Title: Sex-dependent Hemispheric Asymmetries for Processing Frequency Modulated Sounds in the Primary Auditory Cortex of the Mustached Bat

Authors: Stuart D. Washington1 and Jagmeet S. Kanwal1,2

Departments of 1Physiology and Biophysics, and 2Neurology
Georgetown University Medical Center
Washington, DC 20007

Abbreviated Title: Cortical asymmetry in FM processing

Number of Pages: 52
Number of Figures: 13

Corresponding Author Address: Jagmeet S. Kanwal
Room WP09, The Research Building
Georgetown University Medical Center
3900 Reservoir Rd, NW
Washington, DC 20007
Phone: (202) 687-1305
Email: kanwalj@georgetown.edu
Species-specific vocalizations of mammals, including humans, contain slow and fast frequency modulations (FMs) as well as tone and noise bursts. Here we establish sex-specific hemispheric differences in the tonal and FM response characteristics of neurons in the Doppler-shifted constant frequency processing area in the mustached bat’s primary auditory cortex (A1). We recorded single unit cortical activity from the right and left A1 in awake bats in response to the presentation of tone bursts and linear FM sweeps that are contained within their echolocation and/or communication sounds. Peak response latencies to neurons’ preferred or best FMs were significantly longer on the right compared to the left in both sexes, and in males this right-left difference was also present for the most excitatory tone burst. Based on peak response magnitudes, right hemispheric A1 neurons preferred low-rate, narrowband FMs, whereas those on the left were less selective, responding to FMs with a variety of rates and bandwidths. The distributions of parameters for best FMs in females were similar on the two sides. Together, our data provide the first strong support of a sex-specific, spectrotemporal hemispheric asymmetry for the representation of tones and FMs in a non-human mammal. Specifically, our results demonstrate a left hemispheric bias in males for the representation of a diverse array of FMs differing in rate and bandwidth. We propose that these asymmetries underlie lateralized processing of communication sounds, and are common to species as divergent as bats and humans.
INTRODUCTION

A left hemispheric specialization for processing of speech sounds is a defining characteristic of the human brain (Geschwind and Levitsky 1968). Studies of brain damaged (Divenyi and Robinson 1989), learning-impaired (Merzenich et al. 1996; Nagarajan et al. 1999; Tallal et al. 1993; Temple et al. 2000), and healthy passive listeners (Belin et al. 1998; Makela et al. 2005; Schwartz and Tallal 1980) demonstrate high temporal precision of the left auditory cortex (AC) that enables it to process speech sounds that contain rapid formant transitions similar to linear frequency modulated (FM) sweeps (Jamison et al. 2006; Zatorre and Belin 2001). The low temporal precision of the right AC matches its inability to follow fast spectral changes that span a large frequency range within a given time window (Poeppel 2003; Zatorre et al. 2002). This low temporal precision does not, however, constrain the right AC from conducting a refined analysis of small fluctuations in average frequency that may be present in stimuli sampled over relatively long time intervals. This refined spectral precision is presented as an explanation for why the right AC can process steady-state signals, such as vowel sounds, just as well as the left AC and why it is specialized for processing speech-related prosodic variation and voice identity (Robinson and Fallside 1991) as well as musical sounds (Poeppel 2003). This asymmetry tends to be greater in the auditory cortices of human males than in females (Dawe and Corballis 1986; Lansdell 1964; McGlone 1977; Shaywitz et al. 1995).

Neurophysiological studies of the mustached bat (*Pteronotus parnellii*) have revealed that its AC contains functional maps for processing its stereotypic echolocation signals (O'Neill and Suga 1979; Suga and Jen 1976). Multifunctional neurons within its AC respond to constant frequencies (CFs) and FMs that are present not only in echolocation signals, but also in the rich variety of communication sounds (or social calls) emitted by this species (Esser et al. 1997; Kanwal et al. 1999; Kanwal et al. 1994b; Washington and Kanwal 2008).
CFs and FMs in social calls of bats and other mammalian species are acoustically analogous to formants and formant transitions in human speech (Kanwal and Rauschecker 2007; Liberman et al. 1956; Rauschecker and Scott 2009; Suga 1992). Therefore, understanding the auditory processing of combinations of FMs and CFs can provide important clues about common mechanisms that direct hemispheric specializations for processing spectrotemporal information within communication sounds of bats analogous to that of phonemes, the spectrotemporal unit of speech in humans. Furthermore, in the midst of emitting and listening to the echoes of their own biosonar or echolocation pulses, bats may encounter social vocalizations of others in the colony. To simultaneously process, perceive and respond to both types of sounds that occasionally overlap in frequency can be challenging. Here again, an understanding of the mechanisms underlying lateralization of auditory processing can provide important insights into a neural solution to this problem.

We recorded single neuron responses to combinations of CFs and FMs in the right and left primary auditory cortex (A1) of mustached bats with the intent of investigating hemispheric differences in the representation of CFs as well as multiple FM parameters, such as rate (slope) and bandwidth, co-varied with duration and the central frequency of FMs. All recordings were obtained from the Doppler-shifted CF processing (DSCF) area, which encompasses nearly half of A1 in this species (Kanwal 1999; Suga and Jen 1976; Xiao and Suga 2002) and has been shown to process both CFs (Kanwal et al. 1999) and FMs (Washington and Kanwal 2008). We used both male and female bats to test for hemispheric differences within and between sexes. Our results demonstrate sex-specific hemispheric asymmetries for processing FMs and include a hemispheric difference in the response latencies to CFs.
MATERIALS AND METHODS

