity p=0.029, NIf/HVC song-evoked activity during song p=0.019, NIf/HVC song-evoked activity between song p=0.033, HVCleft/HVCright spontaneous activity p=0.000, HVCleft/HVCright song-evoked activity during song p=0.002, HVCleft/HVCright song-evoked activity between song p=0.005; one-way ANOVAs: MMAN vs. NIf vs. HVC spontaneous activity p=0.103, MMAN vs. NIf vs. HVC song-evoked activity during song p=0.003 (Tukey HSD: MMAN vs. NIf p=nonsignificant, NIf vs. HVC $p<0.01$, MMAN vs. HVC $p<0.05$), MMAN vs. NIf vs. HVC song-evoked activity between song p=0.115). The same was true for data collected from areas just outside of these nuclei (Figure 12b; outside of MMAN spontaneous activity n=3, outside of MMAN song-evoked activity during song n =1, outside of

MMAN song-evoked activity between song n=1, outside of NIf spontaneous activity n=3, outside of NIf song-evoked activity during song n =3, outside of NIf song-evoked activity between song n=3, outside of HVC spontaneous activity n=2, outside of HVC song-evoked activity during song n=1, outside of HVC song-evoked activity between song n=1; single sample t-tests versus 0: outside of MMAN/HVC spontaneous activity p=0.037, outside of MMAN/HVC song-evoked activity during song p=N/A, outside of MMAN/HVC song-evoked activity between song p=N/A, outside of NIf/HVC spontaneous activity p=0.007, outside of NIf/HVC song-evoked activity during song p=0.045, outside of NIf/HVC song-evoked activity between song p=0.052, outside of HVCleft/HVCright spontaneous activity p=0.013, outside of HVCleft/HVCright song-evoked activity during song p=N/A, outside of HVCleft/HVCright song-evoked activity between song p=N/A; one-way ANOVAs: outside of MMAN vs outside of NIf vs outside of HVC spontaneous activity p=0.375, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity during song p=0.885, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity between song p=0.901). These results suggest that low-frequency oscillations are not a mechanism behind the synchronous bursting of action potentials seen in this system. To determine whether bursts of action potentials were occurring simultaneously with the oscillations in that nucleus, the offset times of the peak correlation values were plotted (Figure 13). The timing of the peak correlation values in each of these analyses were not significantly different from each other, but were mostly slightly offset from 0, suggesting that the bursting waveform and the oscillation waveform within a nucleus were slightly out of phase (Figure 13; MMAN spontaneous activity n=4, MMAN song-evoked activity during song n =4, MMAN song-evoked

activity between song n=4, NIf spontaneous activity n=4, NIf song-evoked activity during song n =3, NIf song-evoked activity between song n=3, HVC spontaneous activity n=8, HVC song-evoked activity during song n=6, HVC song-evoked activity between song n=6, outside of MMAN spontaneous activity n=3, outside of MMAN song-evoked activity during song n =1, outside of MMAN song-evoked activity between song n=1, outside of NIf spontaneous activity n=3, outside of NIf song-evoked activity during song n =3, outside of NIf song-evoked activity between song n=3, outside of HVC spontaneous activity n=2, outside of HVC song-evoked activity during song n=1, outside of HVC song-evoked activity between song n=1; single sample t-tests versus 0: MMAN/HVC spontaneous activity p=0.045, MMAN/HVC song-evoked activity during song p=0.049, MMAN/HVC song-evoked activity between song p=0.026, NIf/HVC spontaneous activity p=0.001, NIf/HVC song-evoked activity during song p=0.306, NIf/HVC song-evoked activity between song p=0.001, HVCleft/HVCright spontaneous activity p=0.036, HVCleft/HVCright song-evoked activity during song p=0.075, HVCleft/HVCright song-evoked activity between song p=0.105, outside of MMAN/HVC spontaneous activity p=0.105, outside of MMAN/HVC song-evoked activity during song p=N/A, outside of MMAN/HVC song-evoked activity between song p=N/A, outside of NIf/HVC spontaneous activity p=0.428, outside of NIf/HVC song-evoked activity during song p=0.328, outside of NIf/HVC song-evoked activity between song p=0.403, outside of HVCleft/HVCright spontaneous activity p=0.026, outside of HVCleft/HVCright song-evoked activity during song p=N/A, outside of HVCleft/HVCright song-evoked activity between song p=N/A; one-way ANOVAs: MMAN vs NIf vs HVC spontaneous activity p=0.584, MMAN vs NIf vs HVC song-evoked activity during song p=0.257, MMAN vs

