VOCAL TIMING IN THE BAT

A Dissertation

by

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Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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May 2013

Major Subject: Zoology

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ABSTRACT

Bats are social organisms that live in large colonies. However, reliance upon echolocation in order to hunt and navigate, means that bats also face pressing acoustic challenges due to overlap with surrounding noise. Bats also possess fine control over the properties of their echolocation pulses. This study's goal was to determine how bats are able to effectively function in large groups despite the interfering noise generated by conspecifics. Mexican free-tailed bats *(Tadarida brasiliensis*) were exposed to both artificially generated interfering noises and noise generated by conspecifics, and the temporal characteristics of their resulting echolocation calls were analyzed. In addition, bats were given injections of dopaminergic and serotonergic drugs, in an effort to determine which monoamine(s) were capable of altering vocal motor timing and to determine which regions of the brain play a role in regulating the timing of echolocation. I hypothesized that bats would alter the timing of emission of their own echolocation pulses in response to noise, and that drugs affecting the 5HT2A receptor would shift the timing of emission of echolocation pulses.

The first part of this dissertation describes a novel temporal alteration behavior that occurs in response to artificially generated intermittent noise, and is characterized by a period of pulse suppression followed by a gradual return to normal call rates. Bats alter the timing of emission of their echolocation pulses to avoid overlap with noise and call within silent periods. The second part of this study investigated whether dopamine or

serotonin, or both, could alter the timing of this vocal behavior. The results of this study were inconclusive, although I found some evidence that 5HT2A agonists can produce faster responses. Finally, I show that echolocating bats suppress pulse emission in nearby conspecifics. The resulting decrease in call rate leads to an overall increase in information throughput. This study also demonstrates that bats respond to continuous noise by increasing their call rate, and that the switch between the responses to intermittent noise and continuous noise occurs at a duty cycle of 50% or higher. Overall, this dissertation establishes that bats alter the timing of emission of their echolocation calls in response to noise, and that these mechanisms may be regulated by serotoninergic mechanisms.

ACKNOWLEDGEMENTS

I would like to thank Doctor Smotherman whose guidance, support, and patience made completion of this work possible. I would also like to thank the members of my committee, Dr. Zoran, Dr. Lints, and Dr. Abbott, whose feedback and advice were invaluable in the development of this research project and in shaping my time here at Texas A&M. Thanks also to our lab's excellent undergraduates, especially William Jackson, who assisted with the data collection and analysis in Chapter IV. Finally, I wish to thank my family, whose unconditional love and support has always given me the strength to keep moving forward.

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CHAPTER I

INTRODUCTION

Bats are one of the few species on earth that possess the ability to echolocate. They use this unique active sensory behavior in order to hunt for prey and navigate their environment (Neuweiler, 2000; Schnitzler and Kalko, 2001). In order to successfully echolocate, the bat must emit a burst of ultrasonic noise, and then listen for the returning echoes. Any alterations present in the returning signal conveys information about their environment such as the distance from obstacles, the texture of surfaces, and the location of prey (Moss et al., 2006; Neuweiler, 2000). This behavior allows them to function even in low to no light conditions, allowing them to exploit a variety of niches that diurnal animals are incapable of exploiting. As a consequence of this reliance upon echolocation, the presence of interfering noise in the surrounding environment can be highly detrimental to bats' ability to function effectively (Gillam and McCracken, 2007; Obrist, 1995). Further compounding this problem is the fact that many species of bats are highly social, living in colonies that can consist of up to several thousand individuals (Ratcliffe et al., 2004; Simmons et al., 1978). Each individual produces not only echolocation calls, but a diverse array of communication calls (Bohn et al., 2008; Schwartz et al., 2007), leading to highly cluttered acoustic space and the near omnipresence of noise. Furthermore, the calls produced by conspecifics would necessarily possess a similar frequency, bandwidth, and volume as the target echoes of an individual bat, making it even harder to form an accurate representation of the

surrounding environment. This constant acoustic challenge leads to the central question of this research project: how is it that bats are able to echolocate effectively in such large groups, despite the presence of constant interfering noise from conspecifics?

Many animals are capable of altering the frequency, timing, and amplitude of their own acoustic signals, and can alter these properties in an effort to increase signal clarity. There are two general categories of behaviors available through which animals may reduce or avoid overlap with surrounding interfering noise. The first of these methods is to alter the acoustic or spectral properties of the outgoing signal: changing the volume, frequency, or bandwidth of the emitted sound. An example of such a spectral alteration is the jamming avoidance response, or JAR. The jamming avoidance response has been well documented in bats and is also found in weakly electric fish (Bates et al., 2008; Gillam et al., 2007; Ulanovsky et al., 2004). Weakly electric fish emit either a constant electromagnetic field or pulses of electricity and monitor changes to the electric field caused by contact with obstacles or prey, using another form of active sensing called electrolocation (Ulanovsky et al., 2004). In this sense, they face challenges similar to those found in bats, supporting the viability of this method of interference avoidance for species that rely upon active sensing. When in the presence of a conspecific that is producing a signal of similar frequency, neighboring bats or fish will shift the frequency of their own calls or electric field higher or lower so the two signals do not overlap or interfere with each other (Ulanovsky et al., 2004). While this mechanism can be effective in pairs or small groups, this mechanism would be of limited

use in larger collections of animals (Ulanovsky and Moss, 2008). All organisms are restricted to the range of frequencies that their physiology can naturally produce: after a certain point, an individual would be unable to shift their calls any higher or lower than these set boundaries. With so many bats echolocating in a single roost, the available frequencies would quickly be used up, with no further adjustments being possible. Thus the jamming avoidance response alone does not provide a suitable solution to the problem of conspecific interference in large groups.

Another vocal alteration which can be applied in order to avoid interference is to increase the volume of the outgoing signals so that they possess a higher amplitude than the background signals or noise, allowing them to be easily detected by the intended receiver. The ability of animals to increase the amplitude of their own calls in response to background noise is referred to as the Lombard effect, and has been demonstrated in many animals (Brumm, 2006; Egnor et al., 2007; Gillam and McCracken, 2007; Penna et al., 2005) including humans. Just as with the jamming avoidance response however, this technique is constrained by physiological mechanisms: increasing the volume of the emitted signal requires an increased expenditure of energy, and there is a natural upper threshold set by the animal's physical capabilities, beyond which they could not call any louder (Brumm, 2006). Furthermore, this would lead to competition with nearby con- and heterospecifics sharing the same acoustic space: with each individual raising the volume of their own calls, neighboring animals would need to follow suit to transmit their own signals and the constant escalation of volume would ultimately drown out all

attempts at acoustic communication or echolocation (Brumm, 2006). Alterations to either the frequency or amplitude of calls alone do not provide an adequate explanation for how bats can function in large colonies.

A second method for reducing or avoiding interference is to alter the temporal properties of the acoustic signals: to alter the length of the sounds produced, the intervals between calls, or the rate at which signals are produced. A simple method for avoiding overlap with interfering noises is to alter the timing of emission of one's own calls. This strategy is particularly effective if the interfering noise is not constant, and there are intermittent silent periods in which to place one's calls. Some vocalizing animals experience a refractory period after a call (Moore et al., 1989; Zelick and Narins, 1985) or are restricted by respiratory cycles that prevent them from calling constantly, leading to a mandatory period of silence regardless of the surrounding noise. Many bats that rely upon frequency modulated calls leave periods of silence lasting several hundred milliseconds between emissions (Ulanovsky and Moss, 2008). If an animal allowed the interfering intermittent noise to fall into this period of silence, and then called before the onset of the next bout of noise, they would avoid overlap with the sound without an increased expenditure of energy (Popp et al., 1985). Indeed, this type of temporal alteration has been observed in a variety of species including birds (Brumm, 2006; Ficken and Ficken, 1974; Knapton, 1987; Planque and Slabbekoorn, 2008), frogs (Moore et al., 1989; Zelick and Narins, 1985), and primates (Egnor et al., 2007; Versace et al., 2008). Altering the timing of emission of a vocal signal is less energy intensive

than altering volume or frequency, though it may lead to a decrease in overall call rate or total number of calls. Furthermore, calling in silent periods between other's calls reduces competition for the available acoustic space, allowing multiple animals to vocalize in quick succession by alternating their calls (Ficken and Ficken, 1974; Knapton, 1987; Popp et al., 1985). Still, temporal alterations alone have their limitations in large groups, namely, with a large enough number of bats, there would no longer be regular silent intervals available to call within. As a result of this limitation, bats may rely upon a number of discrete mechanisms used in combination to avoid overlap, including spectral, acoustic, and temporal changes. While spectral and acoustic alterations as a means of interference reduction or overlap avoidance have previously been described, until this study no temporal mechanism for avoiding noise overlap or reducing interference had been described within bats, short of remaining silent while flying in the presence of other bats (Chui et al., 2008).

There are a number of potential temporal alterations that bats could use to reduce or avoid overlap with background noise, or to make their own returning echoes easier to detect. The first of these alterations would be to develop a system of antiphonal calling, where conspecifics call out of phase with one another, alternating vocalizations so that no animal is calling at the same time as another. This type of asynchrony has been seen in birds (Brumm, 2006; Ficken and Ficken, 1974; Popp et al., 1985) and frogs (Zelick and Narins, 1985): where individuals reduce the total number of songs in order to avoid overlapping their signals. Typically, however, these behaviors are seen in animals that

use acoustic signals to communicate with conspecifics. For bats, which rely upon acoustic signals to gather information about the surrounding environment, this antiphonal behavior may not be adequate to avoid overlap with both the emitted sound and returning echoes, and the sheer number of animals calling within such close proximity could make coordination difficult. Another potential mechanism is to restrict calling to silent windows, placing echolocation calls between bouts of noise and attempting to avoid overlap altogether. This specific mechanism has been seen in other species (Brumm, 2006; Egnor et al., 2007; Zelick and Narins, 1985), and could provide a simple, low energy avoidance mechanism. Alone, this method might not provide an adequate solution for interference avoidance in large colonies, but it could be used in combination with spectral alterations such as the jamming avoidance response. Based on the available information, I hypothesized that Mexican free-tailed bats confronted with intermittent, interfering bursts of noise would attempt to avoid overlap by altering the timing of emission of their own echolocation pulses.

Building upon this work, I then asked how such a possible behavior might be regulated within the brain. Research into the mammalian brain's control of timing has primarily utilized an interval timing framework: focusing upon the timing of intervals in the seconds to minutes range (Schirmer, 2004). Experiments with human subjects with preexisting speech disorders have indicated that interval timing can form an appropriate contextual framework for the study of both animal vocalizations and human speech. Human speech requires very precise temporal control of the motor programs that

produce syllables and words, and previous researchers have speculated that speech timing may be regulated by the same brain networks that control the expression of other time-dependent motor programs (Mauk and Buonomano, 2004; Schirmer, 2004), though there is still much debate within the field. These timing networks are broadly mediated by the basal ganglia circuits that regulate behavior and the basal ganglia are in turn characterized by their dependence on monoaminergic synapses. There is evidence that the monoaminergic networks of the basal ganglia control speech timing (Meck, 1996; Pastor et al., 2006). Pharmacological studies indicate that speech timing may be regulated through either the dopaminergic or serotonergic systems, or both together (Maguire et al., 2000; Pastor et al., 2006; Salome et al., 2000; Stager et al., 2005). Prior research into this subject was constrained, however, by several factors. Human speech studies are restricted to those volunteers that possess pre-existing speech disorders and the types of experiments that can be performed are limited, especially in regards to pharmacological and surgical studies. While more flexible than human studies, there are no currently accepted animal models for human speech as animal vocalizations are not speech.

However, animal models can be used to study specific parameters of vocal motor control such as onset, vocal duration, and rate of syllable production. Animal research into vocal behaviors using primates or rodents, which typically produce highly stereotyped and limited vocalizations, lacks the spectral and temporal flexibility and wide repertoire of calls that Mexican free-tailed bats possess (Simmons et al., 1978;

Smotherman, 2007). Bats, which are much more temporally specialized due to their reliance on echolocation, would allow for a diversity of pharmacological and surgical studies that were previously unavailable or unfeasible. Since preliminary studies demonstrated that Mexican free-tailed bats possess a specialized mechanism for precisely controlling the timing of their pulse emission, I hypothesized this behavior could be described as an example of interval timing. More specifically, I tested the hypothesis that serotonin, which had already been shown to influence vocal timing in humans and motor timing in rodents (Maguire et al., 2000; Salome et al., 2000) could alter the timing of emission of bats' echolocation calls. Serotonergic antagonists and agonists, binding to the 5HT2A receptor, should phase lag and a phase lead, respectively. If true, this would demonstrate that interval timing circuits similar to those described for other behaviors contribute to echolocation pulse timing in bats. Such a conclusion could guide future efforts to characterize the neural control of echolocation behavior and also help link this research to the broader topics of motor timing and speech production.

Finally, it is necessary to consider the broader ecological significance of the acoustic suppression of pulse emissions. The constant barrage of echolocation calls produced by the thousands of conspecifics present in a single day roost would provide a constant level of background noise, with little to no viable periods of silence to call within. Furthermore, as established in previous studies, bursts of artificial noise can temporarily suppress pulse emission in a single bat (Jarvis et al., 2010). This leads to an

important question: if natural echolocation calls (which share many properties with the artificial stimulus created for use in these experiments) also lead to a period of call suppression in nearby conspecifics, then how do bats in large colonies with hundreds or thousands of echolocating conspecifics ever manage to emit a pulse, instead of being constantly suppressed? For this phase of my research I investigated the temporal properties of bats' echolocation pulses both in groups, and in the presence of constant artificial noise. The goal was to examine how social context affects echolocation pulse production, and to determine how the previously identified behavior could benefit bats echolocating in social situations. I hypothesized that echolocating bats will suppress each other's pulse production, leading to an overall decrease in call rate. How decreasing call rate would benefit the bat operating in social situations was unclear, and a major focus of this research project. However, I also felt that in very large colonies, this suppression behavior and corresponding reduction in call rate would no longer be a viable response to interfering noise. In very large groups, such as is found in many roosts, the presence of noise would be constant, with few to no periods of silence. However, even in such challenging situations, bats are never fully suppressed, and continue to vocalize (Jarvis et al., 2010). Thus, I considered the possibility that bats might have another response when confronted with sustained interfering noise. Specifically, I hypothesized that in the presence of constant noise Mexican free-tailed bats would no longer experience suppression, and would instead increase their call rate in an attempt to reduce the impact of the background noise. Some species of bird

increase their call rate in response to the presence of constant noise such as wind; increasing the number of calls being emitted may increase the chances that the desired signal reaches the intended receiver or increase the chances of the signal falling into a random quiet period (Lengagne et al., 1999; Potash, 1972).

In the following sections, I will discuss the results of my study investigating the ability of bats to successfully echolocate in large groups, and characterizing the temporal mechanisms that bats use to alter the timing of emission of their echolocation pulses in order to reduce overlap with interfering noise. This study also includes an overview of pharmacological experiments designed to identify which monoamines, and thus regions of the brain, are likely to play a role in the temporal control of echolocation. Chapter II details the materials and methods of this study. In Chapter III, I identify and characterize a novel behavior in which bats alter the timing of emission of their echolocation pulses when confronted with intermittent, artificial noise. In Chapter IV, I detail a series of experiments in which I attempted to determine whether or not the behavior discussed in the previous chapter can be described as an example of interval timing, as well as which monoaminergic pathway(s) played a role in the temporal regulation of echolocation pulse emission. In Chapter V, I investigated the ecological significance of the temporal alteration behavior. First, I determined whether or not echolocating bats would suppress pulse emissions in their conspecifics. Following this, I investigated how bats would respond to the presence of constant interfering noise, given that they could no longer call within windows of silence. Finally, I determined the threshold at which bats switch

between their responses to intermittent and continuous noise. Lastly, in Chapter VI, I discuss my conclusions regarding this study, and suggest possibilities for future research.

CHAPTER II

MATERIALS AND METHODS

Animals

All experiments for this study were performed on Mexican free-tailed bats (*Tadarida brasiliensis).* Research animals were wild-caught from the colony present in the Kyle Field football stadium on the Texas A&M University campus in College Station, Texas. The captured bats were primarily male, though some females were captured and used in recording sessions as well. The typical weight range of the bats was 9-13 grams, and animals typically possessed a 40-44 mm forearm length. All newly captured animals were kept in isolation for a period of four to six weeks. During this time, they were taught to take mealworms (*Tenebrio molitor)* from a bowl. Once fully able to feed themselves, individuals were introduced to the captive colony maintained on the A&M campus. Individuals were able to freely fly and roost in this facility, and were maintained on a diet of mealworms supplemented with vitamins and fatty acids provided once a day, with water available *ad libitum.* The colony was kept on reversed 14:10 hour light/dark cycle, with the proportion of light to dark varying seasonally. Temperature and humidity were also controlled in order to simulate natural conditions. All procedures, both for care of the animals and experimental protocols conformed to the National Institutes of Health guidelines, and were additionally approved by the local Institutional Animal Care and Use Committee (protocol #2007-254). During experimental trials the bats were placed in a 10x10x20cm cage composed of 0.25”

plastic-coated steel mesh that was then set in the center of a warm (30°C) anechoic 6.1 x 3.0 x 1.5 meter recording chamber which was soundproofed with four-inch Sonex acoustic foam; model UNX-4 (Pinta Acoustic Inc, Seattle, WA). All animals were habituated to the handling procedures, as all had been hand feed and regularly handled during their initial introduction to the lab.

Acoustic Apparatus

All acoustic stimuli for these experiments were digitally created with the TDT OpenEX software v5.4, and the analog signal was generated by TDT System III RX6 hardware (Tucker-Davis Technologies, Alachua, FL). For the experiments detailed in Chapters III and IV, stimuli were played through a Sony amplifier (model # STR-DE598) driving a 4-speaker array composed of 2 Pioneer Ribbon Tweeters (ART-55D/301080) and 2 Pioneer Rifle Tweeters (ART-59F/301081) juxtaposed and oriented towards the cage holding the bat. The speakers provided a flat (±3 dB) output of 85 dB SPL over the range of 15 to 60 kHz recorded at the position of the bat. In the experiments detailed in Chapter V, Acoustic stimuli were produced with a Vifa 1" Tweeter (model # BC25SC55-04) powered by a Sony amplifier (model # STR-DE598) which provided a maximum output of ≈ 80±6 dBs from 15 to 50 kHz. The speaker was mounted 10 cm from and oriented towards the bat's cage. The microphone and loudspeaker were separated by a piece of sound-absorbing foam adjusted daily to minimize the recorded amplitude of the stimulus relative to the amplitude of the bats' pulse emissions. The bats' echolocation pulses ranged in intensity from 90 to 115 dB

SPL, as measured by a Bruel & Kjær free-field ¼" microphone (Type 4939) placed just outside the cage and surrounded by a cone of acoustic foam which was oriented to minimize the recorded amplitude of the stimuli relative to the bat's pulses.

Incoming signals were digitized with a National Instruments DAQmx card, NI PCI-6251 (200 kHz, 16 bit sample rate), viewed in real time with Avisoft Recorder v3.0, and stored on the computer hard drive for subsequent analysis. On a separate computer both the recorded bat pulses, recorded stimuli and TTL pulses issued by the TDT hardware at the onset of each stimulus were stored on separate channels for analysis with the hardware and software package Datapak 2K2 (Run Technologies, Mission Viejo, CA). Transmission delays between the TDT system's TTL pulses and the actual recordings of sound stimuli at the microphone (4.4 ms) were accounted for in post-hoc analyses. Echolocation pulses were automatically separated from the simultaneously recorded acoustic stimulus based on differences in amplitude and duration: bat pulses were on average 20 dB louder than the stimulus at the microphone and were of shorter duration than the stimuli (mean ± s.e. bat pulse duration was 4.67 ± 0.38 ms, n=10 bats, 300 pulses per bat).