Surgery and Electrophysiological Recordings

All methods were approved by the Georgetown University Animal Care and Use Committee. Surgical and recording methods were previously described (Kanwal et al. 1999; Medvedev and Kanwal 2004; Washington and Kanwal 2008). We used 10 (6 male and 4 female) wild-caught mustached bats. Animals were anesthetized with an initial dose of 0.5 to 1% isoflurane/oxygen mixture (medical grade, Anaquest) followed by a continuous stream of ~0.25% isoflurane. A skin incision was made along the midline of the head of each bat, and a 2-mm-diameter metal post was glued immediately caudal to the intersection of the sagittal and coronal sutures. Each bat was allowed >3 days recovery prior to initiation of electrophysiological recordings. During recordings, each bat was restrained by clamping the metal post, and the body was suspended in a Styrofoam mold by elastic bands in a heated (31°C), sound-proof, and echo-attenuated chamber (IAC 400A). Electrophysiological recordings were made from the AC at a depth of 300-650 μm using custom-made, sharpened, vinyl-coated tungsten-microelectrodes (>1 MΩ). A second microelectrode (< 1 MΩ) was placed onto the dura mater of a non-auditory region of cortex as a reference for differential recording. Electrical signals acquired by the recording electrode were amplified and band-pass filtered between 300 and 3000 Hz.

Successful neural recordings presented here were obtained from a total of 129 penetrations. Of these, thirty-eight penetrations were from the left hemispheres and 26 penetrations from the right hemispheres of male mustached bats. Thirty-nine penetrations were from the left and 26 were from the right hemispheres of female mustached bats. Sampling bias in single-unit recordings may result from differences in electrode location, impedance and tip diameter, which select for a particular size range of the cell body,
dendritic morphology, and spiking properties of neurons (Stone 1973). To minimize the possibility of a sampling bias when making R-L comparisons, we used similar electrodes for recording from the right and left sides. In 4/6 males and 3/4 females, we recorded from both sides of the same animal. We recorded only from the left-hemisphere in a male and in a female, and only from the right-hemisphere in another male. Response characteristics from each side of these animals were comparable to those from the same side and sex of other animals.

Acoustic stimuli

We used constant frequency tone-bursts (or simply CFs) and frequency modulations (FMs) to study response characteristics of DSCF neurons in the mustached bat A1. CFs were generated using custom-made analog function generators. A customized SIGNAL 3.0 script (Engineering Design) was used to generate FMs (Washington and Kanwal 2008). All CFs were 30 ms in duration, including 0.5 ms rise and fall times of amplitude. FMs ranged in duration between 0.4 and 213 ms and were tapered only if their duration exceeded 2 ms. We presented CFs from two custom-made condenser loudspeakers that were flat (± 6 dB SPL) from 20 to 120 kHz. We presented FMs from a leaf-tweeter speaker (Panasonic, Inc.) that was flat (± 3 dB SPL) from 5 to 100 kHz, which did not generate a significantly wideband click at DSCF neurons’ preferred stimulus amplitudes (< 80 dB SPL). Moreover, DSCF neurons are tuned to a narrow range of frequencies and can discriminate between 2 ms long FMs presented in the upward versus downward direction (see data supplement, Washington and Kanwal 2008).

Constant Frequencies: We first determined which frequencies elicited peak responses from a neuron in order (1) to determine if it was a DSCF neuron and (2) to establish a frequency on which to (initially) center FMs. We classified a neuron as a “DSCF neuron” if it
had a peak response to a CF between 57 and 61 kHz (best high frequency, or BF\textsubscript{high}) and this response was facilitated when paired with a simultaneously presented CF between 23 and 28 kHz (best low frequency, or BF\textsubscript{low}). The neural responses to CFs were elicited by CFs (BF\textsubscript{low}+ BF\textsubscript{high}) paired at onset and presented 200 times at best amplitude (BA) for excitation. Neurons generally showed only a small response to BF\textsubscript{low} alone and the criteria for facilitation were the same as previously described (Fitzpatrick et al. 1993; Kanwal et al. 1999). Pairs of CFs in the 23-28 kHz and 57-61 kHz ranges were presented to facilitate responses per the well-studied tuning properties of DSCF neurons (Kanwal et al., 1999). The frequencies and amplitudes of both CFs were adjusted in order to obtain the best frequencies in the 23-28 kHz and 57-61 kHz ranges at their best amplitudes of facilitation. Stimuli were tested at the resolution set by the step value for changing CF, FM and amplitude parameters, e.g., of FM rates or bandwidths within the respective arrays. Frequency adjustments to CFs in the BF\textsubscript{low} and BF\textsubscript{high} range were performed manually using custom-made sweep generator analog equipment with a 0.01 kHz resolution. Frequency tuning curves were obtained by changing the frequency of 30-ms CFs in six 0.25 kHz steps above and below the manually determined BF\textsubscript{high}. Each of the 14 CFs was presented 100 times and decreased in amplitude by 10 dB SPL per every 10 repetitions starting at 97 dB SPL. To obtain representative PSTHs, we recorded single neuron responses to 200 repetitions of best FMs and CFs at their best amplitudes. Examples of 3 single neurons showing facilitation to combinations of CFs (BF\textsubscript{low}+ BF\textsubscript{high}) as well as CFs and FMs (BF\textsubscript{low}+ best FM) are shown in figure 1. Due to potential amplitude-related latency shifts, right-left (R-L) comparisons of temporal response parameters are based on 200 repetitions of the best FMs and/or CFs at best amplitude for excitation (BA).

The term “best” as used here and in the previous literature (Kanwal et al. 1999; Suga et al. 1987; Suga et al. 1983) implies the value of a stimulus parameter at which the response
magnitude is the highest. Response magnitude is described as the total number of spikes in either a 5 or 10 ms window and is estimated by sliding this window in 1 ms steps from stimulus onset. Because of the phasic nature of onset responses in the DSCF area, this response measure is optimal and the most realistic given that this is a widely accepted integration window for postsynaptic neurons. Average firing rate computed over relatively long (>10 ms) response durations is less appropriate because of differences in duration between different stimuli, which ranged approximately from 2 to 200 ms. Response measures over 50 ms or longer time windows are likely to yield erroneous results because a majority of the FM rates tested had durations of under 10 ms. We recorded from neurons representing the same range of BFs from each side. Though the results reported here are derived from summary statistics of single neuron populations, none of the neurophysiological data described here are derived from multi-neuron recordings.