NIf vs HVC song-evoked activity between song p=0.599, outside of MMAN vs outside of NIf vs outside of HVC spontaneous activity p=0.656, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity during song p=0.645, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity between song p=0.877).

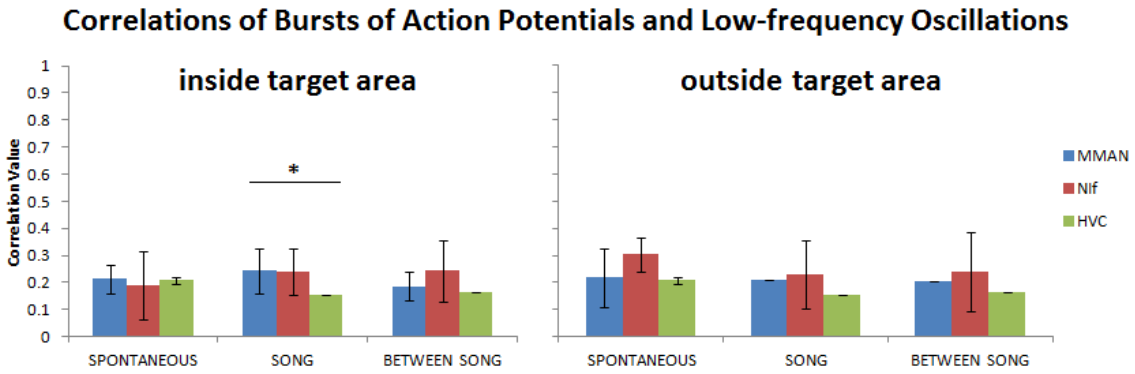

Figure 12. Peak correlation value for the correlation analysis of bursts of action potentials and low-frequency oscillations. In each area, bursts of action potentials and low-frequency oscillations are not very correlated.

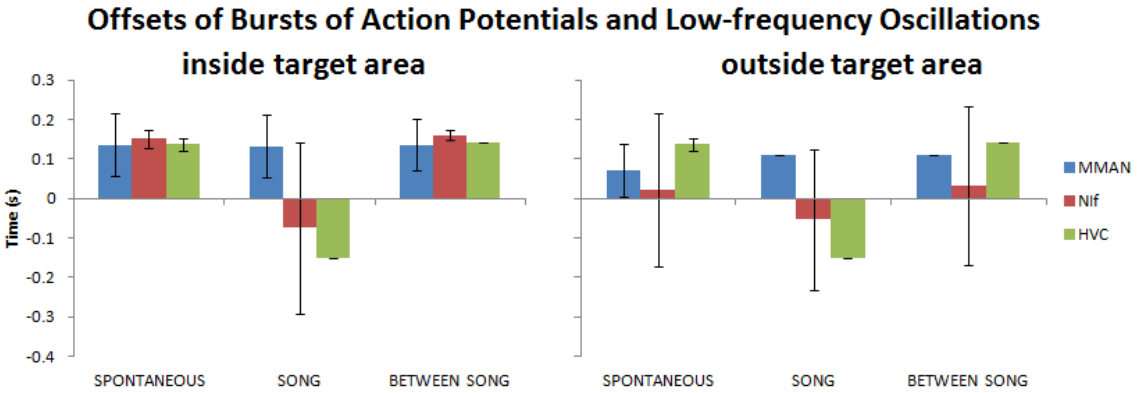

Figure 13. Offset times of peak correlation values of bursts of action potentials and low-frequency oscillations.