Experimental Procedures and Statistical Analysis for Chapter III

I conducted four different primary experiments that included a total of 12 different stimuli including a control. For the control trials I activated the stimulus generating software, recorded bat vocalizations, and performed the experiments as usual

except that the speaker amplifier was turned off and no sounds were generated from the speakers. A total of ten individual bats were used for each experiment.

Experiment 1 tested whether bats modified the temporal pattern of their echolocation pulses differently in response to acoustic stimuli presented at different rates. The stimulus consisted of a continuous train of 10 ms broadband "noise bursts" separated by 50, 100, 200 or 1000 ms periods of silence. Noise bursts were produced by digitally band-pass filtering white noise to a range of 20 to 45 kHz, which completely overlaps with the frequency range of the most prominent harmonic component of the free-tailed bat's echolocation pulses. Onset and offset of all stimuli were gated with a 0.1 ms rise/fall time.

Experiment 2 tested whether the regularity of the intervals between signals influenced the bats temporal patterns of pulse emissions by measuring the responses to noise bursts presented at randomly varying inter-stimulus intervals. As in experiment 1, the stimulus consisted of 10 ms noise bursts repeated at intervals that varied randomly between 200 to 1000 ms. The range of intervals was constrained to allow for a statistical comparison between the response to random intervals versus the responses to regular repeating intervals of 200 and 1000 ms, and because longer intervals required impractically long recording times.

Experiment 3 examined how the bats would respond to stimuli comprised of more complex temporal patterns. The first complex stimulus consisted of a repeating "paired-pulse" stimulus in which two 10 ms noise bursts separated by 50 ms were

repeated every 200 ms. The second stimulus was an artificially generated pulse train mimicking the feeding "buzzes" used by bats during the final stage of prey capture. The artificial buzzes consisted of trains of 15 downward-sweeping frequency-modulated sounds (45 to 20 kHz, 8 to 4 ms duration) produced over a time span of approximately 200 ms. The artificial pulses were designed to shorten in duration and increase in repetition rate similar to natural patterns recorded in the field (Schwartz et al., 2007).

Experiment 4 tested whether the duration or bandwidth of the stimulus influenced the temporal patterns of the bats' vocal response. This experiment was carried out using stimulus repetition rates of 5 Hz, since it was determined that this was the shortest stimulus interval that consistently produced the maximum response. The bats were presented with repeating noise bursts with durations of 5, 10, 25 and 50 ms. To test whether stimulus bandwidth affected the nature or magnitude of the response, I examined the bats' vocal responses to a pure tone also repeated at a rate of 5 Hz. A stimulus of 25 kHz and 20 ms duration was used because this sound approximated the acoustic structure of one of the most common classes of communication syllables used by this species and because 25 kHz is centered within the most sensitive bandwidth of the bats auditory system (Pollak et al., 1978). This choice of stimulus also allowed me to address whether communication sounds in the roost would be as likely to evoke shifts in echolocation call temporal patterns as broadband echolocation calls. I did not examine the effects of changing stimulus frequency or amplitude on the bats' temporal responses

because it was assumed that any changes in the behavior caused by changes in these parameters would primarily reflect the physiological properties of the auditory system.

Statistical analysis

Post-stimulus time histograms (PSTHs) were used to quantify and illustrate the proportion of pulses occurring in successive time windows relative to each preceding stimulus. I tested for stimuli effects using repeated measures analysis of variance (ANOVA) models on the percent of calls per bat in each time bin with stimulus type and stimulus type by time bin interaction effects. The interaction effect of bin and stimulus was the primary target of the ANOVA analysis, as this provided an indication of whether the distribution of pulses across bins differed significantly among conditions. For experiment 1 we ran three tests because different intervals resulted in different ranges in bins. First we examined the 50 ms interval stimulus with the first 50 ms of the 100 ms, 200 ms, 1000 ms and control stimuli. Then we examined the 50 ms to 100 ms range of the 100 ms, 200 ms, 1000 ms and control stimuli. Finally we compared the second 100 ms of the 200 ms and 1000 ms interval stimuli with the control. For the first two comparisons we used 4 ms bin widths, which resulted in 13 time bins. However, for the final comparison which occurred over a 100-ms time period, we had to broaden our bin widths to 8 ms to have sufficient degrees of freedom for analyses. For all remaining experimental analyses (experiments 2,3 and 4), which focused on the 200 ms intervals, we used 20-ms time bins for all statistical analyses.

For presentation purposes PSTH bin counts were normalized such that a value of 1.0 equaled the predicted mean number of pulses per bin in the absence of stimuli (i.e. random chance). For example, for a PSTH comprised of 50 bins the random average number of pulses per bin is expected to be 2.0% of the total number of pulses recorded during the experimental trial, thus experimental data were normalized by dividing actual bin percentages by 2%. Normalization facilitated comparisons across data produced using different stimulus intervals or analyzed with different bin numbers and widths. All data are presented as means ± standard deviations. All statistical procedures were performed utilizing SAS v9.2 and SAS-JMP 8.0 (SAS Institute Inc, Cary, NC).

Experimental Procedures and Statistical Analysis for Chapter IV

Drug injections procedure

All drug injections for this study were given intraperitoneally, using a 27 and a half gauge needle. For control recordings, 0.1 mL injections of saline were administered instead of drugs. Immediately after saline injections, bats were placed into the recording chamber and data acquisition began. For bats given injections of haloperidol, ketanserin, and 2, 5-dimethoxy-4-iodoamphetamine, the animals were given their injections, and then were set aside for five minutes to allow the drug time to take effect before recording began.

Haloperidol injections

Three different dosages were tested during this set of experiments: 0.1 mg/kg, 1 mg/kg, and 10 mg/kg. Each tested dose used an injection volume of 0.1 mL. The initial

stock for the haloperidol injections was created with 5 mg of haloperidol dissolved in 1 mL of 2% acetic acid, diluted with 49 mL phosphate buffered saline (PBS). This stock was used to create the 0.1 and 1 mg/kg dosages. For the 10 mg/kg dosage, 10 mg of haloperidol was dissolved into 1 mL 2% acetic acid, which was then added to 9 mL of either PBS or sodium chloride. Six bats were administered the 0.1 mg/kg and 1 mg/kg dose, and nine bats were administered the 10 mg/kg dose.

Ketanserin injections

For this experiment, two dosages were tested, 1 mg/kg and 10 mg/kg. As with the previous experiments, an injection volume of 0.1 mL was used for each dosage. The initial stock solution for the ketanserin recordings was created by dissolving 5 mg of ketanserin into 1 mL of dimethyl sulfoxide (DMSO), which was then added to 49 mL of phosphate buffered saline (PBS), which was used to create the 1 mg/kg dosage. A second stock of 1 mg ketanserin dissolved in 200 μL of 1,2 propanediol, brought up to 1 mL with deionized water was used to create the 10 mg/kg dosage. Later dosages of the 10 mg/kg concentration were created with 10 mg ketanserin dissolved in 1 mL DMSO added to 9 mL PBS. A third and final dosage of 3mg/kg was created by dissolving 3 mg of ketanserin into 1 mL DMSO, added to 9 mL PBS. The 1 mg/kg and 3 mg/kg doses were each tested on twelve bats total, six in each group. The 10 mg/kg dose was tested on eight bats.

2, 5-dimethoxy-4-iodoamphetamine (DOI) injections

Only the 1 mg/kg dosage of DOI was used during these experiments. This dosage was created by dissolving 1 mg of DOI into 10 mL of dH_2O, and utilized the standard 0.1 mL injection volume. This dosage was tested twice, once with a group of four bats, the second time with a group of eight bats.

Statistical analysis

Statistical analysis was performed using the same methods outlined in the Chapter III section. All statistical procedures were performed utilizing SAS v9.2 and SAS-JMP 8.0 (SAS Institute Inc, Cary, NC).

Experimental Procedures and Statistical Analysis for Chapter V

Experiment 1

Groups of bats ranging from a single individual to 10 animals in total were placed in a 10 x 10 x 20 cm plastic-coated ¼" steel mesh cage which was then positioned in the center of the anechoic recording chamber. The mean pulse emission rate per bat was calculated as the total number of pulses detected divided by total duration of the recording and the number of individuals placed in the cage. To determine whether an artificial stimulus altered pulse emission rates solitary bats were presented with artificial downward frequency-modulated sounds mimicking the echolocation pulses of free-tailed bats (Jarvis et al., 2010) at a repetition rate of 5 pulses per second, similar to naturally behaving bats.

Experiment 2

To determine whether the prevalence of overlapping pulse emissions occurred less frequently than predicted based on random chance we compared the real rate of overlaps occurring between two bats with Monte Carlo simulations of pairs of bats echolocating together. Real rate of overlaps was measured by manually counting the numbers of overlapping pulses occurring in randomly selected 10-second time epochs collected from 141 separate recordings of pairs of bats. We defined an overlap event as any instance when a second pulse appeared in the spectrogram within 10 ms of the onset of a previous pulse. Pulse durations typically varied from 4 to 8 ms and the returning echoes perpetuated in the chamber for at least 5 ms beyond the end of the first pulse. Under natural conditions the period over which another bat's emissions might overlap with the time course of a returning echo likely extends well beyond the 10 ms limit used here, but we will show that the results presented here are easily adapted to reflect more liberal time windows to accommodate different species or habitats.

Monte Carlo simulations of pairs of bats echolocating together were generated using 100 randomly chosen ten-second epochs of acoustic recordings from isolated naïve bats, which gave 4950 discreet simulated cross-pairings. For each real and simulated epoch we measured the mean pulse rate and number overlaps occurring within the 10 second epoch and from this determined the probability distribution of overlaps as a function of mean pulse rate. It was not possible to discriminate between the echolocation pulses of real bats recorded in pairs reliably enough to measure each individual bat's

pulse emission rate. Finally, based on the assumption that simultaneous emissions always have the potential to create ambiguities in the perception and interpretations of succeeding echoes, we defined *pulse efficiency* as the mean proportion of emitted pulses that did not overlap with another bat's emissions and therefore likely produced unambiguous echoes. Pulse efficiency was calculated by subtracting the expected overlap rate from mean pulse emission rate.

Experiment 3

To measure the behavioral response to continuous noise I measured the effects of a prolonged broadband noise stimulus on pulse emission rates. Preliminary experiments indicated that the bat's pulse emission rates typically declined over the twenty to thirty minute time-course of an experimental session regardless of stimulus type, preventing me from directly comparing extended recordings of bats echolocating in noisy versus silent conditions. Furthermore, individual call rates varied significantly across days, making it difficult to achieve statistically significant results when comparing stimulus conditions across days. Therefore to control for daily fluctuations and the systematic short-term decline in emission rates seen over the course of initial recordings, bats were exposed to a time-varying noise stimulus composed of ten-second blocks of white noise alternated with ten-seconds of silence. An iterative process led me to compromise upon ten-second stimulus epochs because this timeframe was at least two orders of magnitude longer than their typical inter-pulse intervals and yet short enough that there was no detectable time-dependent reduction in mean call rate within each epoch. Preliminary

trials with longer epochs of up to 2 minutes produced qualitatively similar results. This stimulus was referred to as the "continuous" noise stimulus to distinguish it from the periodic noise-burst stimuli used in experiment 1 and the stimulus used in Chapter III (Jarvis et al., 2009). For each trial the total number of echolocation pulses uttered was pooled from all experimental (stimulus ON) and silent (stimulus OFF) conditions and both mean emission rate and relative proportion of pulse's uttered was calculated for the noise ON and noise OFF conditions. To test if the bats responded differently to noise when alone versus in the presence of other bats, experiments were conducted in two separate sessions. In the first session, recordings were carried out with groups of either four or eight bats placed in the same cage and collectively exposed to the continuous noise stimulus. Following this, each bat from the group was isolated and recorded individually while being exposed to the same series of stimuli. Data were normalized as the total percentages of pulses occurring in silence versus noise.

Experiment 4

Six solitary bats were exposed to stimuli of varying duty cycles constructed by alternating a 10 ms burst of broadband noise with silent intervals of variable length. For example 10 ms of noise alternating with a 90 ms silent period gave a 10% duty cycle; other silent intervals were 40 ms (20% duty cycle), 10 ms (50% duty cycle, 3.3 ms (a 75% duty cycle) and 1.1 ms (a 90% duty cycle). Each bat was recorded for six twelve-minute exposures to each duty cycle. During these recording sessions, the stimulus was switched on and off every two minutes, allowing the stimulus blocks to be interspersed

with blocks of silence. The total number of echolocation pulses uttered was pooled from all six minutes of experimental (stimulus ON) and silent (stimulus OFF) conditions during each session. Different duty-cycle stimuli were presented in pseudorandom order to balance for time and order effects.

Experiment 5

A total of four bats were used in this experiment. Each individual was recorded separately while placed in a steel mesh cage. Bats were exposed to an auditory stimulus consisting of bursts of white noise with a center frequency of 33 kHz and -6 dB bandwidth of 16 kHz, separated by silent intervals of 200 ms (as used in Jarvis et al 2009). An attenuator (Tucker-Davis Technologies, Alachua, FL) was used to decrease the amplitude of the noise, for a total of four conditions: no attenuation (88 dBs), attenuator set to 10 (78 dBs), to 20 (68 dBs), and finally to 40 (48 dBs). For analysis, the silent interval was broken into ten consecutive 20 millisecond bins, the bats' responses averaged, and the resulting data normalized. T-tests were used to compare the depth of suppression (bin three) and the height of recovery (bin five) between the different conditions. The first data point of each set was compromised due to overlap with the artificial stimulus, and was disregarded.

Statistical analysis

Statistical analyses were performed with Sigma Stat v.9.0 (Systat Software, San Jose, CA). For experiment 1 a Kruskal-Wallis one-way analysis of variance on ranks was used to investigate the effect of population density on average pulse rate, and a

least-squares method was used to determine the best fit. For experiments 2 and 3, a two-way analysis of variance test was performed to investigate the effects of noise and social conditions on pulse emission rates. For experiment 4, a two-way analysis of variance using Holm-Sidak multiple comparison tests was performed to determine the effects of stimulus condition and duty cycle on emission rates.

CHAPTER III

HOW DO BATS ECHOLOCATE IN THE PRESENCE OF INTERMITTENT NOISE?*

Introduction

As animals that function primarily in low light conditions, bats rely upon their ability to echolocate in order to find food, navigate their surroundings, and avoid collisions with obstacles (Neuweiler, 2000). While this behavior allows them to function effectively in a wide variety of environments and situations, it is also highly susceptible from disruption due to the presence of environmental noise (Arlettaz et al., 2001; Gillam and McCracken, 2007; Jones, 2008). Interference from other members of the same species is particularly detrimental, as those signals will necessarily possess a similar bandwidth, frequency, and duration to the bats' own signals. Further complicating the issue is that many species of bat, particularly the Mexican free-tailed bat (*Tadarida brasiliensis*), are highly social, living in colonies and roosts of thousands of individuals (Ratcliffe et al., 2004; Simmons et al., 1978). Possibly as a consequence of this vulnerability, many species of bats possess a diverse and flexible repertoire of vocalizations and are able to alter many spectral and temporal characteristics of their echolocation calls (Bohn et al., 2008; Moss et al., 2006; Obrist, 1995; Schwartz et al., 2007; Ulanovsky and Moss, 2008).

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Given that bats exhibit control over the spectral, acoustic, and temporal properties of their echolocation calls, they have a variety of potential responses to the presence of interfering noise. One of the most well studied of these behaviors is the jamming avoidance response (JAR). In the JAR, bats shift the frequencies of their own echolocation calls to avoid frequency overlap with background noise or the echolocation calls and echoes of nearby conspecifics (Gillam et al., 2007; Ulanovsky et al., 2004). Bats have also been demonstrated to alter the amplitude of their own calls in response to the presence of interfering noise (Tressler and Smotherman, 2009). Less work has been done regarding bats' abilities to alter the temporal properties of their echolocation calls in order to avoid overlap with environmental noise or conspecific calls. One of the only discoveries made regarding temporal control as a mechanism for avoiding overlap found that bats flying in the presence of conspecifics cease calling for periods of time (Chui et al., 2008). More elaborate examples of temporal alteration as a mechanism for avoiding overlap with noise have been documented in a variety of animals including primates (Egnor et al., 2007; Versace et al., 2008), birds (Brumm, 2006; Ficken and Ficken, 1974; Knapton, 1987; Planque and Slabbekoorn) and frogs (Moore et al., 1989; Zelick and Narins, 1985). In these studies, animals alter the timing of emission of their own echolocation calls in order to exploit silent windows, or in order establish an asynchronous calling pattern with neighboring con- or heterospecifics.

In this chapter, I investigate the ability of bats to alter the temporal properties of the echolocation calls to reduce or avoid overlap with bursts of artificial interfering

noise. The first experiment of this study sought to determine if Mexican free-tailed bats could alter the time at which they emitted their echolocation pulses to avoid interference produced by intermittent, repetitive bursts of white noise. For the second experiment the bats were exposed to stimuli which had shorter available silent windows, to determine how this could affect the bats' ability to alter the timing of emission of their own pulse to avoid overlap with the noise. For the third experiment, the bats were presented with a stimulus in which the length of the silent interval varied semi-randomly from 200 to 1000 ms, in order to determine if they could successfully avoid overlap given a less predictable interfering stimulus, and if the resulting response would vary from the response to fixed, predictable noise. In the fourth experiment, the bats were exposed to more complex interfering stimuli to determine how these more complicated noises might alter the previously identified vocal timing behavior. Finally, a set of experiments were performed to investigate whether or not the duration and bandwidth of the interfering noise itself had an impact upon the nature of the bats' response. The overall goals of these studies were twofold: first, to determine if bats were successfully able to avoid overlap with a variety of stimuli of varying spectral and temporal properties, and second, to characterize the resulting patterns of pulse distribution for each stimuli.

Results

These experiments used the program Datapac 2K2 to determine where in the silent intervals between two computer-generated stimuli the bats placed their echolocation pulses. The program broke these silent intervals into 4 ms bins, and the

average number of calls per bin was calculated for each of the various experimental conditions. For the control recordings, the amplifier was disabled, ensuring that the subjects were exposed to only silence during the recording period. The initial control recording series of bats were used as the controls for the 50 ms, 100ms, 200ms, and random conditions, thus there were no more than 20 bats examined in each series of experiments. Repeated measures ANOVAs were used to compare the distribution of calls between the control and experimental recordings. For experiment 2, two statistical approaches were used. The first test utilized larger 20 ms time bins in order to perform pair-wise comparisons of the 50, 100, and 200 ms conditions. When comparing the entire length of each of these stimuli, the 4 ms bins could not be used, as the large number of data points created degree of freedom issues. The second statistical approach used for the second experiment involved running two tests, and the silent interval was broken into two equal halves. One test was run over the first 50 ms of the stimulus for the 50, 100, 200, and 1000 ms conditions. A second test was run for the latter 50-100 ms portion of the 100, 200, and 100 ms conditions. The smaller number of data points (13 total bins) meant that these tests could utilize the 4 ms bin widths. Experiments 1, 3, and 5 utilized the larger 20 ms time bins, with a Bonferroni adjustment so that these later stimuli could be compared to the original 200 ms recordings.