**Frequency Modulations:** FMs used in this study were linear modulations of frequency \( f \) in the 57-61 kHz range. Details of the procedure for studying FM response characteristics were reported elsewhere (Washington and Kanwal, 2008). All linear FMs are defined by four parameters: duration in ms \( \Delta t \), bandwidth in kHz \( \Delta f \), rate of modulation \( \Delta f / \Delta t \) in kHz/ms, and the central frequency of an FM in kHz. To determine the best FM parameters for each neuron, we created a sequence of FM stimuli (or an FM array) and varied one (target) FM parameter (e.g. rate) within a desired range and allowed a nontarget parameter (e.g. duration) to co-vary with the first, while other parameters were kept constant. Each FM array was presented 100 times and decreased in amplitude by 10 dB SPL per every 10 repetitions starting at 91 dB SPL. All stimuli in the FM arrays were paired at onset with a CF at BF\textsubscript{low} for facilitating the response and maximizing peak response magnitudes. Since we needed to determine the FM directional preference for each neuron, there were two types of FM arrays: one where all the FMs were upward and another where all the FMs were downward.
Experimental Design

We first created 14 FMs with rates increasing from 0.04 to 4.0 kHz/ms at a constant bandwidth of either 3.5 or 5.25 kHz to generate an array of different FM rates (Fig. 2). We call this an “FM rate-array”. The peak response magnitude elicited by FMs presented in the rate array indicated the “best FM rate” for a neuron. Keeping the FM rate constant, we then varied the bandwidths of 13 FMs either from either 0.44 to 5.25 kHz or 0.66 to 7.88 kHz to generate the FM bandwidth array. An initial bandwidth range was based on the narrow width of frequency tuning in DSCF neurons, which ranges from ~0.4 to ~5 kHz. Midway through data acquisition, we discovered that DSCF neurons continued to respond robustly to FMs with relatively broad bandwidths (~5.25 kHz) despite their relatively narrow tuning to CFs. To capture this wider range of tuning, we increased the FM bandwidth range to ~7.88 kHz and pooled the data for R-L comparisons. The rates of all FMs in the bandwidth array were set equal to the best FM rate of the neuron under study. Each sequence of FM stimuli (or FM array) was presented 100 times and decreased in amplitude by 10 dB SPL every 10 repetitions starting at 91 dB SPL. Peak response magnitudes to FMs in the bandwidth array allowed us to describe the “best FM bandwidth” at the “best FM rate” for a neuron. All stimuli in the FM rate and FM bandwidth arrays were centered on the BF$_{\text{high}}$ of the neuron under study. FM duration was allowed to co-vary for both of these target parameters.

Finally, we varied the FM central frequency to generate another FM array at best FM rate and best FM bandwidth that contained 13 FMs (with the middle one centered on BF$_{\text{high}}$) that were shifted in frequency by one half the best FM bandwidth of the neuron under study. The rates and bandwidths of all FMs in the “FM central frequency array” were, respectively, equal to the “best FM rate” and “best FM bandwidth” of the neuron under study. All other FM
parameters were kept constant. We used the FM central frequency array to determine where to initiate the best FM in the neuron's excitatory frequency response area. Here, we report only single neuron responses elicited by FMs modulated in a neuron's "best FM direction" as measured by peak response magnitude. An FM modulated at the "best FM rate," spanning the "best FM bandwidth," and centered on the "best FM central frequency" of a particular neuron is referred to as the "best FM" for that neuron.

The range of FM parameters to be tested in this study was carefully selected keeping various issues in mind. First and foremost, FM parameters were meant to be neuroethologically meaningful. Each range of parameters is meant to be meaningful in a species-specific manner. The upper limit of 4.0 kHz/ms for mustached bats represents the modulation rate of the downward FM in the second harmonic of their echolocation signal, which is comparable to the fastest rates encountered in some of the social calls. FMs centered on BF$_{high}$ that have bandwidths broader than 9 kHz can interact with other excitatory frequency ranges (see Kanwal et al 1999 and Kanwal 2006) making it difficult to make conclusive statements about response mechanisms.

Data Analysis

Responses to BF$_{low}$, BF$_{high}$, and best FM were recorded in the form of peri-stimulus time histograms (PSTHs). These histograms, calculated on-line by summation of spike trains over repeated trials, were used to measure the neuronal response that represents a stimulus-locked change in peak response magnitude and latency. Peak response magnitudes and latencies of DSCF neural responses to "best FMs" (final step in figure 2) described below were elicited by CFs and best FMs (BF$_{low}+$ best FM) paired at onset and presented 200 times at their best amplitude of excitation (BA).

Peak response magnitudes to CFs and FMs presented 200 times at BA were calculated
using 5 ms bin widths. Peak response magnitudes to FMs presented within arrays were calculated using 10 ms bin widths to eliminate small differences due to amplitude-related latency shifts. Peak responses to FMs in arrays presented at different amplitudes were collapsed across amplitudes to create single neuron “FM response magnitude curves” (Fig. 3A and 3B). A response magnitude curve, or simply a response curve, is a line plot for values corresponding to peak response magnitudes, expressed as a percentage of the maximum peak response value, to the stimulus being tested. This provided an amplitude-independent measure for comparing response curves, while still incorporating the effect of amplitude on the averaged response measure. In this manner, response curves of individual neurons as well as those representing an average of response curves for all neurons (shown for 2 neurons in figure 3C) on the left versus the right side (referred to as “population response curves” with standard error bars) could be compared and tested for significant differences using repeated measures analyses of variance (ANOVA). Due to a potential for amplitude-related latency shifts, R-L comparisons of temporal parameters in responses to FMs are based on the response to the best FM within the FM rate, bandwidth, or central frequency arrays.