Action Potential Correlations

To determine whether individual action potentials, rather than bursts of action potentials, were correlated across nuclei, event correlations were performed on the action potentials in the pairs of nuclei. These event correlation analyses showed that there were

many more correlated action potentials within all three pairs of the nuclei during spontaneous activity (Figure 14a; MMAN spontaneous activity n=5, MMAN song-evoked activity during song n=5, MMAN song-evoked activity between song n=5, NIf spontaneous activity n=4, NIf song-evoked activity during song n=4, NIf song-evoked activity between song n=4, HVC spontaneous activity n=6, HVC song-evoked activity during song n=5, HVC song-evoked activity between song n=5; single sample t-tests versus 0: MMAN/HVC spontaneous activity p=0.001, MMAN/HVC song-evoked activity during song p=0.036, MMAN/HVC song-evoked activity between song p=0.026, NIf/HVC spontaneous activity p=0.047, NIf/HVC song-evoked activity during song p=0.086, NIf/HVC song-evoked activity between song p=0.094, HVCleft/HVCright spontaneous activity p=0.003, HVCleft/HVCright song-evoked activity during song p=0.006, HVCleft/HVCright song-evoked activity between song p=0.026; one-way ANOVAs: MMAN vs. NIf vs. HVC spontaneous activity p=0.048 (Tukey HSD: MMAN vs. NIf p=nonsignificant, NIf vs. HVC p=nonsignificant, MMAN vs. HVC $p<0.05$), MMAN vs. NIf vs. HVC song-evoked activity during song p=0.084, MMAN vs. NIf vs. HVC song-evoked activity between song p=0.592). Data collected from areas just outside of the nuclei of interest showed few correlated action potentials (Figure 14b; outside of MMAN spontaneous activity n=3, outside of MMAN song-evoked activity during song n =1, outside of MMAN song-evoked activity between song n=1, outside of NIf spontaneous activity n=3, outside of NIf song-evoked activity during song n =3, outside of NIf song-evoked activity between song n=3, outside of HVC spontaneous activity n=2, outside of HVC song-evoked activity during song n=2, outside of HVC song-evoked activity between song n=2; single sample t-tests versus 0: outside of

MMAN/HVC spontaneous activity p=0.116, outside of MMAN/HVC song-evoked activity during song p=N/A, outside of MMAN/HVC song-evoked activity between song p=N/A, outside of NIf/HVC spontaneous activity p<0.0001, outside of NIf/HVC song-evoked activity during song p=0.064, outside of NIf/HVC song-evoked activity between song p=0.032, outside of HVCleft/HVCright spontaneous activity p=0.078, outside of HVCleft/HVCright song-evoked activity during song p=0.102, outside of HVCleft/HVCright song-evoked activity between song p=0.045; one-way ANOVAs: outside of MMAN vs outside of NIf vs outside of HVC spontaneous activity p=0.269, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity during song p=0.626, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity between song p=0.205). This is not surprising, since the regions outside of the nuclei of interest are not involved in the production and maintenance of song and thus have no reason to produce synchronized output with the nuclei in the song system. To determine how synchronously the pairs of nuclei were firing action potentials, the offset times of the peak action potential correlation values were plotted. The timing of the highest action potential count in each of these analyses were not significantly different from each other and most were not significantly different from zero, suggesting that all of the pairs of nuclei produced action potentials rather synchronously (Figure 15; MMAN spontaneous activity n=5, MMAN song-evoked activity during song n=5, MMAN song-evoked activity between song n=4, NIf spontaneous activity n=4, NIf song-evoked activity during song n=3, NIf song-evoked activity between song n=3, HVC spontaneous activity n=5, HVC song-evoked activity during song n=5, HVC song-evoked activity between song n=3, outside of MMAN spontaneous activity n=3, outside of MMAN song-evoked