Experiment 1: Characterizing the bat's temporal response to the presence of artificial, interfering noise

The first objective of these experiments was to determine whether it was possible for a pattern of artificial noise to provoke changes in the timing of emission of the bats' echolocation pulses. In this initial experiment, 10 bats were exposed to an artificial stimulus consisting of 10 ms bursts of white noise separated by silent intervals of 200 ms. A repeated measures ANOVA demonstrated that the presence of the noise caused bats to alter the placement of their echolocation pulses within the silent interval when compared to their pulse distribution in silence ($F = 5.6711$, $P = .0061$, $DF = 18$). As demonstrated in Figure 1A, during the control recordings the bats were equally likely to emit a pulse in any of the available 20 ms bins, that is, anywhere within the silent interval. Upon exposure to a burst of noise, the probability of pulse emission underwent a period of suppression, which reached its depth between 40-60 ms after the onset of the noise. This period of suppression was followed by an increased probability of pulse emission peaking around 100-120 ms, followed by a gradual period of stabilization, during which time the average percentage of calls began to return to the initial values. During this experiment, the question arose of whether or not there was a learned component to this behavior, and whether an individual bat's response to noise might change with repeated exposure to the experimental stimulus. To determine if this was a possibility, three naïve bats were exposed to the 200 ms silent interval stimulus for three consecutive days, and their average responses were analyzed (Figure 1B). The

performance of all three bats was highly consistent over all three days, and no statistically significant difference was seen between the recordings taken on successive days ($F_{8,58} = 1.13$, $P = 0.37$). Thus, the same individuals were able to be used for multiple experiments, as their reactions to the interfering noise would not change over time and with exposure to the artificial noise.

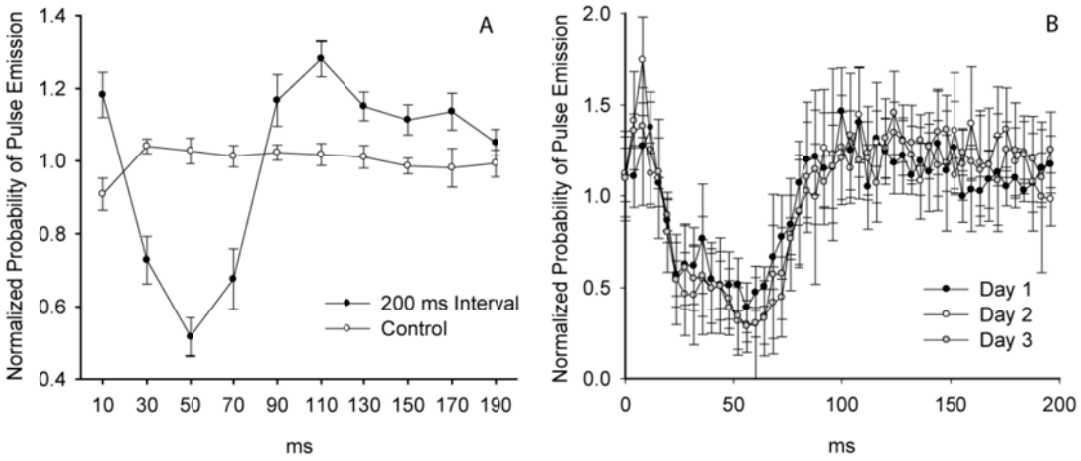

Fig. 1 The Vocal Timing Behavior. **A.** Normalized probability of pulse emission for both the control (recordings done with the amplifier disabled) and 200 ms conditions (10 ms bursts of white noise, occurring once every 200 ms). Each data point represents the mean values for 10 subjects, and the error bars represent the standard error of the mean. Data were normalized so that a value of one represents random chance, bin values falling below one indicate that pulse emissions were suppressed below the value expected by random chance, and values above one indicate that pulse emission occurred more frequently than expected. This graph uses 20 ms bins. **B.** Average data for three naïve bats tested on three successive days with the 200 ms silent interval stimulus. This graph uses 4 ms bins.

Experiment 2: Characterizing the bats' response to stimuli with shorter available silent windows

After determining that bats were capable of altering the timing of their pulse emissions to avoid overlap with the 200 ms stimulus, the question arose of whether or not they would be able to avoid overlapping their pulses with noise separated by shorter silent intervals, as would be expected with faster stimuli. A 1000 ms stimulus was also tested, to determine if the bats' response would change when confronted by noise with longer silent intervals. First, the subjects were presented with bursts of white noise separated by only 100 ms of silence. The bats' behavior during the presence of this stimulus was found to be significantly different from their behavior in silence by a repeated measures ANOVA using 20 ms bins ($F= 10.3992$, $P= 0.0003$, $DF=18$). The same general pattern of suppression followed by recovery is present, though the suppression phase is shallower, and the recovery phase hits its peak immediately before the onset of the next noise burst.

Following this, bats were exposed to a 50 ms silent interval stimulus (Figure 2A). Once again, the pulse distribution in noise was found to be significantly different from that in silence, when tested with 20 ms bins ($F= 48.9054$, $P= <.0001$, $DF= 18$). The observed distribution of calls was visibility different from the patterns observed in response to the 200 and 100 ms interval stimuli, however. During the 50 ms stimulus, bats had a very low probability of emitting a pulse both immediately after and immediately before a burst of noise, while they had the highest probability of emitting a

pulse in the middle of the silent interval. Finally, repeated measures ANOVAs determined that the pulse distribution patterns for the 200, 100, and 50 ms conditions were statistically different from each other (100 vs. 200 (F= 9.2703, P= 0.0006, DF=18); 50 vs. 100 (F= 48.2628, P= 0.0001, 18 DF= 18); 50 vs. 200 (F= 10.0361, P= 0.0013, DF= 18), demonstrating that there was no one consistent response to interfering noise, the response varied as the length of the silent interval varied.

Following this, another set of tests were run, this time using 4 ms bins and breaking the silent interval into two phases for increased precision: the first from 0-50 ms, and the second from 50-100 ms. A repeated measures ANOVA was run using the 0-50 ms section for the control, 50, 100, 200, and 1000 ms intervals found that the type of stimulus used significantly affected the distribution of pulses within the silent interval ($F_{48,133} = 2.6$, $P < 0.0001$). Another repeated measures ANOVA was run on the 50-100 ms period for the control, 100, 200, and 1000 ms stimulus, and again found a significant effect of noise repetition rate on pulse distribution ($F_{33,77} = 4.8$, $P < 0.0001$). Post hoc comparisons demonstrated a significant difference for all stimuli versus controls for both the first 50 ms period ($F_{12,34} = 3.4$, $P = 0.002$, $\alpha = 0.008$) and the second 50 ms period ($F_{11,26} = 8.0$, $P < 0.0001$, $\alpha = 0.008$). Both sets of tests confirm that bats alter the placement of their calls when confronted with interfering noise, and that the distribution of these calls varies with the length of the available silent interval (Figure 2B).

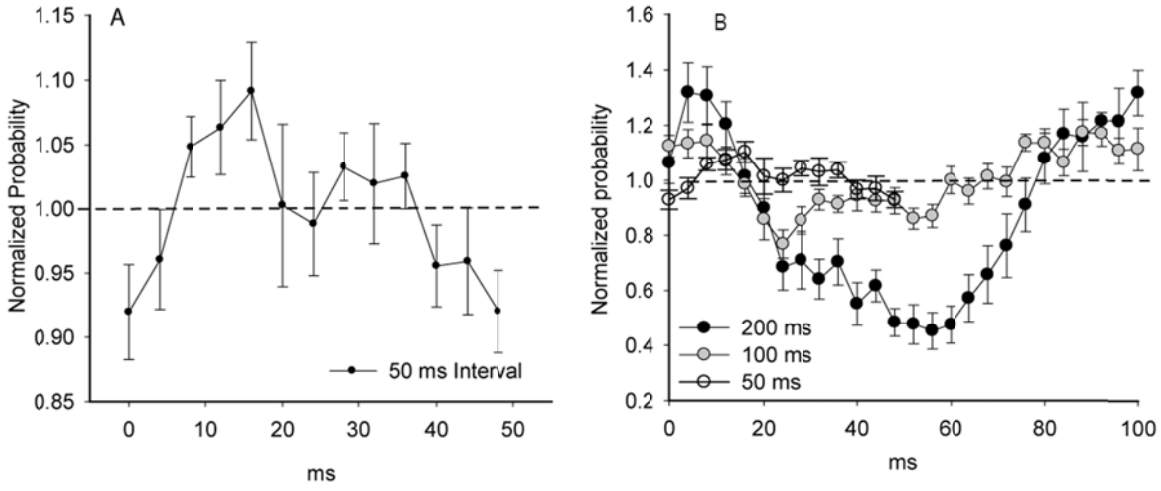

Fig. 2 Bats' Response to 50, 100, and 200 ms Conditions. **A.** Mean probability of emitting a call for 10 bats exposed to 10 ms bursts of white noise occurring once every 50 ms. Error bars indicated standard error of the mean. **B.** Comparison of the 50, 100, and 200 ms conditions. This graph only shows the first 100 ms of the 200 ms condition. Each data point represents the mean probability of emitting a call for 10 bats, utilizing 4 ms bins. The error bars represent standard error of the mean.

Experiment 3: Characterizing the bats' response to a randomly varying stimuli

The previous experiments demonstrated that bats were capable of altering the timing of emission of their echolocation pulses to avoid overlap with bursts of silent noise. However, all the previous stimuli were highly stereotyped, and thus easily predictable. It was important to determine if bats could continue to avoid overlap with interfering noise even when confronted with a less predictable, random stimulus, as one might expect to find in a natural setting. For this experiment, the bats were exposed to a stimulus where the silent intervals between noise bursts varied randomly between 200 and 1000 ms. The resulting distribution of calls was found to be significantly different from the control recordings ($F= 6.2271$, $P= 0.0043$, $DF= 18$), indicating that the bats

were still able to alter the timing of emission to avoid the interfering noise. The response to the random stimulus was compared to a set of 200 ms and 1000 ms silent interval stimuli (Figure 3A); a repeated measures ANOVA indicated that there was a significant difference between the responses to the random, 200, and 1000 ms conditions ($F_{18,38}$ = 3.6, $P < 0.0005$). The overall pattern of call distribution was similar to that observed for the 200 ms stimulus, though both the suppression and recovery phases appeared slightly shallower for the random stimulus when compared to the 200 ms results.

Experiment 4: Characterizing the bats response to more complex acoustic stimuli

The previous experiments used very simple stimuli, designed to mimic another bat's echolocation pulses, which the subjects were successfully able to work around. However, the inferring noise in a bat's environment would not always be so straightforward. Communication calls, for example, would present a much greater challenge to work around, as they tend to be longer and much more spectrally complex. For this experiment, bats were presented with two different, complex stimuli in order to determine how their responses to noise would differ from their reaction to exposure to the simpler 200 ms stimulus. The first of these stimuli consisted of pairs of 10 ms pulses separated by silent intervals of 50 ms (Figure 3B). This stimulus was presented every 200 ms for a total of 20 minutes. The second stimulus consisted of an artificial "feeding buzz" (Figure 3C): a train of downwards FM pulses lasting 200 ms and repeated every 500 ms. A repeated measures ANOVA was used to compare the 200 ms following the onset of the artificial noise for the 200 ms, double pulse, and buzz conditions, and found

that all three conditions were significantly different from each other ($F_{18,38} = 4.6$, $P < 0.0001$). A post hoc comparison further demonstrated that the bat's response to the 200 ms stimulus differed significantly from their response to the two more complex stimuli ($F_{9,19} = 31.$, $P = 0.0003$, $\alpha = 0.008$).

The paired pulse stimulus response initially appeared similar to the 200 ms response, with a period of suppression reaching its deepest part around 50 ms. Rather than heading into the recovery phase, however, the second pulse lengthened the period of this suppression, roughly doubling it. As a result, the recovery phase occurred much later, reaching its peak just before the onset of the next burst of noise. The buzz stimulus caused an immediate suppression of pulse emission, with the lowest probability of pulse emission occurring around 60 ms. Following this was a recovery phase, which featured an abrupt drop-off as the buzz stimulus terminated. From this point, there is a gradual decrease in pulse emission before the onset of the next buzz. Despite these apparent visual differences, a post hoc comparison between the two complex stimuli (Figure 3D) did not reveal a significantly different effect on pulse emission between the two ($F_{9,19} = 4.2$, $P = 0.02$, $\alpha = 0.008$).

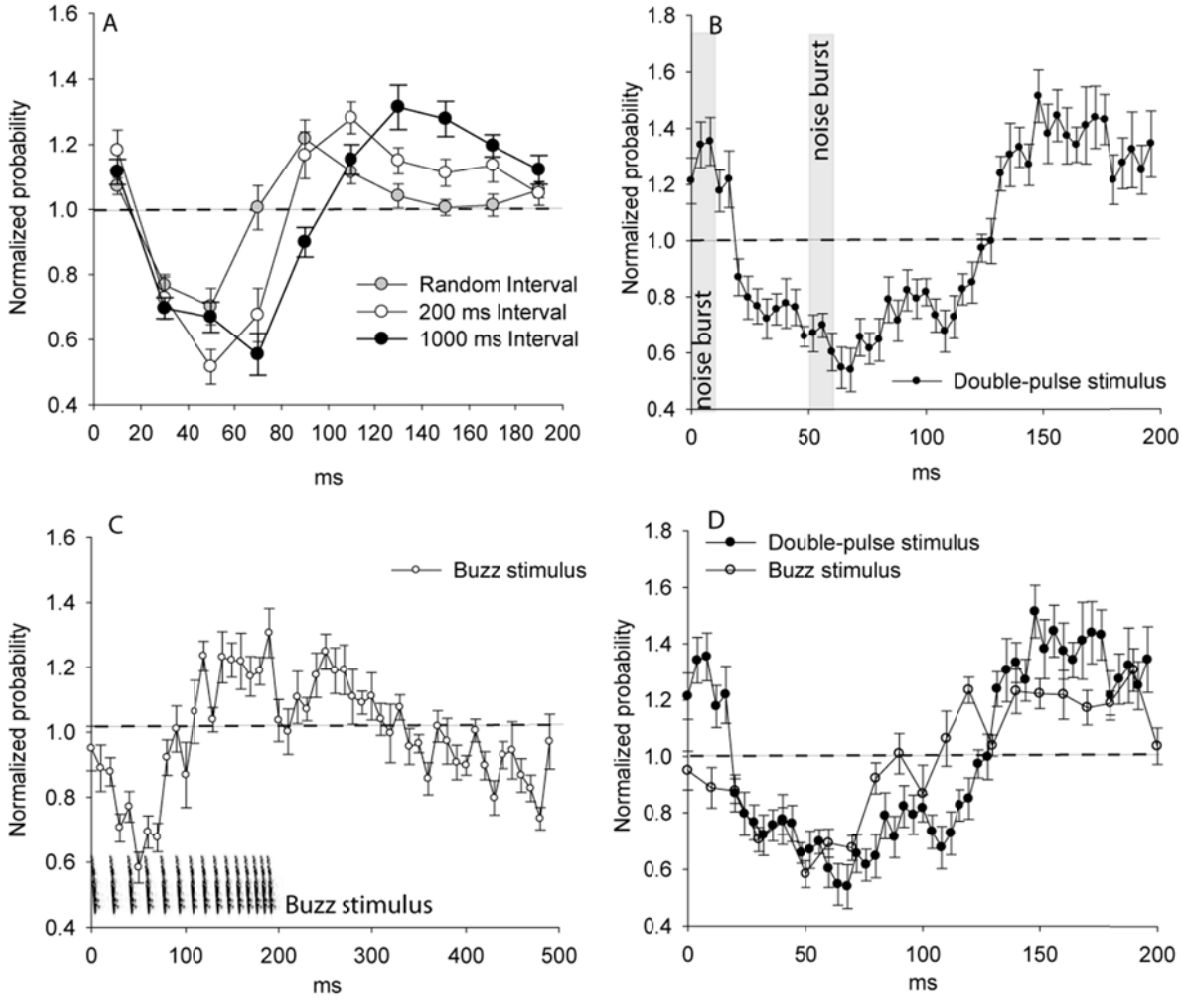

Fig. 3 Bats' Response to More Complex Stimuli. **A.** Comparison of the 200 ms, 1000 ms, and random interval conditions. Each data point represents the mean probability of emitting a call utilizing 20 ms bins. The error bars represent the standard error of the mean. **B.** Mean response plots of 10 bats listening to a paired-pulse stimulus which consisted of two 10 ms bursts of white noise separated by a 50 ms silent interval, repeated every 200 ms. Error bars represent the standard error of the mean. **C.** Mean ± SEM response of 10 bats calling in the presence of an artificial buzz stimulus, consisting of a train of 15 downwards FM pulses which lasted 200 ms and repeated once every 500 ms. **D.** Comparison of the paired-pulse and buzz stimulus responses. Error bars represent standard error of the mean.

Experiment 5: Investigating the effects of altering the acoustic properties of the stimulus on the bats' response

With the exception of the buzz stimulus, all the prior stimuli shared noise bursts with the same duration and bandwidth. In order to determine whether or not the duration of the interfering noise would affect the bats' response, subjects were exposed to bursts of noise (occurring once every 200 ms) with durations of 5, 10, 25, or 50 ms (Figure 4). None of these longer duration stimuli had a significantly different effect when compared to the regular 200 ms stimulus. Following this, the subjects were exposed to a pure tone stimulus with 25 kHz, 25 ms pulses which repeated once every 200 ms (Figure 4). As in the previous experiment, no significant difference was noted between the response to the original 200 ms noise burst stimulus and the pure tone stimulus. Lastly, the bats were exposed to a 200 ms stimulus in which the bursts of white noise were replaced by downwards frequency modulated sweeps, similar to a Mexican free-tailed bat's actual echolocation calls. The response to this downward FM stimulus was virtually identical to the response to the previous 200 ms stimulus.

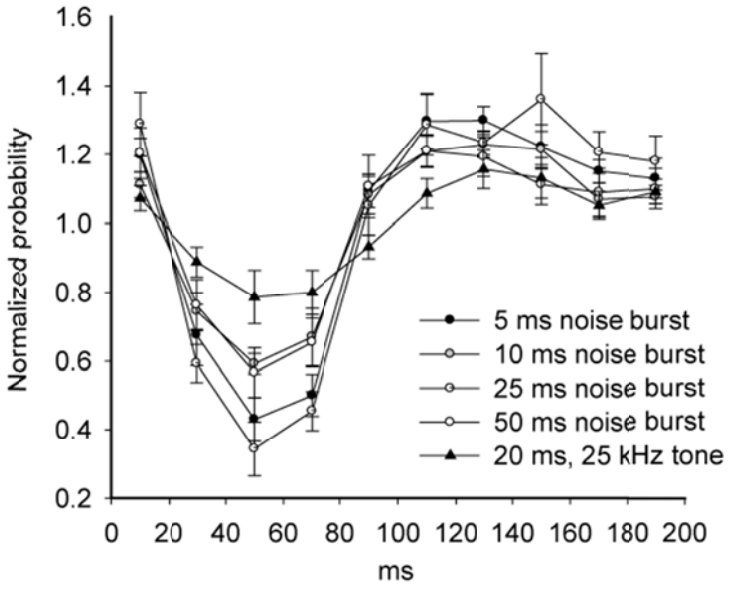

Fig. 4 Bats' Response to Stimuli of Varied Duration and Pure Tones. Comparison of the mean temporal responses to stimuli consisting of bursts of white noise of varying duration and a 25 kHz pure tone stimulus which lasted 20 ms. Each stimulus which repeated every 200 ms. Error bars represent the standard error of the mean.

Discussion

These experiments demonstrated that Mexican free-tailed bats are indeed capable of altering the temporal properties of their own echolocation calls, specifically the timing of emission of the echolocation pulses, to avoid overlap with interfering noise. Experiment 1 conclusively showed that bats distribute their pulses differently in silence and in the presence of interfering noise. Specifically, upon exposure to environmental noise, bats undergo a period of suppression, during which their probability of emitting an echolocation pulse is very low. Following this, there is a period of recovery, during which bats have a high probability of calling before the onset of the next burst of noise.

This behavior appears to be innate and not a learned response, as naïve bats are fully capable of demonstrating the behavior upon first exposure to the stimulus, and show no alterations to the behavior over time or repeated exposure. It would be tempting, therefore, to consider this type of behavior some form of reflex or fixed delay in response to noise.