We measured three types of temporal response parameters: peak response latency (time from stimulus onset to maximum firing rate), response-onset latency (time to first significant deviation 3 standard deviations above the level of ongoing background activity in at least 3 successive 1 ms bins from stimulus onset), and response duration (response-onset latency subtracted from the time of response offset). Peak response latencies were calculated using 1 ms bin widths. For response-onset latencies and response durations, however, we converted our spike train data into spike density functions using 3-ms wide Gaussian kernels and a 1 ms wide sliding window.

Response-onset latencies, peak response latencies and response durations for FMs
were only measured for the best FM at BA and only for tone pairs at BF_{low}+BF_{high} at BA. This avoids the confounding effects of amplitude-related latency shifts. Response latency to BF_{low} alone is typically much longer than that to BF_{high}, so that it does not have a significant impact on the much shorter response latencies either to BF_{high} or an FM traversing through the BF_{high} range (Kanwal et al. 1999). Accordingly, a presentation of this FM with the same onset as BF_{low} does not have a significant impact on response latency. Hence computing response latencies to the best stimulus makes comparisons of this response measure across neurons and between hemispheres viable and independent of specific stimulus parameters, such as FM amplitude, rate, bandwidth and duration.

Unlike the treatment of two ranges of FM bandwidth, FM rate arrays spanning a narrow range (e.g., 0.08 to 0.22 kHz/ms) were not pooled with FM rates spanning a relatively wider (0.04 to 4.0 kHz/ms) range. We used two-way ANOVAs to compare preferred FM parameters and temporal response properties between hemispheres and sexes. We used repeated-measures ANOVAs and multi-level (hierarchical linear) modeling to make similar comparisons between population response curve data.

**RESULTS**

Response measures and terminology

We recorded from a total of 326 DSCF neurons; 178 from the left hemisphere (LH) and 142 from the right hemisphere (RH) in 10 mustached bats. Of these, 158 (LH = 88, RH = 70) were from 6 male bats and 168 (LH = 91, RH = 77) from 4 female bats. *F*-statistics from ANOVAs and means (± std. err.) are only reported when statistically significant or pivotal in arriving at a conclusion.
Response asymmetry for CFs

To test for sex-dependent, hemispheric differences in response to FMs, we first determined the excitatory frequencies for the tone pair (BF_{low} and BF_{high}) for each of the 326 neurons. This enabled us to estimate a central frequency for synthesizing FMs as well as confirm the extent to which the hemispheric asymmetry was reflected in the responses to CFs and emergent properties that were unique to FMs. Since DSCF neurons are spectrally combination-sensitive, we used pairs of CFs (30 ms in duration) presented at the best frequencies (BF_{low}+BF_{high}) and at the best amplitudes (BAs) of each neuron to elicit maximal firing in each neuron. Neurons typically responded phasically at CF onset and activity reached baseline or spontaneous level within 20 to 50 ms.

The frequencies and amplitudes of CF stimuli were similar between hemispheres and sexes. In males, there were no significant hemispheric differences between the values of BF_{low} (LH: 25.36 kHz ± 0.10, n = 88; RH: 25.32 kHz ± 0.10, n = 70) or BF_{high} (LH: 59.21 kHz ± 0.07, n = 91; RH: 59.08 kHz ± 0.06, n = 77), and the same was true for BF_{low} (LH: 26.10 kHz ± 0.09; RH: 25.91 kHz ± 0.08) and BF_{high} (LH: 59.15 kHz ± 0.10; RH: 58.95 kHz ± 0.05) in females. BAs for BF_{low} in males (LH: 87.41 dB SPL ± 0.62; RH: 85.02 dB SPL ± 0.96) and females (LH: 82.17 dB SPL ± 0.76; RH: 83.64 dB SPL ± 1.06) were similar, as were BAs for BF_{high} in males (LH: 56.46 dB SPL ± 1.73; RH: 64.71 dB SPL ± 1.32) and females (LH: 57.70 dB SPL ± 1.27; RH: 64.80 dB SPL ± 0.66).

There were significant differences in temporal response parameters in males, but not in females. Peristimulus time histograms (PSTH) showing responses from 4 different neurons are shown in figure 4A. In males, the peak response latency for the left hemispheric example neuron was 10 ms and was 27 ms for the right hemispheric ones. The right and left hemispheric DSCF neuron shown for females had latencies comparable to that of the male
left DSCF neuron. As a population, average peak response latencies did not differ between
sexes, but were significantly longer for right versus left DSCF neurons (F[1, 322] = 7.08, p <
0.01) (Fig. 4B). Average peak response latencies were significantly longer in males than in
females (F[1, 322] = 9.87, p < 0.01), and longer on the right compared to the left (F[1, 322] =
5.79, p < 0.05). An interaction effect of hemisphere and sex showed that neurons on the
right side in males had significantly longer (and more variable) peak-response latencies than
those on the left, or compared to either side in females (F[1, 322] = 11.82, p < 0.01). A
similar sex-dependent asymmetry held for response-onset latencies (F[1, 322] = 8.73, p <
0.01). Specifically, in males, response-onset latencies on the right showed greater variance
than those on the left (σ²: RH = 26.0; LH = 16.2), whereas the response-onset latencies in
females were more similar between hemispheres (RH = 16.5; LH= 11.5).

The distribution of peak response latencies to CF-pairs on the right is wider than that on
the left in males and compared to either side in females. These data are shown as moving
average (kernel) plots in figure 4C. However, neither response duration nor peak response
magnitude showed hemispheric or sex differences. Overall, our data displayed significant
sex-dependent, hemispheric differences for response latencies to tone pairs. Neurons used
in each of the FM analyses below were also included in this analysis of neural responses to
tone pairs.