activity during song n=0, outside of MMAN song-evoked activity between song n=0, outside of NIf spontaneous activity n=1, outside of NIf song-evoked activity during song n =2, outside of NIf song-evoked activity between song n=1, outside of HVC spontaneous activity n=2, outside of HVC song-evoked activity during song n=1, outside of HVC song-evoked activity between song n=1; single sample t-tests versus 0: MMAN/HVC spontaneous activity p=0.370, MMAN/HVC song-evoked activity during song p=0.102, MMAN/HVC song-evoked activity between song p=0.486, NIf/HVC spontaneous activity p=0.012, NIf/HVC song-evoked activity during song p=0.063, NIf/HVC song-evoked activity between song p=0.065, HVCleft/HVCright spontaneous activity p=0.302, HVCleft/HVCright song-evoked activity during song p=0.152, HVCleft/HVCright song-evoked activity between song p=0.111, outside of MMAN/HVC spontaneous activity p=0.211, outside of MMAN/HVC song-evoked activity during song p=N/A, outside of MMAN/HVC song-evoked activity between song p=N/A, outside of NIf/HVC spontaneous activity p=N/A, outside of NIf/HVC song-evoked activity during song p=0.273, outside of NIf/HVC song-evoked activity between song p=N/A, outside of HVCleft/HVCright spontaneous activity p=0.250, outside of HVCleft/HVCright song-evoked activity during song p=N/A, outside of HVCleft/HVCright song-evoked activity between song p=N/A; one-way ANOVAs: MMAN vs NIf vs HVC spontaneous activity p=0.699, MMAN vs NIf vs HVC song-evoked activity during song p=0.814, MMAN vs NIf vs HVC song-evoked activity between song p=0.076, outside of MMAN vs outside of NIf vs outside of HVC spontaneous activity p=0.421, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity during song p=1, outside of MMAN vs outside of NIf vs outside of HVC song-evoked activity between song p=1). Because the

action potential correlation was performed with the event correlation function and the low-frequency oscillation correlation was performed with the waveform correlation function in Spike2, these correlations could not be directly compared as in the correlation between bursts of action potentials and low-frequency oscillations. Therefore, comparisons were made between patterns in Figures 10 and 14 in order to discuss the relationship between low-frequency oscillations and action potentials.

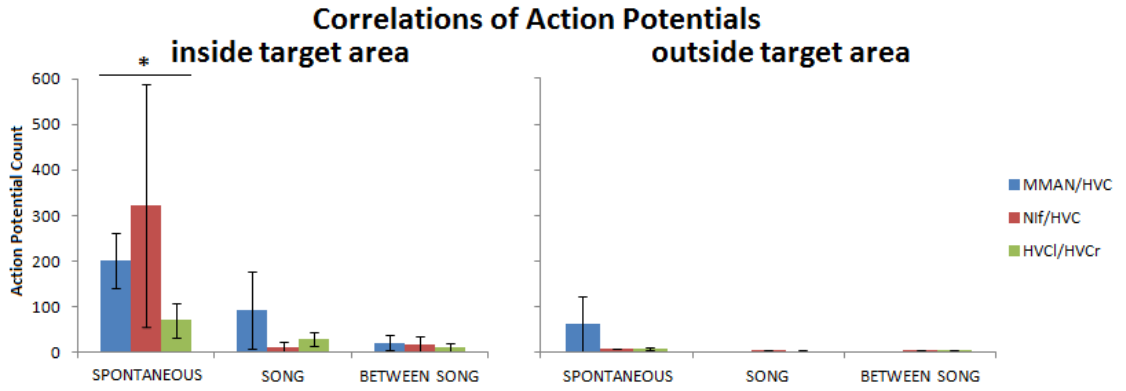

Figure 14. Peak action potential counts are relatively high only within the nuclei and in spontaneous activity.