Experiment 2, however, demonstrated that not only are bats capable of avoiding interference even when confronted with shorter silent windows in which to place their calls, but that the temporal properties of the bats' response to noise varies with the length of the silent interval. When confronted with a 100 ms silent interval stimulus, the overall structure of suppression followed by recovery was maintained, although the depth of suppression appeared to be shallower, and the recovery phase occurred earlier than it did during the 200 ms stimulus. Here, the bats appeared most likely to call immediately before the onset of the next noise burst, as if they were attempting to call at an increased rate before the next stimulus, but the shorter interval between bursts meant that it hit during this bout of increased calling. The response to the 50 ms stimulus was even more dramatic. While there was still a visible suppression phase, it began as soon as the first burst of noise occurred. Instead of waiting until the onset of the next burst of noise, the bats appeared to place the majority of their echolocation pulses in the middle of the silent interval, undergoing a return to a decreased call rate immediately before the onset of the next burst of noise. These results imply that rather than possessing a singular response to interfering noise, bats alter the temporal properties of their own echolocation

pulses in order to best fit the stimulus that they are confronted with. This indicates a greater degree of flexibility than would be expected if the response to noise was a mere reflex or the product of a fixed delay. This flexibility was further supported in Experiment 3, which confirmed that bats are capable of utilizing this method of interference avoidance even when the stimulus is less predictable in nature. Taken together, these results indicate that the vocal timing behavior demonstrated in this study cannot be simply classified as a reflex or startle response; the bats alter their behavior in order to best suit the temporal characteristics of the interfering noise.

While this behavior is indeed flexible, some characteristics of the vocal timing behavior remained consistent across all trials. The presence of both a suppression and recovery phase was noted for each of the stimuli that were tested. The suppression phase is particularly notable, typically occurring immediately after the onset of the first burst of noise. Experiment 4 provided some important insights into this phase of the behavior. The double pulse stimulus showed that the length of this suppressive phase could be extended, that a second burst of noise kept the suppression going longer than those stimuli with single pulses. The buzz stimuli, however, showed that this suppressive effect could not be kept up indefinitely: eventually, despite the noise still being active, the bats will undergo a recovery phase and begin calling once more. These results are important, in that they show that the suppression experienced as a response to noise is likely involuntary, as all tested stimuli showed some evidence of suppression. Despite this, vocalization was not be suppressed permanently: eventually, the bat began to call

again, when this actually occurs is based upon the temporal characteristics of the stimulus that the bat was currently exposed to. This latter fact is particularly important: otherwise, in the presence of dense acoustic clutter bats would be continuously suppressed and unable to echolocate at all. Finally, the fifth experiment demonstrated that this same behavior could be used for a wide variety of noises, regardless of their duration or bandwidth. This was especially important for the last stimulus tested, the downwards FM sweeps, as this is the structure that a conspecifics signals or the bat's own echoes would take. The ability to successfully avoid overlap with the FM sweeps indicates that bats should have no trouble avoiding overlap with another bat's echolocation calls.

Taken all together, the results of these studies demonstrate the existence of an innate, flexible behavioral pattern that can be used to avoid echo overlap with a wide variety of interfering noises. As previously stated, Mexican free-tailed bats live in large social groups, dense colonies who both roost and often fly in close proximity. While this behavior alone may not be enough to fully avoid overlap in very dense roosts, the ability of bats to alter the timing of emission of their own echolocation pulses in order to avoid overlap with their conspecifics would provide one more tool in the arsenal of responses to noise, in addition to their ability to alter the amplitude or other spectral characteristics of their calls. The behavior examined in this study is particularly well suited to bats, as they are known for their consistent, predictable echolocation patterns that result from echolocation being entrained to the respiratory period as well as the wing beat pattern

while flying. By calling in the silent window between conspecific calls, multiple bats could presumably share the same acoustic space without overlapping with one another or suffering major decreases in their call rate. This type of antiphonal calling behavior has been observed in other species, particularly frogs, birds, and primates, but this study is the first evidence of this kind of temporal alteration in bats.

CHAPTER IV

WHICH MONOAMINES PLAY A ROLE IN THE REGULATION OF VOCAL TIMING?

Introduction

The discovery that Mexican free-tailed bats alter the timing of emission of their echolocation pulses in order to avoid overlap with intermittent noise (Jarvis et al., 2010) established a model behavior that could be used to explore the neuropharmacology of vocal timing within the bats' brain. Generally, the mammalian brain regulates and monitors a variety of timed intervals. These time periods can range from circadian rhythms which cover hours or months of time to much shorter time periods: seconds, minutes, milliseconds, and microseconds (Buhusi and Meck, 2005; Buonomano, 2007; Mauk and Buonomano, 2004). Of these time periods, the one most associated with the timing of vocalizations is interval timing. Interval timing focuses on the detection and production of durations in the seconds to minutes range (Schirmer, 2004), and is used to characterize several behaviors, including time estimation, foraging behaviors, and decision making (Buhusi and Meck, 2005). A theoretical processing model for interval timing that has been proposed is broken into three distinctive stages: the clock, memory, and decision stages (Malapani and Fairhurst, 2002; Schirmer, 2004). This model posits the existence of an internal clock which generates neural oscillations or pulses, which are gated by a switch into an accumulator which integrates pulses over time (Meck, 1996). This accumulated number of pulses is compared to a value stored in reference

memory representing a previous duration of interest, and a comparator determines whether or not a behavioral response will be made (Malapani and Fairhurst, 2002; Meck, 1996). Information gained from such trials can be transferred into reference memory, allowing temporal information gained to be stored for later replications (Buhusi and Meck, 2002; Meck, 1996).

Examples of interval timing are characterized by several distinct properties. The first of these traits is the scalar property, which states that the longer the duration being timed, the greater temporal variability is seen in the final behavioral response, that is, the standard deviation of the interval being timed is proportional to the mean of that interval (Buhusi and Meck, 2005; Matell and Meck, 2000; Meck, 1996). Another key property of interval timing is the importance of dopamine, and the D2 receptor in particular, to the regulation and perception of timed intervals (MacDonald and Meck, 2005; Malapani and Fairhurst, 2002). Drugs which act as antagonists for the D2 receptor alone, particularly haloperidol, lead to a rightwards shift or phase lag in peak response times for interval timing behaviors (Buhusi and Meck, 2005; MacDonald and Meck, 2005; Meck et al., 2008). These drugs are thought to act through the alteration of the internal clock (clock stage), with dopamine antagonists causing it to slow down (Buhusi and Meck, 2002) while stimulants and dopamine agonists cause it to speed up (Mauk and Buonomano, 2004; Meck et al., 2008). As a result of this sensitivity to dopamine, many typical antipsychotics and other D2 antagonists are used during interval timing studies (Buhusi

and Meck, 2002; MacDonald and Meck, 2005), to the point where sensitivity to these drugs can be considered a defining characteristic of interval timing behaviors.

Finally, the responsitivity of interval timing behaviors to dopaminergic drugs has led to hypotheses regarding which regions of the brain play a role in interval timing. Within the literature there is especially strong support for involvement of the basal ganglia (Buhusi and Meck, 2005; Matell and Meck, 2000, 2004; Schirmer, 2004). The basal ganglia consist of several subcortical nuclei which possess intricate connections to each other, as well as afferent and efferent connections to the cortex and thalamus (Alm, 2004; Wisniecki et al., 2006). They also house dense clusters of dopaminergic neurons, primarily in the substantia nigra pars compacta (Alm, 2004; Wisniecki et al., 2006), which project to the forebrain and anterior cingulate cortex (Alm, 2004; Porrino and Goldmanrakic, 1982). The basal ganglia are rich in both D1 and D2 receptors (Feng et al., 2009), which are associated with the excitatory direct pathway and the inhibitory indirect pathway, respectively (Alm, 2004). Furthermore, the basal ganglia provide output to the supplementary motor area, a region that is thought to aid in the production of internal timing cues, along with the presupplementary motor area (Alm, 2004; Pastor et al., 2006). This suggests that the basal ganglia play a role in the generation of timing cues for motor sequences, which would naturally possess implications for its role in speech production and temporal control (Meck, 1996; Pastor et al., 2006). Finally, lesions or disorders of the basal ganglia are thought to be partially or wholly responsible

for stuttering, further supporting the importance of this area for vocalization (Ludlow and Loucks, 2003; Stager et al., 2005).

There is also support within the literature for the involvement of corticostriatal circuits (Balsam et al., 2009; Matell and Meck, 2000; Meck et al., 2008), with an emphasis on involvement of the prefrontal cortex (Balsam et al., 2009; Buhusi and Meck, 2005; Pastor et al., 2006). There is debate within in the literature regarding whether or not the cerebellum is involved in timing in the seconds to minutes range (Matell and Meck, 2004; Schirmer, 2004) though it is often associated with timing in the millisecond range, which may be a result of a separate internal clocks for the two time scales (Buhusi and Meck, 2002; Matell and Meck, 2000).

Human speech research, which has served as the primary model for studying vocal timing in the mammalian brain, has turned up parallels to interval timing studies, suggesting that the interval timing literature might serve as a framework for studying speech disorders and the regulation of vocal timing. A major focus of human speech studies are the various diseases and disorders that disrupt the ability of speakers to properly control the timing of their speech, and the effects of various drugs that can influence speech fluency. Included amongst these disorders are Parkinson's disease, Huntington's disease, schizophrenia, and stuttering (Wisniecki et al., 2006). Stuttering in particular has been closely studied, and there is an extensive body of work covering the subject. Stuttering is defined as a motor control disorder characterized by the repetition or lengthening of syllables as well as the extension of the duration of words (Ludlow

and Loucks, 2003; Maguire et al., 2000). As previously stated, this disorder is currently hypothesized to be a result of a dysfunction of the basal ganglia, resulting in hyperactivity of the dopaminergic system which in turn leads to a loss of motor inhibition via overstimulation of the indirect pathway (Alm, 2004; Stager et al., 2005). Consequently, D2 antagonists such as haloperidol can lead to an increase in fluency or a lessening of the severity of symptoms (Alm, 2004; Maguire et al., 2000; Stager et al., 2005). Both the sensitivity to dopamine and involvement of the basal ganglia support a connection to interval timing mechanisms.

Further support for an interval timing framework for human speech disruptions comes from studies of schizophrenia. The speech disfluency associated with schizophrenia is thought to be the result of a dysfunctional dopaminergic system (Salome et al., 2000), and can be alleviated through the use of atypical antipsychotics that affect both the dopaminergic and serotonergic systems (Salome et al., 2000). Finally, the effects of brain lesions upon speech fluency still more support for a connection to interval timing mechanisms; as an example, lesions or damage of the basal ganglia can lead to acquired stuttering (Alm, 2004; Ludlow and Loucks, 2003). Taken together, this research supports the idea that human speech could be regulated by interval timing mechanisms, with a notable involvement of both the basal ganglia and the dopaminergic system.

Dopamine is not the only monoamine thought to play a role in speech timing. While there is strong support for the involvement of dopamine within basal ganglia

circuitry regulating speech motor control and vocalization timing, there have also been studies implicating a potential role for serotonin in vocal timing. Atypical antipsychotics such as risperidone, which affect the 5HT2A receptor as well as the D2 receptor, have been shown to lead to improvements in speech fluency in those suffering from speech disorders (Maguire et al., 2000; Salome et al., 2000). Speech disorders associated with schizophrenia and major depression have also shown improvement in response to atypical antipsychotics or antidepressants which act upon the dopaminergic and serotonergic systems (Salome et al., 2000; Wisniecki et al., 2006). Serotonergic neurons are most densely concentrated in the raphe nuclei midbrain nuclei which may also play a role in speech timing. This area possesses projections to the forebrain, including the anterior cingulate cortex, which plays a proposed role in the vocal control pathway (Jurgens, 2009; Paus, 2001; Porrino and Goldmanrakic, 1982). There are three major possibilities that account for the effectiveness of such neuroleptics. Firstly, it is possible that the D2 receptor component alone is responsible for the observed effects. As typical neuroleptics such as haloperidol (which is a strict D2 antagonist) can lead to an improvement in symptoms, this is a definite possibility (Maguire et al., 2000; Stager et al., 2005). In addition, dopamine is thought to play a major role in the control of speech and motor behaviors, and aberrations in the dopamine system are suspected to play a role in many neurodegenerative or speech disorders such as schizophrenia or stuttering (Salome et al., 2000; Stager et al., 2005). There has also been research connecting dopamine specifically to the perception of time intervals (Pastor et al., 2006). A second

possibility is that atypical antipsychotics' function as a serotonin antagonist is responsible for the improvement of symptoms, with serotonin acting indirectly by modulating the activity of dopaminergic neurons. Stahl proposes a model for this mechanism in his 2008 book on psychopharmacology. In dopaminergic neurons, 5HT2A receptors serve as a dopamine inhibitor: that is, when serotonin is bound to the 5HT2A receptor on either the neuron itself or a GABAergic interneuron, the release of dopamine is blocked. When these same channels are occupied by an antagonist, serotonin cannot bind and the dopamine flows freely (Stahl, 2008). The final possibility is that serotonin is acting via modulation of pyramidal neurons at the level of the anterior cingulate cortex. In this model, the 5HT2A receptor stimulates the release of glutamate, which will then excite the cells downstream (which can be dopaminergic, as in the prefrontal cortex) (Stahl, 2008). The use of serotonin antagonists would prevent or reduce this release of glutamate and the resulting excitation.

I had two goals for this project. The first goal was to determine whether the vocal timing behavior I had previously identified (Jarvis et al., 2010) could be described as an example of interval timing. To test this, it was necessary to determine whether or not the behavior expressed the two characteristics that are used to identify interval timing behaviors: a scalar nature and sensitivity to D2 antagonists, especially haloperidol. Building upon this I wanted to determine whether either serotonin or dopamine, or both, could alter the time at which bats would emit their echolocation pulses in response to the artificial stimulus. To this end, I tested various drugs that act via the D2 and/or 5HT2A

receptors and determined whether or not the bats' vocal timing behavior underwent a phase lag or phase lead in response to the drugs. Preliminary trials (figure 5) indicated that drugs that affected the 5HT2A receptor, and not the D2 receptor, were capable of altering the timing of emission of echolocation pulses. In these studies, I planned to conduct more rigorous tests, to determine whether or not the preliminary data was accurate. My first hypothesis for these experiments was that the vocal timing behavior could accurately be described as an example of interval timing. My second hypothesis was that serotonin, acting via the 5HT2A receptor, would alter the timing of emission of echolocation pulses; producing a phase lag in response to 5HT2A antagonists and a phase lead in response to 5HT2A agonists.

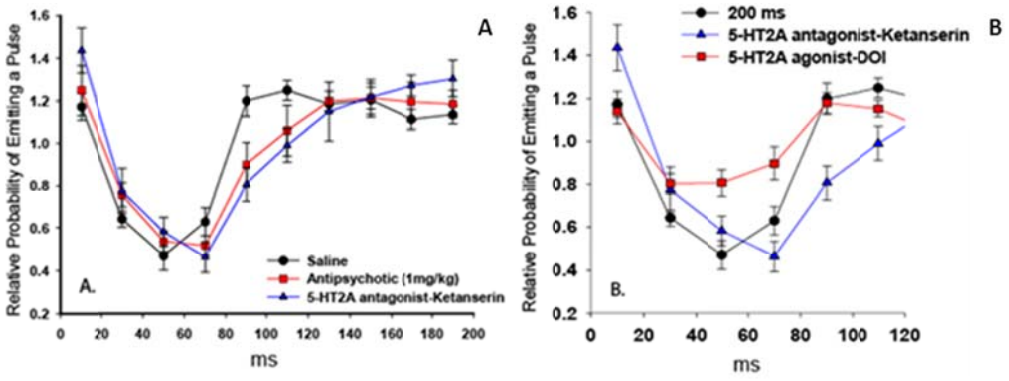

Fig. 5 Results of Preliminary Drug Trials. Error bars represent the standard error of the mean. **A.** Comparison between resulting effects of injections of saline (control), ketanserin, and the antipsychotics risperidone and clozapine. The values for the two atypical antipsychotics have been combined in this graph. **B.** Comparison of the typical response to the presence of bursts of interfering noise separated by silent intervals of 200 ms, the response when dosed with 5-HT2A antagonist ketanserin, and the lastly the response when dosed with 5-HT2A agonist DOI.

My first hypothesis for these experiments was that the vocal timing behavior could accurately be described as an example of interval timing. My second hypothesis was that serotonin, acting via the 5HT2A receptor, would alter the timing of emission of echolocation pulses; producing a phase lag in response to 5HT2A antagonists and a phase lead in response to 5HT2A agonists

Results

Experiment 1: Determining if the vocal timing behavior is scalar in nature

In order to determine whether or not the vocal timing behavior was scalar in nature, the breadth of the time window in which postponed calls reappeared following the period of suppression was examined. This period was designated the "window of recovery" (Figure 6A). As previously stated, the scalar property holds that the longer the interval being timed, the greater the standard deviation that is seen around the time of response (Buhusi and Meck, 2005). According to this definition, the vocal timing behavior was indeed scalar in nature, as the width of the window of recovery varied with the length of the silent interval (Figure 6B). This finding supports the hypothesis that the vocal timing behavior is an example of interval timing.

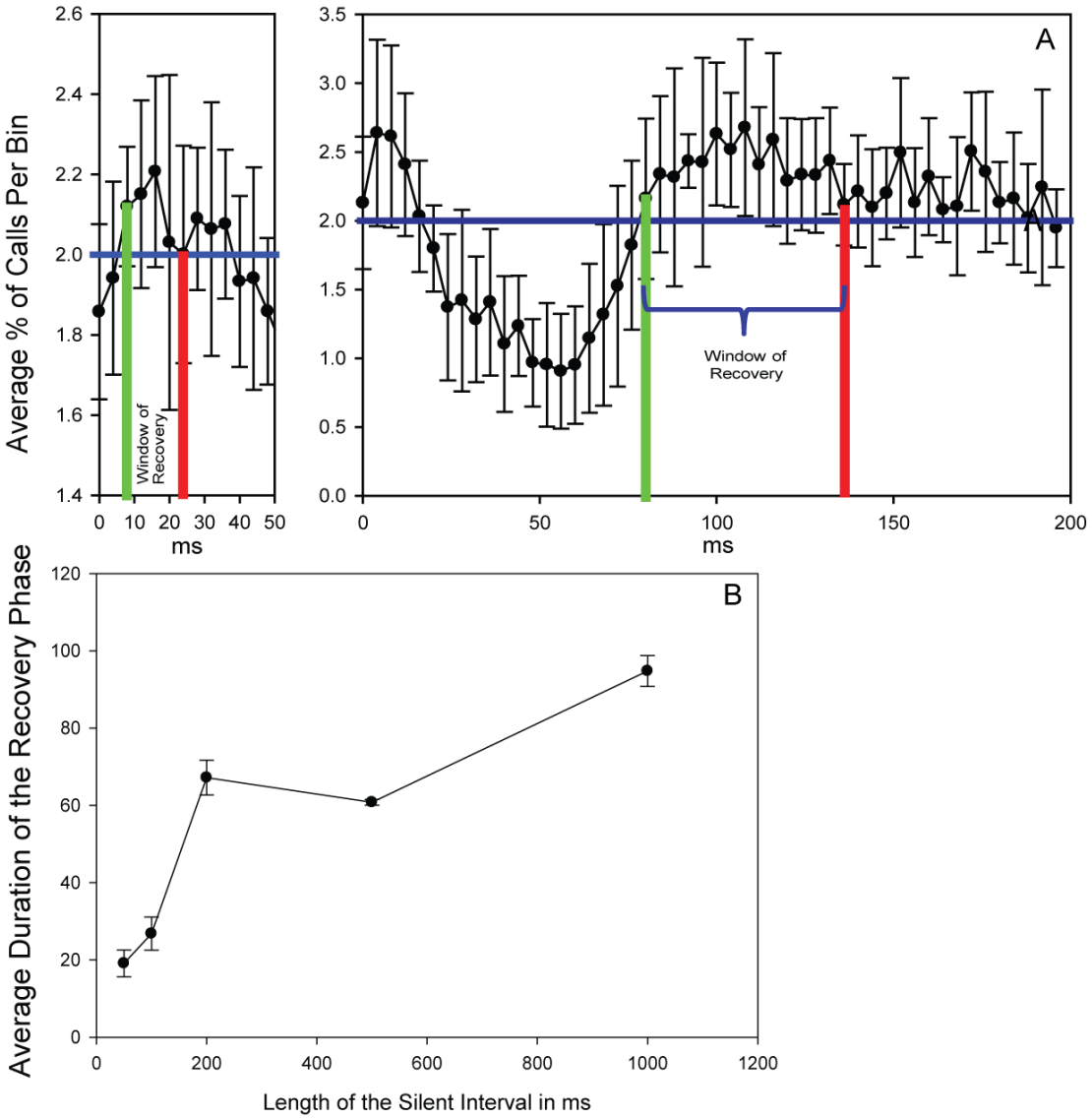

Fig. 6 Demonstration of the Scalar Nature. **A.** Examples of the "window of recovery" in the vocal timing behavior, illustrating how the accuracy of the bats' vocal timing at different stimulus repetition intervals was measured. Both an example of the 50 ms silent interval and the 200 ms silent interval are included. **B.** Analysis of the mean recovery window durations at a range of five different stimulus intervals, error bars represent standard error of the mean.