Selectivity for FM rate and bandwidth within individual neurons

We first present data on R-L differences in DSCF neuron selectivity for FM parameters
based on peak response magnitudes, and then examine the temporal characteristics of
neurons’ response patterns to their best FMs. Figure 5 shows raster and PSTH plots of a
sequence of single unit responses obtained for slow to fast FM rates from DSCF neurons in
the left (Fig. 5A-C) and right (Fig. 5D-5F) hemispheres. The rate of change of each FM was
fixed. Figure 6A shows examples of two types of response curves that are present in both the left and the right hemisphere but in different proportions (see Fig. 6B). One type of response curve (shown on the left in figure 6A), illustrates sharp tuning to a fast FM rate (0.34 kHz/ms), whereas the other type illustrates broad tuning to a relatively slow FM rate (1.87 kHz/ms).

We divided response curves into two groups according to their preferred FM. The boundary between these two groups was the average of best FM rates for all neurons, regardless of the side from which they were recorded or the sex of the animal. The percentage of best FM rates and best FM bandwidths below versus above the mean rate for each side in each sex are plotted as pie charts (Fig. 6B). In males, the percentage of slow versus fast rates was dissimilar between hemispheres, such that 54% of left DSCF neurons responded best to slow rates (<0.6 kHz/ms), but 87% of right DSCF neurons did the same. In females, the percentages of each group were relatively even with 60% of left and 67% of right DSCF neurons responding best to slow rates. The preferred or best FM bandwidths were divided into two groups as well, separated by the mean value of best bandwidths (4.5 kHz) obtained for all neurons. Percentages of these in each hemisphere in each sex are also shown as pie charts (fig. 6C). The percentage of best FM bandwidths below versus above the mean bandwidth in males was dissimilar between the two sides, such that 51% were narrowband (<4.5 kHz) in the left hemisphere and 66% were narrowband in the right. In females, the division of best FM bandwidths across the two sides was similar.

Population-level selectivity for FM rates:

We averaged response-curves of single neurons to create “population response-curves,” and compared these response-curves using repeated-measures ANOVAs and multi-level modeling. Values for peak response magnitudes declined more steeply with increasing FM
rate for the mean response on the right compared to the left for data averaged across males and females (Fig. 7A) and particularly in males (Fig. 7B). Overall, neurons on the left, particularly in males were generally more responsive to fast FM rates relative to those on the right (Figs. 7A and 7B). The distributions of response curves for both sides in females were similar to those on the right side of males (Fig 6C). Mean peak response magnitude was greatest to the slowest FM rate (0.04 kHz/ms) in both hemispheres, and declined monotonically with increases in FM rate ($F[13, 2379] = 63.83, p < 0.01$). The results showed a significant main effect of hemisphere ($F[13, 2379] = 7.33, p < 0.01$) but not sex ($F[13, 2379] = 3.19, p = 0.08$). An interaction effect between the factors of hemisphere, sex, and FM rate ($F[13, 2379] = 2.63, p = 0.01$) was also significant and provided statistical evidence of a sex-dependent, hemispheric asymmetry for FM rate preference. This provides quantitative evidence indicating a preference for relatively rapid rates in left hemispheric DSCF neurons compared to those on the right in males or compared to neurons in either hemisphere in females.

We also performed multi-level (hierarchical linear) modeling on the response curve data since this type of analysis is (1) more sensitive than the repeated-measures ANOVA and (2) unlikely to result in significance if the data were biased by one or two animals. Figure 7D – 7F is a set graphical representations of predicted outcomes of DSCF neural responses to increasing FM rate from 0.04 to 4.0 kHz/ms. Figure 7D shows the predicted percent of peak response to increasing FM rate for neurons from male and female bats (LH = 96; RH = 91), figure 7E shows this same relationship in male bats alone (LH = 46; RH = 45), and figure 7F shows this relationship in female bats alone (LH = 50; RH = 46). Table 1 shows the correlational coefficients and, in parentheses, the t-values associated with the outcomes predicted by the multi-level model. Specifically, the multi-level model shows that, over the total range (0.04-4.0 kHz/ms), there are significant main effects of FM rate, hemisphere, and
sex on peak response magnitude but not strong interaction effects between hemisphere and rate on peak response magnitude. Such an interaction effect does exist, however, within the range of 0.04-1.26 kHz/ms. Further, when separated by sex, males, but not females, show strong main effect of hemisphere on peak response magnitude. In the 0.04-1.26 kHz/ms range, males show an interaction effect between hemisphere and FM rate on peak response magnitude. This more refined analysis, like the repeated-measures ANOVAs above, showed that FM rate affected peak response magnitudes in significantly different ways in the two hemispheres in males, but not for females, were significantly affected (p < 0.05) by hemisphere's interactions with FM rate. Furthermore, this analysis shows that the strongest hemispheric differences in FM rate selectivity lie between 0.04 and 1.26 kHz/ms.

Population-level selectivity for FM bandwidth

Peak response magnitudes increased with FM bandwidth in both hemispheres in females, but in only on the left in males (Figs. 8A to 8C). Right DSCF neurons in males responded maximally to FMs between 1.75 and 3.06 kHz, whereas on the left they reach their maximum at or above 7.88 kHz. Conversely, on the left, neurons respond least to FMs <0.88 kHz, whereas on the right they show a steady decline in peak response magnitude for bandwidths >3.1 kHz. In females, left and right hemispheric DSCF neurons exhibit a pattern more similar to that on the left in males.

We used two repeated-measures ANOVAs (one per bandwidth range presented; 0.44-5.25 kHz and 0.66-7.88 kHz) to test whether the factors of hemisphere and/or sex impacted the shape of FM bandwidth population response curves. For each range, FM bandwidth had a significant (p < 0.01) main effect such that peak response magnitudes increased with FM bandwidth as previously reported (Washington and Kanwal 2008). We observed a significant interaction effect of FM bandwidth, hemisphere, and sex along both the 0.44-5.25 kHz (F[11,
1661] = 3.67, \( p < 0.01 \) and 0.66-7.88 kHz (\( F[11, 1232] = 3.06, p < 0.01 \)) ranges. This interaction effect indicates that there were significant hemispheric differences in the preference of DSCF neurons for FM bandwidths in males but not in females.