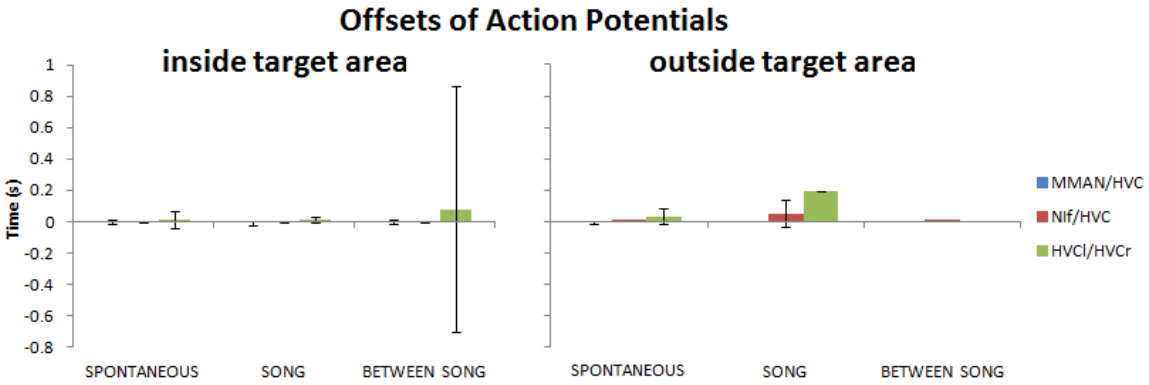

Figure 15. Offset times of peak action potential correlation values.

DISCUSSION

The goal of this project was to determine whether low-frequency field potential oscillations were a mechanism behind the synchronous activity observed by Shayna Williams in the zebra finch song system. To do this, I needed to compare activity between pairs of nuclei in the zebra finch brain. Specifically, I needed to test that the bursts of action potentials or just the individual action potentials were synchronous between pairs of nuclei. I also needed to test that the low-frequency oscillations were correlated between the pairs of nuclei. Finally, I needed to compare the bursts of action potentials and the individual action potentials with the low-frequency field potential oscillations within a single area. If the bursts of action potentials and the individual action potentials were synchronous between pairs of nuclei in the song system, I will have confirmed Shayna's observations. If the bursts of action potentials or individual action potentials are then correlated with the low-frequency oscillations within these nuclei, I will have strong evidence that the low-frequency field potential oscillations do play a role in the mechanism behind that synchrony.

The burst correlation data showed that bursts were highly correlated and simultaneous between MMAN and HVC and NIf and HVC, but not necessarily between HVCl and HVCr (Figure 8a). This result could simply be because MMAN and NIf are monosynaptically connected to the ipsilateral HVC, while the two HVCs are polysynaptically connected. This theory is supported by the fact that the data from just outside of NIf and MMAN do not exhibit correlated bursting with HVC (Figure 8b). However, the highest correlation value occurred when there was no temporal offset between the bursts in the pairs of nuclei, meaning that the bursts were synchronous

(Figure 9). It has been shown that there is a 12 ms delay between NIf and HVC (Coleman et al., 2004) and an ambiguous, yet nonzero, delay between MMAN and HVC (Vates et al., 1997, Seki and Okanoya, 2008). These delays may not show up in this data because of the small sample size or because there actually is some synchronizing phenomenon, allowing no delay between these nuclei.

The low-frequency oscillation correlation data showed that the oscillations in NIf and MMAN were correlated with those in HVC, but HVCleft was not correlated with HVCright (Figure 10a). Also, the data from just outside of these areas followed the same pattern (Figure 10b). This result could simply reflect whether or not the two nuclei in question are on the same side of the brain. A high oscillation correlation value could indicate ipsilateral nuclei, while low correlation values could indicate contralateral nuclei. The offset data for the oscillations showed that they were in phase in all recorded pairs, except in NIf and HVC (Figure 11). This result is important as it suggests that there is a difference between being in the feedback loop (MMAN and HVCs) and not being in it (NIf). The architecture of the feedback loop, therefore, seems to play an important role in the observed synchronous activity in the nuclei.

The action potential correlation results showed more firing of action potentials in the spontaneous activity than in song or between song contexts (Figure 12). This is because the trial for spontaneous activity much longer than the others and this data needs to be normalized. The action potential offset data showed that the individual action potentials, like the bursts of action potentials, were also synchronous between the pairs of nuclei (Figure 13). This data supports Shayna's observation that MMAN and HVC exhibit synchronous activity. However, these results may be flawed. It is very possible

that the thresholds chosen to define action potentials in the different trials were not consistent, skewing the results.