Experiment 2: Determining if the vocal timing behavior is responsive to haloperidol

The initial series of haloperidol injections was performed on a group of six bats using a dosage of 0.1 mg/kg haloperidol. This tested dosage failed to produce a significantly different alteration of the bats' vocal timing behavior when compared to control injections of saline ($F = 1.9438$, $P = 0.3856$. $DF = 10$) (Figure 7A). The dosage was then increased to 1 mg/kg haloperidol, and administered to the same six individuals. This higher dose also failed to produce any alterations in the observed behavior ($F = 0.2696$, $P = 0.9331$, $DF = 10$) (Figure 7A). At this time, all but one of these bats were dropped from the study. These dropped bats were failing to consistently produce the vocal timing behavior, necessitating their replacement. A new group was formed, consisting of nine new bats in addition to the remaining individual from the previous tests. This new group of ten bats was given a dose of 10 mg/kg haloperidol. Even with this higher dose of the drug, the bats' response to interfering noise was statistically indistinguishable from their response after injections of saline ($F = 1.4142$, $P = 0.2976$, $DF = 18$) (Figure 7B). Finally, t-tests and Mann-Whitney Rank Sum tests determined that none of the three tested dosages of drug had any significant effect upon call rate. As none of the tested doses of haloperidol resulted in any form of alteration to the bats' overall behavior or acoustic properties of their calls, I concluded that haloperidol had no effect upon the bats' vocal timing behavior. As my vocal timing behavior had met the first criteria for an interval timing behavior, but had not met the second, my initial hypothesis was not confirmed.

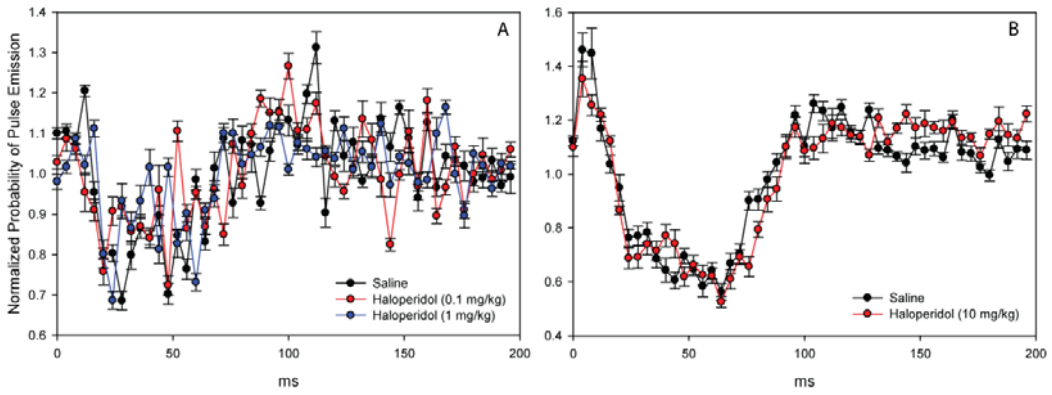

Fig. 7 Comparison of Saline Controls and Haloperidol. **A.** Mean probability of emitting a call for six bats, for saline, 0.1 mg/kg haloperidol, and 1 mg/kg haloperidol injections. Error bars represent standard error of the mean. **B.** Mean probability ± SEM of emitting a call for ten bats, for saline and 10 mg/kg haloperidol injections.

Experiment 3: Determining if the vocal timing behavior is responsive to ketanserin

As with the haloperidol injections, it was necessary to test several dosages of ketanserin in order to determine an effective dosage. An initial six bats were tested with a dosage of 1 mg/kg ketanserin, which failed to provoke a significant response when compared to saline ($F = 3.2525$, $P = 0.2573$, $DF = 10$) (Figure 8A). The dosage was then increased to 10 mg/kg ketanserin, as with the haloperidol study. This higher dosage was administered to eight bats, yet did not produce a significant effect upon probability of pulse emission ($F = 1.5673$, $P = 0.3009$, $DF = 14$) (Figure 8B). Lastly, a dosage of 3 mg/kg was administered to a new set of six bats. As with the previously tested dosages, no effect of ketanserin on call emission was found ($F = 3.7524$, $P = 0.2281$, $DF = 10$) (Figure 8C). All three dosages of ketanserin had a statistically insignificant effect upon call rate, as determined by t-tests and Mann- Whitney Rank Sum tests. Due to the failure

of all tested dosages to produce any alteration in the bats' response to interfering noise, I concluded that ketanserin had no effect upon the vocal timing behavior.

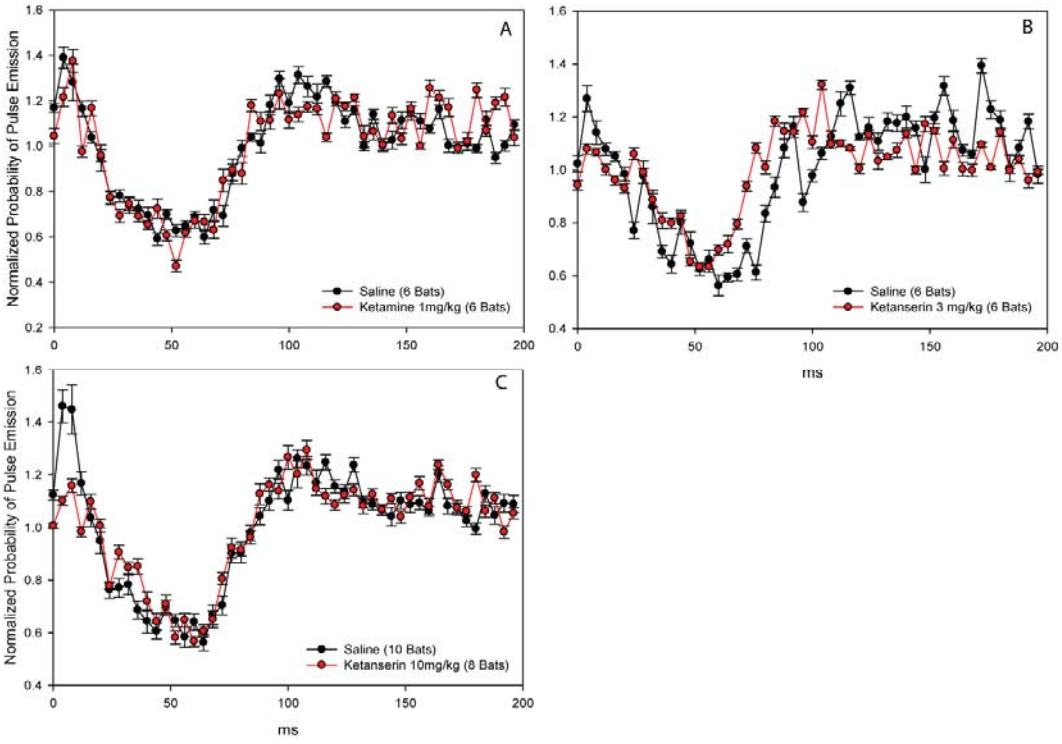

Fig. 8 Comparison of Saline Controls and Ketanserin. **A.** Mean probability of emitting a pulse for 6 bats, injected with either saline or 1mg/kg ketanserin. Error bars represent the standard error of the mean. **B.** Mean probability ± SEM of emitting a pulse for 10 bats, injected with either saline or 10 mg/kg ketanserin. **C.** Mean probability ± SEM of emitting a pulse for 6 bats, injected with either saline or 3 mg/kg ketanserin.

Experiment 4: Determining if the vocal timing behavior is responsive to 2, 5-dimethoxy-4-iodoamphetamine

The initial dosage of DOI tested was 1 mg/kg. This dosage was tested on four individual bats, and when the resulting data was graphed, there was a visibly apparent difference between the saline and DOI recordings (Figure 9A). A repeated measures ANOVA test could not be run on the data, presumably due to the small number of individuals tested. A two way ANOVA, however, found a significant interaction effect between the drug treatment (DOI vs. saline used) and the average percentage of calls per bin ($F = 3.743$, $P = <0.001$, $DF = 9$). These same four bats were tested again with an additional four bats, for a total of eight subjects. For this next set of recordings, each bat was recorded for a full 20 minutes, as the concern had arisen that previous recordings were not allowed to run on long enough to find a discernible effect of the drug, or were running on so long that such an effect might be weakening or wearing off. During these more standardized files, while there still appeared to be a visible phase lead, there was no longer a significant difference between the two drug conditions ($F = 1.5985$, $P = 0.2924$, $DF = 14$) (Figure 9B). In addition, the bats' call rate was unaffected by DOI in both sets of recordings, as determined by t-tests and Mann- Whitney Rank Sum tests. These latter experiments led us to conclude that DOI does not ultimately have a significant effect upon the bats' vocal timing behavior.

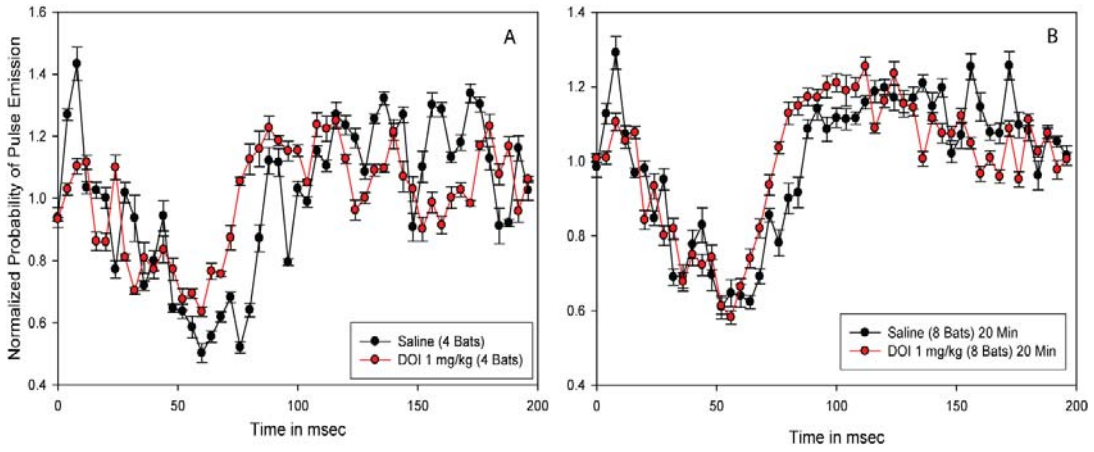

Fig. 9 Comparison of Saline Controls and DOI. For all graphs, error bras represent standard error of the mean. **a.** Mean probability of emitting a pulse for 4 bats given intraperitoneal injections of either saline or 1mg/kg DOI. **b.** Mean probability of emitting a pulse for 8 bats given intraperitoneal injections of either saline or 1mg/kg DOI. All recordings done in this round of tests lasted 20 minutes.

Discussion

My initial hypothesis for these experiments was that the described vocal timing behavior could be characterized as an example of interval timing. To test this hypothesis, I examined two criteria that are used to identify interval timing behaviors: a scalar nature and sensitivity to D2 antagonists. First, I determined that the behavior was indeed scalar in nature: the width of the window of recovery increased as the length of the silent interval increased. This indicates that as the silent interval grows longer, bats become less precise in their timing, either because there is ambiguity in when the silent period will end, or because there is less pressure on them to call with precision, as they have a longer interval in which to work. However, my experiments with haloperidol

demonstrated that the behavior was unresponsive to the typical antipsychotic, with even the highest dose of 10 mg/kg failing to elicit a response. As a result of this, I was unable to demonstrate that that bats' behavior fit the description of an interval timing behavior. The failure of haloperidol to elicit a response, however, turned my attention to other monoamines which could play a role in the temporal regulation of echolocation pulse emission.

Preliminary trials performed by this lab indicated that the atypical antipsychotics risperidone and clozapine (which affect both serotonergic 5HT2A and dopaminergic D2 receptors) as well as the 5HT2A antagonist ketanserin and the 5HT2A agonist 2, 5-dimethoxy-4-iodoamphetamine could affect the temporal pattern of the bats' vocal behavior (Figure 5). The antipsychotics and 5HT2A antagonist ketanserin seemed to produce a rightwards shift in the timing of call emission, while the agonist DOI produced a leftwards shift, as well as decreased the overall depth of the suppressive phase (Figure 5). These data, combined with background information gathered from the literature, led me to hypothesize that serotonin acting via the 5HT2A receptor could play a role in the regulation of the bats' vocal timing behavior. More specifically, I expected 5HT2A antagonists to produce a phase lag in the vocal timing behavior, while 5HT2A agonists would produce a phase lead. However despite the success of the preliminary trials in altering the timing of emission of the bats' echolocation pulses, I could not replicate the success of the initial experiments in the three sets of drug trials described in this Chapter. Based on these results, I was unable to support my hypothesis that

serotonin acting via the 5HT2A receptor was responsible for altering the timing of emission of the bats echolocation pulses in response to intermittent noise. A more detailed breakdown of the results follows.

The results of the initial trials suggested that it was the 5HT2A component of the antipsychotics, and not the D2 component, that was altering the timing of emission of echolocation pulses, as the strict 5HT2A antagonist ketanserin produced similar results to the tested antipsychotics. Our first goal for these experiments was to confirm or deny a role for dopamine in regulating the vocal timing behavior. We did this through the use of haloperidol, a classical antipsychotic which functions as an antagonist for D2 receptors only. All three tested dosages of haloperidol did not produce a statistically significant difference in the timing of pulse emission when compared to saline trials. These results supported our initial hypothesis that it was serotonin and not dopamine that plays a role in altering the timing of emission of echolocation pulses during the vocal timing behavior.

The drugs ketanserin and DOI were tested in order to confirm the results seen in the preliminary trials with a larger number of bats and under more controlled conditions. Both drugs strongly act upon the 5HT2A receptor, making them excellent choices to test the role of the 5HT2A receptor in the vocal behavior. All three tested dosages of ketanserin failed to provoke a significant difference in pulse emission timing when compared to saline injections, in stark contrast to the results seen in the preliminary trials. The results of the DOI experiments were more ambiguous. The first round of

experiments used a dosage of 1 mg/kg and was tested with a group of four bats. This round of tests demonstrated a significant effect of DOI upon the vocal timing behavior, producing a visible phase lead which was significantly different from the response observed during saline trials. However, this same dosage tested upon a larger group of eight bats failed to replicate the phase lead effect. It is important to note that these latter files were allowed to run for a full 20 minutes, instead of the recording being ended when a sufficient amount of data was collected (at least 1000 calls), as had been my standard procedure prior to this set of tests. These longer files were taken as concern arose over whether enough time was being allocated for the drugs to either take effect or potentially wear off. Under these more stringent conditions, the drug failed to significantly alter the bats' behavior, so I am led to conclude that given these results, a role for the 5HT2A receptor in the regulation of our described vocal timing behavior cannot be demonstrated at this time.

Given the results of these trials, I was unable to support my initial hypothesis, that serotonin acting through the 5HT2A receptor plays a role in the temporal regulation of the bats' vocal response behavior. As neither dopamine nor serotonin could be demonstrated to have any consistent effect upon the vocal timing behavior, these results cannot confirm involvement of either the substantia nigra pars compacta or the dorsal raphae nuclei in its regulation. This is not to say that neither the 5HT2A receptor nor the D2 receptor can be conclusively stated not to play a role in vocal timing whatsoever. There is still strong evidence in the literature that one or both of these neurotransmitters

are capable of altering the temporal characteristics of human speech. Our bats' vocal timing behavior simply may not be analogous to human speech for the purposes of these experiments, and as the next chapter will demonstrate, the bats' response to interfering noise is much more complex than initially suspected. Another possible explanation for these results is the sheer amount of factors that may affect the bats' response to the drugs: the age of the drug dose (and whether or not it expires or weakens over time), the bats' innate call rates and behavioral performance (as well as whether or not they perform consistently throughout the trial period), the bat's body weight and the exact site of the injection, an individual's responsiveness to the given drug, the time of day/season, and how long it takes for the drug to take effect and to wear off, among others. The sheer number of potential interfering factors could have made it difficult to get consistent results. In the future, studies investigating the effects of neurotransmitters upon vocal timing behavior should strive to find ways to correct or account for these factors in order to hopefully generate more consistent results. In addition, future projects examining the effects of drugs upon the control of vocal timing in the bat may wish to additionally investigate norepinephrine, which has been hypothesized to play a role in the regulation of neural activity, and is also present in the ACC (Paus, 2001). This drug has also been used experimentally to treat stuttering, a motor speech disorder (Alm, 2004).

CHAPTER V

HOW DO BATS ECHOLOCATE IN THE PRESENCE OF CONSPECIFICS?

Introduction

Vocal communication is a vital part of life for many animals, but acoustic signals are vulnerable to degradation by environmental noise (Brumm and Slabbekoom, 2005; Jones, 2008; Marten et al., 1977; Penna et al., 2005). Such noise can degrade the quality of both incoming and outgoing signals, resulting in incomplete or missed information for the receiver. To mitigate the detrimental effects of noise many animals display behavioral mechanisms to reduce or avoid the impact of interfering noises such as changing the acoustic characteristics or shifting the timing of their acoustic signals (Brumm, 2006; Egnor et al., 2007; Ficken and Ficken, 1974; Knapton, 1987; Popp et al., 1985; Versace et al., 2008). One of the most prominent sources of noise for many animals are the vocalizations of nearby conspecifics, and many animals adjust the timing and acoustic properties of their vocalizations in response to the sounds of their neighbors (Egnor and Hauser, 2006; Egnor et al., 2007; Gillam et al., 2007; Manabe et al., 1998; Penna et al., 2005; Scheifele et al., 2005; Tressler and Smotherman, 2009). These adaptations can at least partially mitigate the degrading effects of noise on communication within a social context (Brumm and Slabbekoom, 2005; Planque and Slabbekoorn, 2008). However, social context changes, and vocal adaptations that serve well in one social context may be ineffectual in another. It is unknown whether any animals exhibit different strategies for dealing with noise in different social contexts.