We also used multi-level modeling to analyze the response curves elicited by the 0.44-5.25 kHz and 0.66-7.88 kHz FM bandwidth arrays as a single data set. Figure 9D to 9F is a set of graphical representations of predicted outcomes of DSCF neural responses to increasing FM bandwidth from 0.4 to 7.9 kHz. Figure 8D shows the predicted percent of peak response to increasing FM bandwidth for neurons from male and female bats (LH = 150; RH = 104). Figure 8E shows this same relationship in male bats alone (LH = 74; RH = 45), and figure 6F shows this relationship in female bats alone (LH = 76; RH = 59). Table 2 shows the correlational coefficients and, in parentheses, the t-values associated with the outcomes predicted by this multi-level model. Specifically, when both sexes are taken together, there is a significant main effect of FM bandwidth and hemisphere on peak response magnitude but not an interaction effect of FM bandwidth and hemisphere over the entire 0.44-7.88 kHz range. For the broadest bandwidths (4.8-7.9 kHz), not only do FM bandwidth, hemisphere, and sex affect peak response magnitude, but hemisphere and bandwidth interact to affect peak response magnitude as well. In males, peak response magnitude is affected by an interaction between FM bandwidth and hemisphere over the entire 0.44-7.88 kHz range. Though such an interaction effect is not seen over the entire range in females, at the broadest bandwidths, peak response magnitude in females is affected not only by FM bandwidth and hemisphere but also by an interaction between these factors. This interaction effect of FM bandwidth and hemisphere suggests that, in females, right DSCF neurons prefer broader bandwidths than left DSCF neurons. Overall, this analysis, like the repeated-measures ANOVAs above, showed that peak response magnitudes were significantly affected by interaction between hemisphere and FM bandwidth.
such that neurons in the RH of males preferred narrower FM bandwidths than neurons in the LH of males or both hemispheres in females.

**Population-level selectivity for FM central frequency:**

We also performed analyses of population response-curves for selectivity of the central frequency in best FMs (data not shown). Though every neuron did not respond maximally when centered on BF$_{\text{high}}$, the central frequencies of best FMs were close enough to BF$_{\text{high}}$ in the vast majority (>80%) of DSCF neurons in both hemispheres and in both sexes so that the best FM always contained BF$_{\text{high}}$, even if it was not necessarily centered on it. Further, the bell-shaped population response-curves for FM central frequency peaked similarly at BF$_{\text{high}}$ in both hemispheres in males as well as females. Although changes in FM central frequency alone have a profound impact on the peak response magnitudes of DSCF neurons (Washington and Kanwal 2008), ANOVAs revealed that neither sex (F[1, 114] = 0.178, $p = 0.647$) nor hemisphere (F[1, 114] = 0.597, $p = 0.441$) had an effect on peak response magnitudes for values of FM central frequency. Further, no interaction of sex and hemisphere (F[1, 114] = 0.390, $p = 0.533$) or interaction between FM central frequency, sex, and hemisphere (F[1, 114] = 0.525, $p = 0.470$) had an effect on peak response magnitude.

**The duration parameter in best FMs**

Since FM duration co-varies with FM rate and bandwidth, we tested the effect of FM duration on peak response magnitudes. Figure 9A and 9B shows response curves in two neurons for FM rate and bandwidth, respectively. The tuning for both of these FM parameters is unimodal and that for FM rate is relatively sharp. The two neurons, however, do not show a systematic preference for the same FM duration. Rather the preferred duration shifts from $> 131.3$ ms to $65.6$ ms for the L-H neuron, and to $32.8$ ms for the R-H neuron when tested across different FM rates versus different FM bandwidths.
A scatterplot of shifts in best FM duration for those computed from FM bandwidth versus FM rate tuning for the population revealed no clear trends for either side or sex (Figs. 9C and 9D). Average shifts in best FM duration were the smallest in the RH of females (mean = -0.61) and the LH males (mean = -1.34). However, the spread (standard deviation) of shifts was nearly 5 times larger in the RH of females (53.59, n= 41) compared to the LH of males (11.61; n = 27). In the LH of females, the mean shift was 11.8 (std. dev. = 34.68, n = 36), and in the RH of males it was -33.22 (std. dev. = 38.94, n = 25). R-L differences were significant in males ($p = 0.0003$, two-tailed t-test, n = 77), but not in females ($p = 0.1133$, two-tailed t-test, n = 52). In males, the best duration computed from a presentation of different FM rates is longer than that computed from the response to different FM bandwidths in the same neuron. In females, the shifts in best FM duration obtained from FM bandwidth tuning can be towards either shorter or longer duration than those obtained from FM rate tuning. This suggests that overall, neurons in males, especially in the RH, prefer narrower FM bandwidths and/or relatively slow FM rates compared to females.

In some neurons, such as those in the LH that prefer wide FM bandwidths when optimized for FM rate, best FM durations cannot shift much because fast FMs sweep over relatively short durations (< 10 ms). In other words, it is very uncommon for neurons tuned to similar FM rates or bandwidths to be also tuned to a best FM duration unless the entire FM is very short. This makes the presence of FM (unlike CF) duration tuning within either single neurons or the population in the AC unlikely. There are a few neurons (5/52 or ~10% in females but none in males), however, in which duration may be the primary constraint influencing response magnitude, i.e., there is virtually no shift in best FM duration tuning to FM rates that are < 0.6 kHz/ms. Finally, a plot of averaged relative response magnitudes to different FM durations, similar to the ones plotted for FM rate and FM bandwidth in figures 7 and 8, also reveals a complete lack of tuning for particular FM durations within both
hemispheres in either sex (not shown). Thus, there is a lack of either tuning to or preference for particular FM durations both at the single unit and population level within the DSCF area in mustached bats.