Oscillatory Activity May Not Be a Mechanism Behind Synchronous Activity in this System

The low correlation values resulting from the correlations between bursts of action potentials and low-frequency oscillations within each nucleus (Figure 14a) suggest that the oscillations do not in fact drive the synchronized bursting between nuclei. It appears that the same is true for individual action potentials and low-frequency oscillations (compare Figures 10 and 14), although a direct statistical comparison of action potentials and ocillations was not possible since event correlations and waveform correlations could not be compared in Spike 2. Rather than the rough comparison of graphs used in this study, future studies may include a direct comparison of spiking and oscillations, similar to the correlation analysis between bursts of action potentials and low-frequency oscillations performed in this study. The fact that oscillations do not seem to be driving the synchrony in this system leaves two possibilities. Either the synchronicity is driven by some other mechanism or the synchronicity observed is not real, but rather some side effect of the recording procedures used in these experiments.

Possibility 1: Some Other Mechanism

If field potential oscillations are not a mechanism behind the synchronous activity in the nuclei included in this study, then what is? One possibility is that the synchrony is somehow a result of the structure of the feedback loop. The offset data in this study and the previously reported complex timing between MMAN and HVC (Williams, 2009) may

be a result of the bilateral connection from each DMP to each MMAN. Because of this connection, both HVCs can provide information to MMAN. This structure allows MMAN to fire before the ipsilateral HVC or after the same HVC (Figure 16). This structural feature may, through some unknown mechanism, also cause MMAN and HVC to be excited at the same time. Although this is not necessarily the only possible alternative mechanism for the complex timing of excitation in the song system, it does seem quite possible. To test this hypothesis, a future study will create a lesion somewhere in the feedback loop preventing information from the song nuclei in one hemisphere to reach the other. If the synchrony is lost, it would suggest that the feedback loop's structure is responsible for the synchrony and other complex timing observed in this system. Other work including contralateral recordings between MMAN and HVC and other nuclei has the potential to provide further support for this hypothesis.

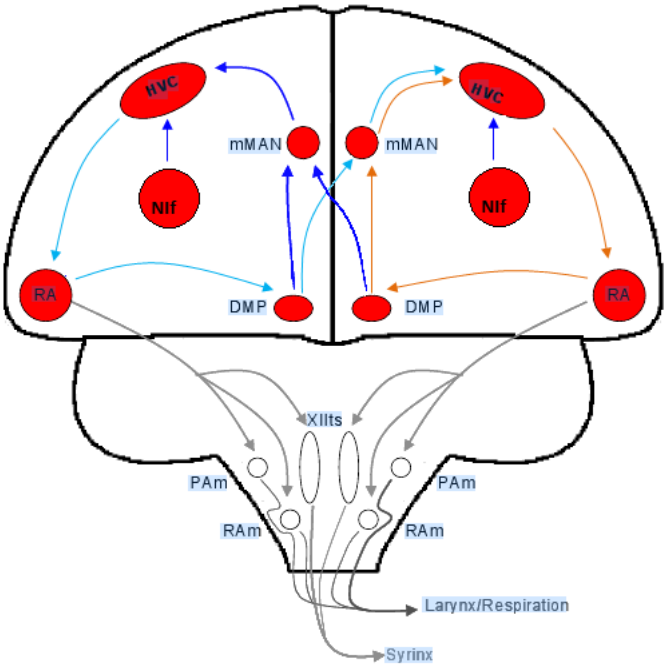

Figure 16. Two pathways provide ways for MMAN on the right to fire before (light blue, starting at HVC on the left) and after (orange, starting at HVC on the right) HVC on the right.

Possibility 2: No Real Synchrony

The idea that synchrony does not in fact exist in this system is also a possibility. The data presented in this study seems to indicate that synchronous activity does exist in this system; however, that result could be some function of dual recordings and not real synchrony. A small amount of noise running through the entire system, if it reached the electrodes, would show up synchronously within each recording and shift the peak correlation values' offset times toward zero in the analyses. However, many precautions were taken against outside noise in these experiments. It is therefore unlikely that the synchronous activity we observed is an artifact of dual recordings.

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