Echolocating bats need to clearly hear their own returning echoes to hunt and navigate (Neuweiler, 2000; Schnitzler and Kalko, 2001). While many bats forage alone or in small groups, they also share day roosts with large numbers of conspecifics. To echolocate efficiently bats maintain precise control over the acoustic and temporal properties of their echolocation pulses (Smotherman, 2007), and in some cases this includes adaptations for echolocating in the presence of other bats (Bates et al., 2008; Bohn et al., 2008b; Gillam and McCracken, 2007; Tressler and Smotherman, 2009; Ulanovsky et al., 2004). Several bat species have been shown to change their outgoing call pitch in order to minimize overlap in call bandwidth (Bates et al., 2008; Gillam et al., 2007; Necknig and Zahn, 2011; Ratcliffe et al., 2004; Tressler and Smotherman, 2009; Ulanovsky et al., 2004), and free-tailed bats increase their call amplitude in the presence of background noise (Tressler et al., 2011; Tressler and Smotherman, 2009). However, less work has been done exploring temporal strategies for minimizing acoustic interference (Chui et al., 2008; Jarvis et al., 2010). Like some birds (Brumm, 2006; Ficken and Ficken, 1974; Planque and Slabbekoorn, 2008), frogs (Moore et al., 1989; Penna et al., 2005) and primates (Egnor et al., 2007; Versace et al., 2008)), when confronted with repetitive, intermittent noise Mexican free-tailed bats shift the timing of emission of their own echolocation pulses in order to avoid overlap with the next oncoming burst of noise (Jarvis et al., 2010). While the benefits of this behavioral strategy are apparent for pairs of bats or even small groups, it is difficult to imagine that such a mechanism would work well within the very large and dense colonies that the

highly social Mexican free-tailed bats form during the day (Ratcliffe et al., 2004; Simmons et al., 1978).

In such dense populations it seems unlikely that small changes in syllable acoustics or timing could effectively mitigate the surrounding din. Thus this series of experiments were created in order to investigate whether free-tailed bats responded differently to the noise of one or a few individuals as opposed to when in the presence of many individuals. We hypothesized that bats may possess separate mechanisms for mitigating the effects of continuous versus periodic noise, enabling them to function even in very noisy environments. Specifically, we predicted that pairs or small groups of bats using temporal shifts would be forced to call less often as bat density increased, and that a threshold density would exist above which bats would abandon attempts to coordinate their calling in time and simply try to call louder and more frequently than their neighbors. We tested this hypothesis both by measuring mean call rates for progressively larger groups of bats and also by exposing individual bats to progressively increasing temporal ratios of noise to silence. The results of these experiments indicate that small groups of bats calling in close proximity do suppress each other's calling, and that bats possess two discreet behavioral responses when confronted with either periodic or continuous interfering noise.

Results

Experiment 1: Do echolocating bats suppress the pulse emissions of their conspecifics?

Experiments with an artificial stimulus had demonstrated that intermittent bursts of white noise can suppress a bat's echolocation briefly. We wondered, however, if any suppression of calling occurred when bats called in the presence of a conspecific as a result of overlap with the other bat's echolocation pulses. To test this, we compared the call rate for individual bats with bats calling in pairs (Figure 10A), and found a significant difference in call rate, as determined by a Mann-Whitney rank sum test ($T = 930$, $n_1 = 28$, $n_2 = 57$, $p = 0.011$). This decrease in mean call rate was similar to the significant decrease in call rate observed when comparing a bat calling alone to an individual calling in the presence of a speaker playing periodic, interfering white noise ($t = 2.045$, $df = 35$, $p = 0.048$). From this, we are able to conclude that the artificial stimulus (as seen in Chapters III and IV) effectively mimics the presence of another bat, leading to the same alteration of behavior as seen with two conspecifics calling together. As two bats undergo some degree of suppression whilst calling together, the question arose as to whether individuals would be able to continue to engage in this pattern of behavior in the presence of increased numbers of conspecifics.

Figure 10b demonstrates that as the number of bats in the recording group increased, the average call rate per bat decreased, indicating that echolocating bats do suppress pulse emission in neighboring conspecifics, and that this effect is more severe in larger groups. A Kruskal-Wallis One-Way Analysis of Variance on Ranks demonstrated a statistically significant difference between the different groups (i.e. the various experimental conditions) of bats ($H = 90.199$, $df = 7$, $P = < 0.001$), demonstrating that population density has a significant effect on call rate. Furthermore, a pairwise multiple comparison procedure (Dunn's method) demonstrated that at least three bats are required for there to be a statistically significant amount of suppression when compared to an individual bat calling alone ($Q = 5.033$, $p < 0.05$). The relationship between the number of bats in the group and the resulting call rate per bat can be described as an inverse first order nonlinear regression $y = 0.92 + 3.82/x$ (Figure 1B): as the number of bats in a group increases, the resulting average call rate will decrease in a mathematically predictable fashion. The relationship between number of bats and call rate was found to be statistically significant ($F_{1,6} = 93.97$, $p < 0.0001$, $R^2 = 0.94$). It is important to note that pulse emission was never suppressed entirely. The decrease in the average call rate reached a plateau around roughly one hundred calls per minute per bat, or about 30% of the maximum call rate, and did not appear to decrease or increase past this point, regardless of the number of bats in the group.

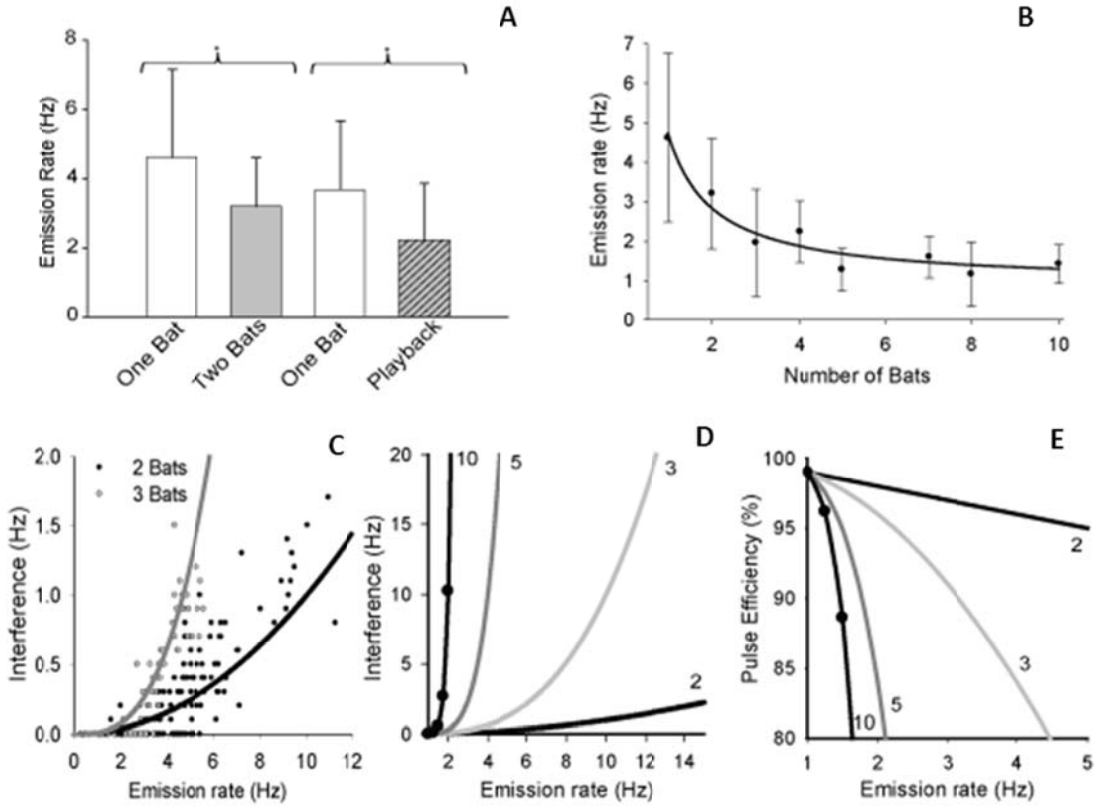

Fig. 10 The Effect of Group Size on Pulse Emission Rates. **A.** Bats' mean pulse emission rates recorded alone versus when echolocating in pairs, and then again for alone versus while echolocating with a speaker simulating the presence of another bat echolocating (stimulus). Error bars represent the standard deviation; brackets and asterisk represent statistically significant differences. **B.** Mean call rate per bat in relation to the total number of bats in the group, fitted with a first order linear regression (solid line, $y = 0.92 + \frac{3.82}{x}$). Error bars represent the standard deviation. **C.** Plot of mean pulse rates versus the rate at which overlaps occurred (interferences) for pairs (n=141) and triads (n=56) of bats. Both sets of data were well fit by the same simple power function of the form $y=r\tau^n$, where r = mean emission rate (Hz), τ = overlap window duration (ms) and n = number of bats. ($r^2=0.71$, $F_{(1,140)}=344.9$, $P<0.001$)., **D.** Illustrates the expected effect of pulse emission rates on mutual interference rates for groups of 2, 3, 5 and 10 bats, expanding the functions derived from C. **E.** These functions were then used to predict the effect of pulse emission rates on pulse efficiency (the proportion of pulses expected to generate unambiguous echoes, or $y=1-r\tau^n$) for different group sizes.

Experiment 2: Do pairs of bats call out of phase with one another to reduce overlap prevalence?

Comparing real groups of bats to Monte Carlo simulated groups of bats revealed that the bats' echolocation behavior was strongly altered by social context. Real pairs of bats emitted significantly fewer pulses per second than simulated pairs (4.6 ± 2.1 Hz versus 6.0 ± 3.1 Hz, respectively, $P<0.0001$) and also emitted overlapping pulses significantly less frequently than simulated pairs (0.29 ± 0.37 Hz versus 0.38 ± 0.38 Hz, $P<0.0001$). Analyses also revealed that real pairs produced a higher percentage of epochs with no instances of overlap (48%) than simulated pairs (15%) suggesting that real pairs of bats were successfully avoiding overlaps better than expected by chance alone. However this observation could simply be a product of reduced pulse emission rates. To investigate this we examined whether the reduction in interferences was independent of pulse emission rates. It was hypothesized that if bats actively avoided overlapping with one another's emissions, then the data from real bats should reflect a downward shift in the prevalence of overlaps independent of pulse emission rates. Alternatively if the probability of two or more bats' emissions overlapping in time was random, then the interference rate was predicted to follow a simple power function of the form $r\tau^n$, where r is the mean emission rate, τ is the empirically defined overlap window duration (10 ms), and n is the number of bats.

Figure 10C plots how frequently real bats echolocating in pairs or triads emitted overlapping pulses (labeled *Interferences*, quantified as overlaps per second) as a function of the mean pulse emission rate. Both data sets were well fit by the function $r\tau^n$ (r^2=0.71, $F_{(1,140)}$ =344.9, P<0.001), indicating that interferences had occurred randomly and their propensity was predictably based on mean emission rates and population density and that the bats were not timing their pulse emissions to avoid overlaps with one another. Figure 10D extends this function to illustrate how pulse emission rates are predicted to influence interference rates for groups as large as ten bats. The graph demonstrates that bats in modest group sizes of 5 or more are faced with a daunting increase in the probability that their pulse emission will overlap with those of neighboring bats. Figure 10F uses the same functions to estimate pulse efficiency ($1- r\tau^n$) as a function of pulse emission rate. This provides an estimate of the relative proportion of emitted pulses that would likely return unambiguous echoes over a natural range of pulse emission rates, illustrating that pulse efficiency is expected to decrease steeply with increasing population density and faster emission rates.

Experiment 3: How do bats respond to the presence of continuous noise?

This experiment measured the effects of a continuous noise stimulus on pulse emission rates. Upon exposure to "continuous" blocks of white noise, the bats called more frequently when the noise was present than during the intervening periods of silence, regardless of whether they were recorded individually or in groups (Figure 11A). A Two-Way Analysis of Variance revealed a statistically significant effect of the noise condition (whether the noise was on or off) upon the relative distribution of calls occurring in each condition ($F_{1,40} = 143.8$, $p = <0.001$). It also demonstrated the presence of a significant interaction effect between the group/individual conditions and the noise conditions ($F_{1,40} = 8.937$, $p = 0.005$). The group/individual condition alone had no significant effect upon the percentage of calls occurring in each condition, supporting the conclusion that it is the presence or absence of noise that has an effect upon the bats' calling, regardless of whether the subjects are calling alone or in groups. In addition, the average call rate increased from 1.4612 ± 0.9469 calls/sec in silence to 1.8195 ± 1.1753 calls/sec in noise for the group condition, and from 1.5305 ± 0.8317 calls/sec in silence to 2.3279 ± 1.0397 calls/sec in noise, though these differences were not statistically significant.

Experiment 4: At what ratio of noise to silence do bats switch from being suppressed to emitting pulses more frequently?

The results of previous experiments demonstrated that free-tailed bats respond differently based upon whether the noise stimulus was continuous or periodic, calling less frequently in the presence of periodic noise and more frequently in the presence of continuous noise. To better estimate the temporal pattern at which bats distinguish between a periodic and continuous noise, the bats were exposed to a series of noise burst stimuli presented at a range of different duty cycles (Figure 11B). A Two-Way Analysis of Variance revealed that variations in noise stimulus duty cycle had a statistically significant effect upon the bats' call rate ($F_{1,70} = 14.888$, $p = <0.001$). A Holm-Sidak multiple comparison test further determined that while there was no significant difference in call rates among the 5%, 10%, and 20% conditions, each of the duty cycles at or above 50 % caused a significant increase in call rate (50% ($t = 2.652$, $p = 0.05$); 75% ($t = 4.613$, $p = 0.05$); 90% ($t = 3.355$, $p = 0.05$)) ($F_{5,70} = 8.872$, $p = <0.001$). Finally, there was a statistically significant interaction effect between the noise on/off and duty cycle conditions ($F_{5,70} = 5.123$, $p = <0.001$), demonstrating that the presence or the absence of the noise was indeed responsible for the observed differences between the silent and experimental conditions.

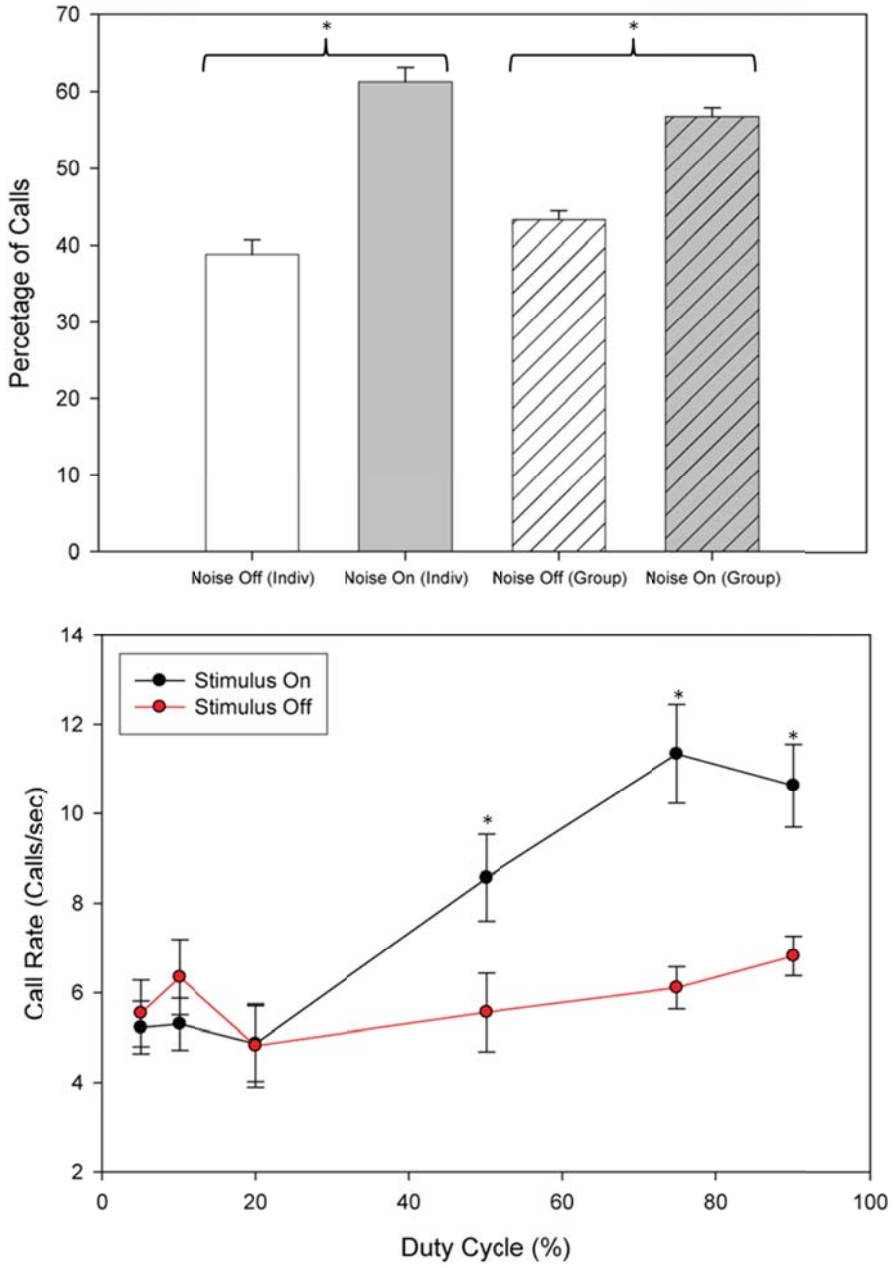

Fig. 11 Demonstration of Variation of Call Rate and Percentage. **A.** The average percentage of calls occurring during silence (Off) and during the presence of blocks of white noise (On), for both individual bats (Indiv) and groups of four or eight bats (Group). Error bars represent standard error of the mean for each condition, brackets and asterisks represent statistically significant differences. **B.** The effect of duty cycle upon the bat's average call rate, measured in calls per second. Error bars represent standard error of the mean, asterisks represent statistically significant differences.

Experiment 5: Does the amplitude of the interfering noise have an effect upon the bat's response?

Finally, we set out to determine the effect of the amplitude of the interfering noise upon the bats' response (Figure 12). An attenuator was used to decrease the amplitude of the intermittent artificial pulse stimulus. Recordings were taken at normal amplitude (88 dB), and then at amplitudes in decreasing units of 10 dB (78 dB, 68 dB, and 48 dB). An insufficient number of data points were available for the 58 dB recordings, and it was dropped from the analysis. As the amplitude of the interfering noise decreased, the effect of the noise upon the bats' behavior diminished (Figure 12). The normalized probability of pulse emission for the two major areas of interest (the depth of suppression at bin three and the height of recovery at bin five) both decreased as the amplitude of the sound was lowered. At 48 dB, the bats' response became virtually indistinguishable from silence. Two sample t-tests confirmed a significant difference between the two most extreme conditions, for both the depth of suppression ($t = -4.209$, $df = 6$, $p = 0.006$) and the height of recovery ($t = 6.813$, $df = 6$, $p = 0.001$).