Best FM parameters and their right-left distribution

Next we examined distributions of best FM parameters in order to (1) test if the overall representation of rate, bandwidth and center frequency is significantly different between the two hemispheres, and (2) obtain a graphical representation of how each parameter is distributed between hemispheres given the FM preference of individual neurons. Figures 10A - 10D shows bar graphs and kernel density plots for the distribution of the rate, bandwidth, duration and central frequency, respectively, of best FMs within each hemisphere of males and females. Bar graphs show the overall hemispheric differences for each FM parameter as mean and standard error. Best FM rates were slower in the RH (0.22 ± 0.05 kHz/ms, n = 73) compared to the LH (0.99 ± 0.13 kHz/ms, n = 51) in males, but not in females (0.41 ± 0.51 kHz/ms, n = 65 for LH and 0.47 ± 0.83 kHz/ms, n = 58 for RH) (Fig. 8A).

There was also a slight, but significant ($p < 0.02$) difference in best FM bandwidth in males (but not females) such that neurons on the right preferred narrower FM bandwidths (3.49 ± 0.29 kHz) than those on the left (4.39 ± 0.26 kHz). RH neurons preferred longer FMs (34.12 ± 4.94 ms) than those on the left (14.03 ± 1.96 ms) in both sexes ($p < 0.01$). Nearly a quarter (18/75) of right DSCF neurons in females had best FM rates of 0.04 kHz/ms and best FM bandwidths ≥4 kHz, thus these FMs exceeded 100 ms. Indeed, some right DSCF neurons in females had best FMs that approached 200 ms in duration (Fig. 10C). FMs with durations ≥100 ms represented only about 10% (7/63) of the best FMs for left DSCF neurons in females.

Tests of ANOVA revealed a significant main effect of hemisphere on best FM rate (F[1,
Kernel density plots show that more than twice as many neurons respond to low FM rates on the right versus the left side of males, but this relationship is reversed in females. Differences in the distribution of FM bandwidth are apparent beyond 3 kHz in males and between 2 and 5 kHz in females (Fig. 10B). Distribution of central frequency within the best FM was similar on the two sides in both males and females (Fig. 10D).

Temporal response parameters of single neurons to their best FMs

A single unit’s temporal response parameters of peak response latency and response duration obtained by 200 presentations of the “best FM” (FMs optimized for rate and bandwidth and paired at onset with a CF at BF_{low}) at BA were less affected by sex than they were by hemisphere (Figs. 9A and 9B). Statistical comparisons of temporal response parameters for best FMs revealed a significant main effect of hemisphere on response-onset latency (F[1, 253] = 15.38, p < 0.01), peak response latency (F[1, 253] = 21.65, p < 0.01) and response duration (F[1, 253] = 10.76, p < 0.01). In response to best FM, peak response latency was significantly (F[1, 253] = 4.68, p < 0.05) affected by sex, such that DSCF neurons in both hemispheres had longer peak response latencies to best FMs in females compared to those in males. Sex and hemisphere had no significant interaction effects with response-onset latency, peak response latency, or response duration. Despite significant differences in temporal response parameters, neurons responded to best FMs with similar magnitudes on both sides and in both sexes. Comparisons of FM central frequency showed
no significant hemispheric or sex differences.

Response latencies to FMs versus CFs

Longer latencies observed in right versus left hemisphere (see Fig. 11) can result from a number of sources. These sources include an intrinsic difference in the membrane properties, such as integration time constants, asymmetric delay lines at the cortical and/subcortical levels (Gordon and O'Neill 1998; Razak and Fuzessery 2006), the organization of inhibitory response areas relative to excitatory response areas (Kanwal et al., 1999; Sadagopan and Wang 2010), differences in the timing of inhibitory and excitatory interactions that initiate spiking and maximally excite the neuron, (e.g. rebound excitation) (Razak and Fuzessery 2006; Gittelman and Li 2011; Gittelman and Pollak 2011), and/or differences in the duration of FMs preferred by each neuron as a result of duration tuning (Fuzessery et al. 2006). To address the role of one or more of these parameters, we compared response latencies of each neuron to its best CF versus best FM.

Figures 12A and 12B are scatterplots of response-onset latencies for FMs versus CFs for right and left hemispheric neurons in males and females, respectively. Overall, response-onset latencies to FMs tended to be longer than those to CFs in females, but less so in males. However, in both sexes, latencies to FMs were longer for DSCF neurons in the right than those in the left hemisphere as shown in figure 11. Not surprisingly, in a majority of the neurons in females and in roughly half in males, response-onset latencies to FMs were longer than those to CFs. In the other half of neurons in males and several neurons in females, response onset latencies were shorter for FMs than they were for CFs. In some neurons (plotted above the dashed horizontal line) that prefer relatively slow and long duration FMs, response onset latencies to FMs can be 4 to 5 times longer than that to their preferred CF. Such neurons are more abundant in females than in males.
Influence of FM parameters on peak response latency

To further investigate the effect of FM stimulus parameters on peak response latency, we plotted peak response latencies to the best FMs of each neuron across hemispheres and sexes for FM bandwidth (top), FM rate (middle), and FM duration (bottom) in the left and right hemispheres of males (Fig. 13A-13C) and females (Fig. 13D-F). Our data show that best FM rates pooled from neurons within each hemisphere ($\sigma^2$: RH = 0.06; LH = 1.26) exhibited a wider variance in the LH compared to the RH. Centroids (99% confidence intervals) show the center-of-mass for data points representing left and right hemispheric DSCF neurons. None of the centroids in either of the 3 scatter diagrams in figures 13A-13C intersect, and all of the best FM parameters significantly ($p < 0.05$) differ between hemispheres. In figures 13D-13F, all of the centroids intersect, and the only best FM parameter to significantly differ between hemispheres was FM duration (see figure 10C).