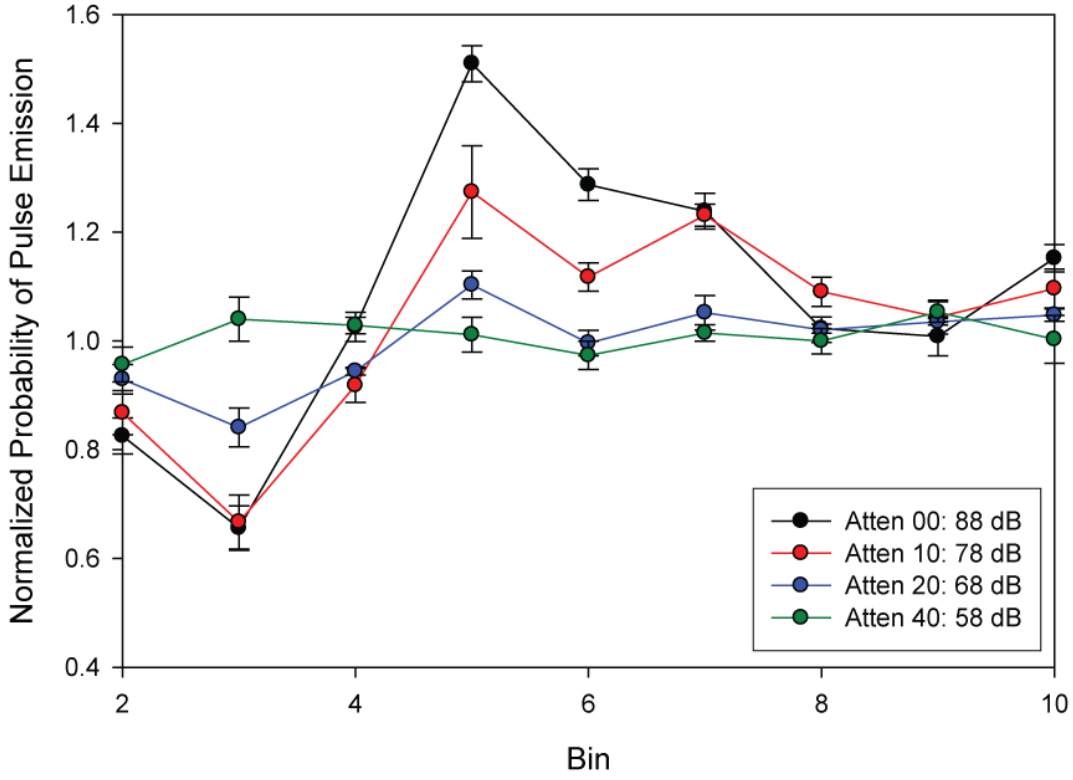

Fig. 12 The Effect of Amplitude Upon Vocal Response. The two primary areas of interest were bin three, representing the maximum depth of suppression, and bin five, the maximum height of recovery. Error bars represent the standard error of the mean.

Discussion

Like many other species, Mexican free-tailed bats have been shown to alter the timing of emission of their own echolocation calls, in order to avoid overlap with predictable, repetitive noise (Jarvis et al., 2010). The bats alter their probability of calling within the available silent interval between bursts of noise, leading to a period of suppression where they are highly unlikely to call and which occurs immediately after

the onset of the interfering noise and lasts for around 50 ms. Following this phase, they undergo a recovery period during which time they have a much higher probability of calling, typically placing their calls before the onset of the next burst of noise. The existence of this period of suppression led to further questions regarding the viability of this vocal timing behavior in large groups of animals. The previous experiments had all been performed with a single individual responding to an artificial stimulus, and even these fairly simple conditions lead to a significant decrease in call rate (Figure 1A). This decrease in call rate was theorized to be a result of the suppressive phase. If the number of bats in the recording group was increased, the greater amount of noise being produced could lead to greater opportunities for call overlap and suppression. If even two naturally echolocating bats calling together experience a significant decrease in their mean call rate (Figure 10A), how would bats be affected when exposed to larger numbers of conspecifics?

Experiment 1 sought to examine this question in depth. The data from this experiment show that as the number of bats in the recording group is increased, the mean call rate per bat decreases in a mathematically predicable fashion. These results strongly suggest that naturally echolocating bats do suppress each other's calling, leading to a significantly lower call rate overall. The greater the number of bats, the more opportunities for call overlap and suppression, and the more strongly the resulting call rate is affected, up to a point. A notable result of this experiment was that regardless of group size, pulse emissions are never entirely suppressed, that is, the mean call rate

never reaches zero. After the group reaches a size of five individuals, the minimum call rate settles into a plateau at roughly 30% of the maximum call rate, regardless of further bats introduced into the group. From this, we can hypothesize that even in huge colonies, bats would never cease vocalizing entirely, they will continue calling even in very chaotic acoustic environments. These results left us with an important question: how does undergoing periods of suppression and experiencing a resulting decrease in call rate provide any benefit to the bats' ability to echolocate in large groups?

While the answer to this question couldn't be found within natural acoustic systems, communication networking literature provided an explanation for how slowing pulse emission may benefit the bat's echolocation performance within large groups. The *ALOHA* system was an inaugural experiment in computer networking designed to link multiple independent users spread across the Hawaiian Islands to a central mainframe computer via a shared UHF radio channel (Abramson, 1970). Signals were randomly transmitted to and from a central computer in time-limited bursts or "packets" of information in a completely unsynchronized manner which led to "collisions" among users transmitting at the same time, causing the loss of both signals. Error detection algorithms were instituted that allowed users to know when their signals had collided, and a simple re-transmission protocol was incorporated independently by users that continually resent signals until a successful transmission occurred. This resulted in an uncoordinated competition for channel time than degraded the overall flow of information for all users. To improve efficiency ALOHAnet's architects (Abramson,

1970) investigated how often collisions occurred and how to best to guide user behavior to optimize information flow through the network while also improving transmission efficiency for each user. Network performance was characterized by its total information *throughput* as a function of overall *traffic load*.

Abramson and colleagues showed that as channel traffic increased the rate of collisions among user transmissions increased exponentially and consequently the probability of a successful transmission decreased exponentially (Abramson, 1970). For any single user the immediate probability (p) of a successful transmission was predicted by $p = e^{-2\lambda}$, where λ was a product of the number of users (n), mean transmission rate (r) and signal duration (τ). Channel throughput (S) was used as a measure of how efficiently information is transmitted through a shared communication channel. Maximum possible throughput for any shared channel is achieved only when all user transmissions are perfectly coordinated to utilize 100% of the channel time without any collisions, and is effectively unachievable without comprehensive central coordination. Since a channel's capacity to transmit information can also be underutilized, S is ultimately a function of both channel usage and p, thus $S = \lambda e^{-2\lambda}$, reflecting the compromise between transmission rate and interference rate. Figure 13A illustrates how this function could be applied to a group of bats sharing a common acoustic space, except that in this analogy the acoustic space represents a shared communication channel. All the bats sharing the space are transmitting and receiving their echolocation pulses over the same shared channel, and each bat is likely to lose information when its transmissions collide with

another bat's transmissions. For analytical purposes we assume that any overlapping pulse emissions result in total loss of the both transmitted signals, but this may not be entirely true for bats. For free-tailed bats we define r = mean pulse emission rate, τ = overlap window (10 ms), and then $\lambda = n_{bats}\, r\tau$. For any given population density greater than 1 it can be shown that there is an optimum mean pulse emission rate where at all bats would presumably benefit from increased pulse efficiency, deriving the most information possible from their echolocation pulse stream with the least amount of wasted emissions. Increasing pulse emission rates beyond this optimum rate rapidly degrades information throughput of the common airspace because the relative proportion of pulses generating unambiguous echoes steeply declines for all individuals.

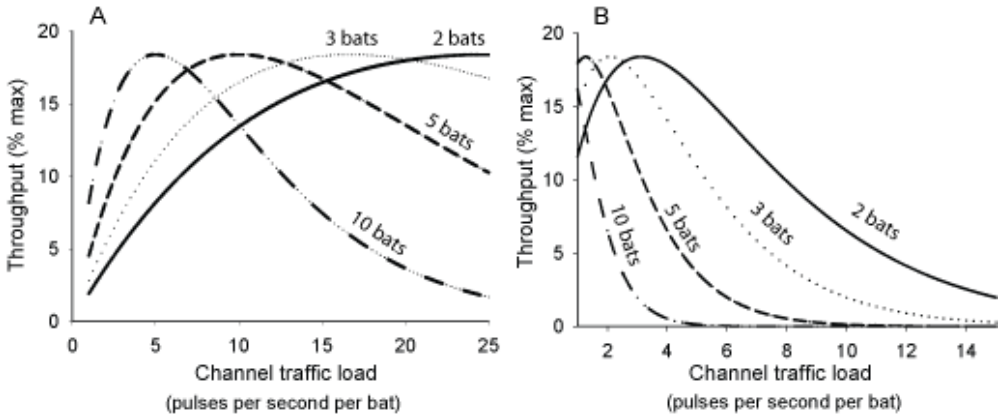

Fig. 13 The Effect of Population Density Upon Channel Traffic Load and Information Throughput. These figures were created using the function $S=\lambda e^{-2\lambda}$, where $\lambda=r\tau n_{bats}$, and where S represents information throughput. **A.** Information calculated assuming an overlap window (τ) of 10 ms. **B.** Information calculated assuming an empirically determined overlap window of 80 ms (the overall length of the suppressive phase). The peak values for information throughput occur at the optimum pulse emission rates of 3.25 Hz/bat for pairs of bats, 2.0 Hz/bat for triads, 1.25/bat Hz for groups of five, and 1 Hz/bat for groups of ten.

The random-access nature of a "pure ALOHA" network such as the one described above was found to constrain network throughput to a maximum value of 0.5/e, or roughly 18.4% of the theoretical maximum achievable capacity (Abramson, 1970; Kleinrock and Tobagi, 1975). Since interferences automatically trigger re-transmissions, such random-access networks are inherently unstable due to a positive feedback loop wherein retransmissions lead to a progressively increasing traffic load and consequently more frequent collisions or interferences. For bats this means that if all the bats in the group increased pulse emission rates to compensate for lost information due to mutual interferences as might be expected based on their known response to cluttered acoustic environments (Petrites et al., 2009), then their net sonar performance would decline rather than improve. Instead, to maintain even modest throughput efficiency bats would be better off reducing emission rates as *n* increased, else the number of pulses generating unambiguous echoes would rapidly diminish. To combat this phenomenon in ALOHAnet, regulatory protocols were applied to constrain when and how often users retransmitted their data. One of these, known as the "carrier sense multiple access" protocol (CSMA) is relevant to bats because CSMA incorporated a "listen-before-send" algorithm, in which transmitters first checked to see if the channel is free before transmitting, and if not briefly postpone transmissions. This greatly reduced traffic load by reducing the number of collisions and retransmissions, and thereby increased network utilization and information flow for all users. We now hypothesize that acoustic suppression of pulse emission exhibited by free-tailed bats serves a function similar to

CSMA in wireless communication networks, effectively improving sonar performance in social settings by optimizing pulse emission rates relative to population density.

The optimum range of pulse emission rates predicted by figure 13A is significantly higher than the emission rates we observed for similarly sized groups of bats (Fig. 10B). This may be accounted for by differences in the predicted and actual overlap window durations. We used a conservative estimate of 10 ms in our analyses, however our previous studies indicate that hearing another bat's echolocation pulses can suppress echolocation pulses for up to 80 ms, suggesting that the effective overlap window is somewhere closer to 80 ms. The actual time window over which returning echoes may be interfered with should vary predictably with habitat and target distances, but it is possible that in free-tailed bats the general behavior is tuned to a specific range represented by 80 ms. This range was taken from the overall length of the suppressive period, as described in Chapter III. When we recalculated information throughput values using an 80 ms value for τ (Fig. 13B) we found optimum pulse emission rates more closely aligned with the empirically obtained emission rates for groups of different sizes. This supports the hypothesis that free-tailed bats are reducing their pulse emissions to optimize information throughput of their shared acoustic channel.

The results of the first two experiments led to the question of how exactly bats would alter their echolocation calls in order to cope with the presence of continuous noise. In this situation, they could no longer alter the timing of emission of their echolocation pulses, as there would be few or no silent intervals in which to place their

calls. If they cannot avoid overlap with artificial noise though this behavior, how would they attempt to cope with the signal degradation that results from a noisy environment? Experiment 3 exposed bats to alternating blocks of 10 seconds of continuous, solid noise, and 10 seconds of silence. When the percentage of calls occurring in silence was compared to the percentage of calls occurring in noise using a Two Way ANOVA, the results demonstrated that a higher percentage of calls occurred when the noise was present, regardless of whether the bats were calling alone or in groups of four or eight individuals. The bats also had an apparently higher call rate in noise than in silence for both the individual and group, although the standard deviations for these call rates were so high that they could not be demonstrated to be significantly different. These results indicate that in the presence of continuous noise, when decreasing call rate through altering the timing of emission of one's own echolocation pulses is no longer a viable mechanism bats instead undergo an excitation of calling. They call more often in constant noise than they would in quiet periods, while demonstrating no evidence of suppression or reduced call rate. This form of response to noise has been seen in various bird species: in response to continuous background noise, these species increase their call rate in order to increase the probability that their calls will be detected against the background noise, or that one of the calls will fall into an unpredictable moment of quiet (Lengagne et al., 1999; Potash, 1972). For bats, this tactic of producing a greater number of calls may help to reduce the severity of the signal degradation occurring as a result of the presence of interfering noise. Though the individual calls may be degraded, the sheer

number of returning echoes could provide enough information for the animals to produce a more accurate map of their surroundings, or for one of the calls to fall into an unpredictable quiet period.

The results of these previous experiments indicated that bats possessed two different responses to noise, one which they utilized when exposed to periodic, discontinuous noise, and the other when exposed to constant noise. This led to the question of what ratio of noise to silence was required in order to generate an excitation of calling. For Experiment 4, the bats were exposed to noise of varying duty cycles (ratios of noise to silence). Lower duty cycles (5%, 10%, and 20%) demonstrated no difference between the bats' call rate in silence and in noise. These duty cycles were apparently insufficient to provoke either a strong suppression or excitation of calling. Bats exposed to the duty cycles at or above 50% (50%, 75%, and 90%) possessed significantly higher call rates than when recorded in silence. From these results, we conclude that as long as the interfering noise takes up the same amount of time as the silent periods, the bats will switch from altering the timing of emission of their echolocation pulses, and instead proceed to undergo an excitation of calling. A duty cycle of 50% seems to be considered the threshold for "continuous" noise, where decreasing call rate will no longer be able to adequately prevent overlap with interfering noise.

Lastly, the results of Experiment 5 demonstrated that the lower the amplitude of the noise, the less impact it has upon the bats' echolocation behavior, until it becomes

indistinguishable from their behavior in silence. Both the depth of suppression and height of recovery decreased in response to gradually diminishing loudness. Returning once again to the problem of bats living and hunting in close proximity to one another, one can see how this experiment is relevant to this topic. The echolocation calls of bats further away from the target individual would obviously be less detrimental than the calls of another individual in close proximity. Bats spaced closely together, as in a roost or during emergence flights, would have a strong impact upon one another's ability to echolocate, and may need to alter the spectral, acoustic, or temporal characteristics of their calls. Bats that are further away from one another would pose less of a threat to a conspecific's ability to echolocate. Mexican free-tailed bats have an average call amplitude of about 100 dB, and their search call frequency is around 30 kHz (Tressler et al., 2011; Tressler and Smotherman, 2009). At a temperature of 25° Celsius and a humidity level of 50%, as might be expected for bats foraging in the open air, a 100 dB call could travel for 30 meters (or 88 ms) before the pulse attenuated to a point where a bat would not undergo suppression in response to the sound (Lawrence and Simmons, 1982). This means that bats need to be closer than 30 meters to one another in order to affect each other's echolocation calls, assuming this set of atmospheric conditions.

One issue of note that arose during these three experiments was the various stimuli's effect on call rate. Experiments 1 and 3, as well as preliminary work done for these studies (Figure 10A) show a strong effect of interference on call rate. A single bat calling alone typically emits 4 to 6 calls per second, although there is a great deal of

natural variation within and between individuals. During experiment 2, however, the subjects possessed much lower call rates than in any prior work. When the stimulus was active, even during the silent periods calls rates were much lower than expected for both the individual (0.89 call/s) and group (0.57 calls/sec) conditions. From these drastically lowered call rates, one can only conclude that the presence of the stimulus had an overall suppressive effect when compared to bats calling in total silence and with the amplifier disabled. Despite this lowered call rate, the conclusion drawn as a result of this experiment are still sound: the bats still emitted more calls when the noise was active than in the silent downtime between bouts of noise. Additionally, while the applied t-tests failed to find a significant effect of stimulus on call rate, as opposed to all the other experiments described here, the alpha values for these t-tests were much lower than the desired power of 0.800 (individual condition: $\alpha = 0.276$; group condition $\alpha = 0.050$). This failure to meet the desired power indicates that these t-tests were likely to overlook a difference between the call rates in noise and silence, even if one existed. Thus, one should be careful before declaring that neither the silence nor noise periods of the tested stimuli were capable of altering call rate.

Overall, these experiments demonstrate that Mexican free-tailed bats possess two distinct responses to the presence of interfering noise. When confronted with periodic noise, smaller groups of conspecifics, they can alter the timing of emission of their echolocation calls in order to reduce or avoid overlap. This behavior would be beneficial to animals roosting or flying in small groups, allowing them to alternate calling and

share the same acoustic space. When the interfering noise is continuous, however, bats can simply call more often. This strategy would perhaps best be of use in very large, dense colonies, where there are little to no periods of silence available for the bats to utilize, and antiphonal calling is no longer an option. While this behavior may not be ideal, at least it would allow the bats to gather some information about the surrounding environment, with the larger number of calls compensating for the signal degradation that the overlap with noise would produce. In addition, these techniques could also be combined with or substituted by spectral alterations of echolocation calls, as is seen with the Lombard effect (Penna et al 2005; Gilliam & McCracken 2007; Egnor et al 2006; Brumm 2006) or the jamming avoidance response (Gillam et al., 2007; Ulanovsky et al., 2004).These experiments further demonstrate that bats have a large, flexible repertoire of potential responses to noise, all of which should allow them to continue using their sophisticated echolocation systems even in very large, very dense colonies, which are the hallmark of many social bat species.

CHAPTER VI

CONCLUSIONS

Acoustic interference and signal degradation have played an important role in shaping animal communication. A wide variety of species rely upon acoustic signals to locate conspecifics, establish territories, search for food, warn of incoming predators, and attract mates. Overlap with interfering biotic or abiotic noise can reduce the sound's signal-to-noise-ratio, decreasing its active space or broadcast area and making it harder for the intended receiver to identify the signal (Brumm and Slabbekoom, 2005). As a result of this degradation due to overlap with masking noise, clear acoustic channels of communication are a valuable commodity, one that can be quickly used up in dense communities of animals (Planque and Slabbekoorn, 2008). As a result, it is vital to the continued survival of such animals that they find methods to reduce or avoid overlap with both con- and heterospecifics' own signals, while getting their own emissions across as clearly and consistently as possible (Brumm and Slabbekoom, 2005). For few species is this acoustic conflict more pressing than with bats, which rely upon their acoustic signals not only for communication, but in order to actively sense their environment. Bats who cannot properly detect their own returning echo pulses would be unable to make accurate judgments regarding the locations of prey or obstacles in their environment (Masters and Raver, 1996; Neuweiler, 2000). Many species of bats are also highly social, roosting in colonies of thousands or even millions of individuals (Ratcliffe et al., 2004; Simmons et al., 1978). During evening emergence flights, each of these

individual bats exit the roost in preparation for the night's foraging. The complex acoustic environments that bats face during such flights, as well as in the roosts, led me to ask the primary question of this research project: how are bats capable of echolocating in such large groups?

Previous research (Bates et al., 2008; Gillam and McCracken, 2007; Gillam et al., 2007; Ulanovsky et al., 2004) into mechanisms through which bats could avoid overlap or reduce signal degradation in the presence of interfering noise focused on spectral or amplitude changes in which bats alter the frequency, bandwidth, or amplitude of their calls. While these methods might be effective in pairs or small groups, none of the currently described mechanisms alone provided an adequate explanation for bats' ability to function in high-density populations. Altering the timing of emission of echolocation calls could provide bats alternate solutions to the problem of acoustic overlap, providing another tool in the bats' arsenal of potential solutions to the presence of noise. Prior studies had established that other animals, including birds (Brumm, 2006; Ficken and Ficken, 1974; Knapton, 1987; Planque and Slabbekoorn, 2008), frogs (Moore et al., 1989; Zelick and Narins, 1985) and primates (Egnor et al., 2007; Versace et al., 2008) alter the timing of emission of their acoustic signals in response to interfering noise. However, no similar studies in bats had been conducted. Echolocation is unique when compared to the acoustic signals examined in these previously studied lter speech timing in humans, as atypical antipsychotics which affect both the D2 and receptors can lead to improved speech fluency in those suffering from speech disorders

Failure to avoid overlap with interfering noise could lead to missed prey captures, collusions with obstacles, and an overall failure to function effectively within the environment. Possibly as a consequence of this pressing need, bats' possess fine control over the temporal properties of their echolocation pulses, making them an ideal model animal for investigating control of vocal timing in response to noise. In this dissertation, I have characterized two novel mechanisms in which Mexican free-tailed bats alter the timing of emission of their echolocation calls in response to noise.

The first of these novel behavioral mechanisms may be observed in their response to intermittent interfering noises, particularly those resulting from echolocating conspecifics. My research is the first study to demonstrate that in response to intermittent interfering noise, bats alter the timing of emission of their echolocation pulses. This behavior is characterized by an initial period of suppression followed by period of recovery, leading to echolocation pulses occurring later within the silent interval. The suppressive period appears to be involuntary, and its' length can be extended through exposure to subsequent, longer, or more complex acoustic stimuli including paired pulses and simulated feeding buzzes. While the length of the suppressive phase can be extended, it cannot be prolonged indefinitely, as bats will resume echolocating even in continuous noise arising from larger groups. Persistent intermittent noise will ultimately decrease call rate, as result of suppression of pulse emission.

There are two potential ways in which this suppressive effect could benefit bats' ability to echolocate in groups. The first such possibility is that these periods of suppression would encourage antiphonal calling in groups of bats. The first animal would emit a pulse, suppressing echolocation in nearby conspecifics, before undergoing a natural refractory period as a result of their own respiratory cycle. During this refractory period, a neighboring bat, newly recovered from its own period of suppression, would be able to emit its own call. The alteration of suppression and recovery would allow bats to call asynchronously, alternating calls and reducing echolocation pulse overlap. While this mechanism alone may not fully be able to account for the ability of bats to echolocate in very large groups, antiphonal calling in combination with other spectral, acoustic, or temporal alterations could explain the ability of bats to echolocate even in very noisy areas. Antiphonal or asynchronous calling has previously been observed in frogs (Moore et al., 1989; Zelick and Narins, 1985) and birds (Brumm, 2006; Ficken and Ficken, 1974; Popp et al., 1985), animals that also often call in large groups, supporting the viability of antiphonal calling for reducing or avoiding call overlap. If this hypothesis were true, one would expect to see a lower rate of call overlap than would otherwise be anticipated from two bats echolocating together.

Another potential explanation for how the suppressive phase may improve the ability of bats to echolocate in noise is that it could provide some benefit to sonar efficiency. The Monte Carlo simulations discussed in Chapter V were undertaken as an

attempt to determine if real pairs of bats calling together overlapped their calls less than would be predicted by examining pairs of simulated bats. While these simulations demonstrated that real pairs of bats do produce significantly less overlaps than would be expected via simulations, they were unable to demonstrate that this decrease in pulse overlap happened regardless of call rate, as could be expected of antiphonal calling strategies. That is to say, the bats were not actively avoiding pulse overlap with one another. Instead, these simulations demonstrated that the decreased incidence of pulse overlaps is strictly due to decreased call rates in the presence of other bats. The lower the call rate, the lower the probability of pulse overlap occurring, and the fewer pulse overlaps are seen. Thus, the Monte Carlo simulations support the hypothesis that the suppressive period benefits bats' ability to echolocate in noise by reducing call rates, and thus increasing pulse efficiency. Decreasing the overall call rate of each conspecific sharing the same acoustic space increases the overall efficiency of each bats' echolocation by reducing the number of pulse overlaps and thus optimizing information throughput for all the animals sharing the acoustic channel. This technique is similar to the one developed to handle information packet overlaps in the ALOHA system, where overlapping packets of information undergo a random period of delay before attempting retransmission (Abramson, 1970). This delay period, while slowing the overall rate of transmission, decreases the probability of packet overlap, allowing a number of users to use the system simultaneously, and without needing to consciously coordinate their

transmission times. This method thus provides a fast and efficient means of avoiding call overlap, and improving echolocation precision in crowded environments.

In the presence of constant noise, such as that caused by abiotic environmental factors or high levels of noise as may be found in a day roost, altering the timing of emission of echolocation pulses would no longer allow the bats reduce or avoid overlap with interfering noise. The prior behavioral mechanism requires that there be some silent intervals for bats to place their calls within. My research into the bats' vocal timing behavior, as described in Chapter III, determined that while the length of the suppressive period could be increased by more complex stimuli, calling could not be postponed indefinitely. This led me to hypothesize that bats must possess another mechanism for coping with the presence of constant interfering noise. My experiments have demonstrated that in the presence of continuous noise, bats increase their call rate, undergoing an excitation of calling rather than suppression. Other species have been shown to increase their call rate in response to noise, primarily birds (Lengagne et al., 1999; Potash, 1972) and frogs (Penna et al., 2005), but this is the first time that such a behavior has been demonstrated in bats. While the inevitable overlap with the interfering noise may degrade the signal quality of the individual echoes, the increased number of pulses being generated may assist the bats in one of two ways. Firstly, the increased number of calls being generated may increase the probability that the echoes will be detected against the background noise, or that one of the calls will fall into an unpredictable moment of quiet (Lengagne et al., 1999; Potash, 1972). Another

possibility is that while the individual echoes themselves may suffer from signal degradation, bats can use cognitive mechanisms to integrate the auditory information from the many sequential returning echoes to form a better picture of the surrounding environment. There is some evidence from the human auditory system that only a portion of the spectral structure of a sound needs to be detected through masking noise to be intelligible (McDermott and Oxenham, 2008). Bats are known to be capable of using information gleaned from trains of separate returning echoes in order to form an accurate picture of their surrounding environment (Moss and Surlykke, 2001; Schnitzler et al., 2003).

In addition to characterizing both of these behaviors, I was also able to determine at what ratio of noise-to-silence Mexican free-tailed bats switch from the suppression of calling to excitation of calling. A stimulus duty cycle of 50% or greater prompts bats to switch between the responses to intermittent noise and continuous noise. This indicates that when at least half of the available time window in which calling is possible is occupied by noise, bats may no longer be able to reliably call within silent periods, or that decreasing their call rate in response to interfering noise is no longer providing any benefit or increase to information throughput. Under these circumstances, the bats' consider any interfering stimulus with a duty cycle of 50% or greater as "continuous" noise, and will adjust their call rate accordingly. Finally, I was able to establish the effect of stimulus amplitude upon the strength of the bats' response to noise. Both the suppression period and the recovery period decrease in response in quieter stimuli: at or

below 48 dB, the animal's behavior in noise is statistically indistinguishable from their behavior in silence. This would have ramifications for the ability of one bat's echolocation pulses to interfere with their conspecifics' calls:, a bat would need to be within 30 meters of a conspecific for their echolocation pulses to interfere with one another's echolocation, assuming a temperature of 25° degrees Celsius and a humidity of 50% (Lawrence and Simmons, 1982) . In the roost, where many bats perch packed close together, echolocation calls would be of high amplitude and thus extremely disruptive. However, while out in the field and foraging the amplitude of the emitted pulses would attenuate as it passed through the atmosphere, meaning that bats echolocating further away would be less disruptive to their conspecifics.

Future projects investigating bats' abilities to alter the timing of emission of their echolocation calls to avoid overlap with conspecifics should focus on the ability of flying bats to utilize these behavioral mechanisms. My research project focused entirely upon temporal alterations as a method of overlap avoidance in stationary bats. It remains to be seen whether or not the same or similar behaviors would be observed in flying bats, which may face a different set of complications. Once Mexican free-tailed bats have emerged from the roost, they tend to fly to their own individual territories, where they spend the night foraging. Once in the field, they should no longer face the extreme acoustic competition that they must cope with during emergence flights and in the roost. This may mean that the mechanism of temporal alteration described in this project may not be as beneficial to bats in flight as it is in stationary bats. On the other hand, foraging

bats can and do encounter conspecifics from neighboring territories (Obrist, 1995) while hunting. Bats in flight may also face increased pressure to echolocate precisely, as they need to be able to track prey and avoid environmental obstacles in their environment. For these reasons, it is likely that flying bats will perform the temporal avoidance behaviors while in flight, just as flying bats perform JAR and alter the amplitude of calls (Tressler and Smotherman, 2009; Ulanovsky et al., 2004). Future research into temporal alterations as a means of avoiding overlap with noise should investigate the behaviors in flying bats facing navigational challenges. These studies could easily be performed within a laboratory setting and would provide a valuable look into the problems facing, and the solutions available to, free flying bats.

One of the original goals of this project was to identify and describe the brain networks that regulated vocal timing in the bat. Using the vocal timing behavior seen in response to intermittent noise and described in Chapter III as a model system, I set out to determine which monoaminergic drugs could alter the timing of the bats' behavior, and from there, which regions of the brain were likely to be involved in the regulation of echolocation. As previously stated, much of the research surrounding the timing of human speech has ties to interval timing literature. Thus, my first goal was to determine whether or not my vocal timing behavior could be classified as an example of interval timing. I tested two prominent criteria of interval timing behaviors: a scalar nature and sensitivity to D2 antagonists, especially haloperidol. I was able to determine that my vocal timing behavior did have scalar properties. As the length of the silent interval

increased, so did the length of the "window of recovery" (see Chapter IV, Figure 1). Essentially, bats working within shorter durations were more precise in the placement of their calls, or made fewer errors, than bats who called within longer silent periods. The confirmation that my vocal timing behavior was scalar in nature supported the hypothesis that it could be described as an example of interval timing. However, the vocal timing behavior was not responsive to the D2 antagonist haloperidol, regardless of the dosage tested. Thus, I was unable to demonstrate that the bats' vocal timing behavior is an example of interval timing.

Further research into the interval timing literature has led to a possible alternate hypothesis. The brain is hypothesized to keep track of numerous timescales, each of which may have their own neural mechanisms (Buhusi and Meck, 2005; Mauk and Buonomano, 2004). A prominent example of these alternate timescales is circadian timing. Circadian timing deals with intervals ranging from days to months and relies upon the suprachiasmatic nuclei to act as a clock (Buhusi and Meck, 2005). Some researchers have posited the existence of another timescale for durations smaller than the seconds to minutes range, millisecond timing (Buhusi and Meck, 2005; Mauk and Buonomano, 2004). In comparison to interval timing, millisecond timing is poorly understood, though some researchers have proposed that it plays a role in the regulation of human speech as well as basic motor control (Buhusi and Meck, 2005; Mauk and Buonomano, 2004). The only region of the brain that is currently hypothesized to play a role in millisecond timing is the cerebellum, which may play a role in both sensory and

motor timing (Balsam et al., 2009; Buhusi and Meck, 2005; Ivry and Spencer, 2004; Mauk and Buonomano, 2004). Lesions to the cerebellum can lead to deficits in precise timing (Ivry and Spencer, 2004). If the bats' vocal timing behavior is an example of millisecond rather than interval timing, this could explain the failure of the dopaminergic and serotonergic drugs to shift the timing of emission of the bats' pulses. Drugs that may strongly affect the neural circuitry that regulates interval timing may have no effect upon the structures which regulate millisecond timing (Mauk and Buonomano, 2004). Some studies have demonstrated that examples of millisecond timing are not vulnerable to alteration from dopaminergic antagonists (Mauk and Buonomano, 2004). Furthermore, while considered to be important for interval timing, the basal ganglia may play no role in millisecond timing behaviors (Mauk and Buonomano, 2004). Future studies examining the neural control of echolocation in the bat would do well to attempt cerebral lesions or inactivation studies, and determine what effect, if any, results upon the bats' ability to regulate the timing of their echolocation pulses. These studies would be difficult due to the cerebellum's importance to motor control in general, but the rewards may be worth the risks.

Regardless of whether or not the vocal timing behavior could be described as an example of interval timing, the second goal of the psychopharmacology experiments remained the same: to determine which monoamines might play a role in regulating vocal motor timing of echolocation pulses. Both dopamine and serotonin have been implicated as monoamines which could potentially alter speech timing in humans, as

atypical antipsychotics which affect both the D2 and 5HT2A receptors can lead to improved speech fluency in those suffering from speech disorders (Maguire et al., 2000; Salome et al., 2000). My hypothesis, based upon preliminary tests and a review of speech timing literature, was that serotonergic drugs that had an affinity for the 5HT2A receptor could alter the timing of emission of the bats' echolocation pulses. As the preliminary experiments and many human speech studies strongly implicate a role of dopaminergic drugs acting via the D2 receptor for vocal timing, I felt it was necessary to first rule out the possibility that it was the dopaminergic component of the atypical antipsychotics that produced the visible phase lag in our preliminary experiments. The three tested dosages of haloperidol (including the highest dosage, 10 mg/kg) failed to elicit any alteration of timing. For this reason, I can confidently state that D2-affecting drugs have no impact upon a bats' vocal timing ability. The tested serotonergic drugs, ketanserin and 2, 5-dimethoxy-4-iodoamphetamine (DOI), produced more ambiguous results. The only statistically significant effect of these drugs upon the vocal timing behavior was a phase lead resulting from injections of a one mg/kg dosage of the serotonin 5HT2A agonist DOI. While later trials with DOI failed to reach statistical significance, I believe it would be a mistake to simply dismiss the results of the first set of DOI trials and the earliest preliminary experiments. Further testing with these and other serotonergic drugs could still yield valuable results. For this reason, I believe future experiments regarding the control of timing of echolocation pulses should focus upon serotonergic antagonists and agonists, rather than dopaminergic drugs. Here I will

outline a potential mechanism through which serotonin could regulate vocal timing, a hypothesized pathway governing the control of echolocation, and discuss potential sources of error and provide alternative hypotheses.

In his book on psychopharmacology (Stahl, 2008), Stahl describes two mechanisms through which serotonergic drugs with an affinity for the 5HT2A receptor could modulate vocal timing. In the first of these mechanisms, serotonin released from 5HT neurons bind to receptors on GABAergic interneurons, causing them to inhibit their associated dopaminergic neurons and decreasing the release of dopamine. As previously stated, dopamine is commonly thought to play a role in models of speech timing, especially in those models that describe human speech timing as a form of interval timing (Schirmer, 2004). These models propose that the substantia nigra pars compacta regulates the activity of the striatum though the release of dopamine (Meck, 1996). If the dopaminergic cells in the substantia nigra pars compacta can have their activity altered through exposure to serotonergic drugs, this would provide an explanation for the efficacy of atypical antipsychotics in treating speech disorders while still maintain ties to interval timing. The second mechanism through which serotonergic drugs could modulate vocal timing involves serotonergic interactions with cortical pyramidal neurons. In this model, serotonin released from serotonergic cells in the dorsal raphe nucleus binds to 5HT2A receptors on pyramidal glutamatergic neurons, triggering the release of glutamate which will excite cells further downstream (Stahl, 2008). A 5HT2A antagonist would bind to the receptor and prevent the release of glutamate, retarding

excitation, while the agonists would trigger further excitation. This latter model is attractive for a number of reasons. Pyramidal cells are abundant in the prefrontal cortex; a region which contains oscillating cells which other researchers have suggested may serve as the internal pacemaker or clock which governs time-sensitive motor and vocal responses (Buhusi and Meck, 2005; Meck et al., 2008). The prefrontal cortex has also been recognized as being important for speech timing, with cortical damage resulting in timing defects (Schirmer, 2004). Increasing the firing rate of these pacemaker cells would speed up the internal clock and thus the animal's perception of the passage of time, leading to behavioral responses that occur earlier.

These mechanisms fit in with one of the two major pathways currently hypothesized to govern the neural control of voluntary vocalizations (Jurgens, 2009). Echolocation is both an innate behavior, and one that is under voluntary control of the bat, and would thus fit into the limbic cinguluo-periaqueductal pathway described by Jürgens (Jurgens, 2009). This pathway begins with the anterior cingulate cortex, which is thought to be responsible for the voluntary initialization of vocalization. The ACC has connections to three major regions which place it in a unique position to voluntarily initiate calling. First, there are projections to the both the motor cortex and spinal cord, and the ACC itself possesses two distinct motor regions (Paus, 2001). The ACC also has reciprocal connections to the prefrontal cortex, giving it access to higher-level executive functions and cognition (Paus, 2001). Finally, the ACC receives projections from the midline thalamus and brain stem regions, which govern motivation (Paus, 2001). These

three connections enable the animal to first have the drive to call, then the will to initiate the call, and finally the proper connections to produce the vocal signals themselves (Paus, 2001). Further supporting the importance of the ACC to echolocation are brain stimulation studies in which electrical stimulation of the anterior cingulate cortex in bats generated echolocation calls in the mustached bat (Gooler and O'Neill, 1987). This area also receives serotonergic input from the dorsal raphe nuclei, which ties in with idea that serotonergic mechanisms, and not dopaminergic, play a role in control of vocal motor timing (Paus, 2001; Porrino and Goldmanrakic, 1982). The ACC also connects to the next structure which is thought to play a role in vocal timing, the periaqueductal grey.

Lesion and pharmacology studies have demonstrated that the PAG, when stimulated, produce natural vocalizations in all mammalian species thus tested (Jurgens, 2009). Lesions of this area result in mutism, though other motor behaviors are undamaged (Jurgens, 2009; Jürgens and Lu, 1993). Neural recordings from the PAG indicate that it most likely serves a gating function, and is responsible for the initiation of vocalizations, but not their patterning (Jurgens, 2009; Jürgens and Lu, 1993). The patterning of calls would thus have to come from the ACC, supporting the idea that it is the beginning of this vocal motor pathway. The PAG itself then projects to the reticular formation, which possesses direct connections with all the phonatory motor nuclei (Jurgens, 2009) which will ultimately produce the final echolocation call. Surgical studies have determined that lesions to the ACC, PAG, and reticular formation can either lead to deterioration of vocalizations, or abolish them entirely (Jurgens, 2009). In

addition, electrical and pharmacological stimulation of these three regions leads to the production of natural vocalization sounds (Gooler and O'Neill, 1987; Jurgens, 2009). For all these reasons, I feel that this pathway is indeed behind the control of echolocation in bats, and that it is serotonergic input from the dorsal raphae nucleus that alters vocal timing at the level of the anterior cingulate cortex.

The primary difficulty that I faced within this psychopharmacology study is that little to no information on drug responsivity exists within bats. When determining correct dosage, it was necessary to turn to studies done in rodents, as their body weight and brain physiology should be similar to that found in my model organisms. It is entirely possible, however, that the tested dosages were simply too low or too high to elicit a shift in vocal timing. Too low of a dose, and the bats' calling may be entirely unaffected. Too high of a dose, and the bat may cease calling altogether, making them useless for the purposes of this study. Another major unknown factor that impacted this study was the time course of the drugs' effects on the bats' behavior. Recording sessions that were too long or too short would not give accurate results, and it was partly as an attempt to correct for this that the second set of 20 minute DOI trials was conducted. However, there was no way to accurately gauge whether or not the bat was reacting to the drugs, as test subjects showed little to no outward symptoms of drug exposure, not even to the antipsychotics which are known to result in severe side effects (Stahl, 2008). Future studies focusing on psychopharmacology and its effects on echolocation should take these factors into consideration. A great deal of trial and error will be needed in

order to determine the correct parameters to use in these studies, but the high cost of drug trials prohibited further exploration of these issues within the study at hand.

In this dissertation I have established and characterized two novel behaviors in which bats alter the timing of emission of their echolocation pulses in response to the presence of interfering noise. In intermittent noise, bats decrease their call rate as a result of pulse suppression, resulting in a lower probability of pulse overlap with conspecifics and thus increased pulse signal efficiency. In constant noise, bats increase their call rate, either increasing the odds of a pulse falling within an unpredictable silent period or allowing them to integrate information from an increased number of degraded echolocation pulses. I have also established that bats switch between these two behavioral responses when the interfering noise has a duty cycle of 50% or higher. These temporal alteration behaviors, in addition to the previously established spectral and acoustic alterations previously recorded in bats such as the jamming avoidance response and the Lombard effect, provides an explanation for how bats continue to echolocate effectively in even very large, noisy groups.

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