Finally, we correlated the best FM parameters of our neurons with the latencies of their peak responses to best FMs. In LH, we observed significant correlations when comparing peak-response latency to best FM rate in males ($r = 0.30$, $p = 0.02$) and females ($r = 0.28$, $p = 0.02$). In females but not males, we observed significant correlations when comparing peak-response latency to best FM bandwidth in RH ($r = 0.31$, $p = 0.02$) and to best FM duration in LH ($r = 0.36$, $p = 0.02$). Most importantly, there was a strong correlation between peak-response latency and best FM duration in the RH of both males ($r = 0.73$, $p < 0.0001$) and females ($r = 0.56$, $p < 0.0001$). Figures 13C and 13F demonstrate that RH neurons in both sexes take longer to respond to FMs that have longer durations because correlations between FM duration and peak-response latency were stronger for right DSCF neurons. These data suggest that peak response latency is strongly influenced by FM duration.
DISCUSSION

Linear FM sweeps overlapping in bandwidth with the CF tuning of DSCF neurons represent spectrotemporally dynamic components of species-specific social calls (Kanwal and Rauschecker 2007; Washington and Kanwal 2008). Unlike different call types, however, artificially synthesized FM sweeps are quantifiable on a continuous scale. This makes them attractive for understanding the neural mechanisms underlying call processing and perception (Washington and Kanwal, 2008). The data presented in this study show sex-specific hemispheric differences in neural responses to both CF and FM stimuli. Specifically, in males, neurons in the RH (1) exhibited relatively long peak response latencies to CF as well as FM stimuli, and (2) responded more strongly to stimuli with slow rates of modulation and narrow bandwidths approaching a CF than to broadband, fast rate FMs. Except for response latency to best FM stimuli, pronounced R-L differences were not observed in females using the same procedure. This makes recording bias a highly unlikely explanation of the observed asymmetry.

Hemispheric asymmetry

The proposed asymmetry for the simultaneous perception of two classes of behaviorally significant events is not unique to bats. Studies of other nonhuman mammalian auditory systems also report left hemispheric lateralization for the representation of social calls and communication-related elements within sounds. Macaques (Hauser and Andersson 1994; Heffner and Heffner 1984) and rodents (Ehret 1987; Fitch et al. 1993) exhibit a right-ear-advantage (REA) for perception of social calls. Functional imaging and 2-DG metabolic studies from rhesus macaques also report a left lateralization in the temporal pole (Poremba et al. 2004) for perception of social calls and a right lateralization of the superior temporal gyrus (Poremba and Mishkin 2007) for perception of environmental sounds. In the common marmoset, the organization of responses to pure tones in A1 was not significantly lateralized.
when tested within the same individual (Philibert et al. 2005), but FMs were not tested in this study. Studies combining foot-shock within a fear conditioning paradigm and cortical lesions in gerbils show that perception of global versus local features within FMs is different between the two sides (Wetzel et al. 2008). In these animals, the right side plays a greater role in determining the direction of an FM sweep, whereas the left side plays a dominant role in gap detection between segments of the same FM. These findings together with the results of this study suggest that lateralized processing is common within the auditory cortex of mammals, and constitutes an important operational principle for simultaneous processing of multiple parameters within auditory signals.

In humans, neuroimaging results indicate that the right AC prefers slowly as opposed to rapidly modulated acoustic signals (Belin et al. 1998; Boemio et al. 2005), which are similar to the CFs and slow FMs used in the present study. Steady-state signals represent vowels in speech sounds and are also important for the perception of pitch, prosody, and speaker identity (Robin et al. 1990). Inertness to rapidly changing broadband FMs gives the RH an advantage over the left for detecting small frequency differences over a relatively long time scale. Conversely, the LH exhibits a stronger response than the RH to formant transitions or rapid changes in the predominant frequency in speech sounds. This underlies the classic REA reported for comprehension of speech sounds (Schwartz and Tallal 1980) and is also consistent with multiple time scales of integration proposed as a basis of lateralization of speech perception in humans per the “asymmetric sampling in time” or AST hypothesis (Poeppel 2003). Accordingly, it is thought that the left AC processes rapid signal changes, such as consonant-vowel combinations, whereas the right AC is better at detecting slight changes in pitch or vocal intonation (Schonwiesner et al. 2005; Zatorre and Belin 2001; Zatorre et al. 2002). In patients with lesions in the left temporo-parietal junction the ability to perceive temporal, but not spectral, information is impaired and vice-versa in those with
lesions in the right temporo-parietal junction (Robin et al. 1990). This type of double-
dissociation between spectral and temporal information processing is consistent with our
findings.

Sex-specific asymmetries

An REA for processing rapidly changing spectral information as in tone sequences is
prominent in male rather than female rats (Fitch et al. 1993). Sex differences in hemispheric
lateralization for vocalization are often reported in songbird studies, which also show more
lateralization in males than females (DeVoogd and Nottebohm 1981; Nottebohm and Arnold
1976). Lateralization of song perception in zebra finches changes if the song is from a
conspecific (LH) or is the bird's-own-song (RH) (Poirier et al. 2009). It was found that male
zebra finches use both spectral and temporal information to classify long call stimuli by
gender of the bird producing the call, but that females use only temporal information (Vicario
2004).

In humans, an interaction of sex hormones with developing neurons (Geschwind and
Galaburda 1985a; 1985b) accounts for a less frequent and smaller extent of cerebral
lateralization for speech and language in the auditory cortices of females (Dawe and
Corballis 1986; Lansdell 1964). Specifically, prenatal exposure to testosterone affects the
development of left lateralized brain areas for processing temporal information in speech
(Beech and Beauvois 2006). Changes in testosterone levels in infants have been implicated
in dysfunctional processing of this information (Geschwind and Galaburda 1985a; Tallal et al.
1988; Tallal et al. 1993). Thus, sex differences in auditory lateralization are present within
many species in different taxonomic groups.

Computational and cellular underpinnings of hemispheric asymmetry

Next we consider which parameters of an FM may play the biggest role in generating
hemispheric asymmetry. For this, we need to examine the cellular basis of a neuron’s response to an FM and how it is different from that to a pure tone. In FM processing neurons, spatial summation of the input frequency range is a function of FM bandwidth (Fuzessery and Hall, 1996), whereas temporal summation would result from the rate of an FM sweep. The broad bandwidths and slow rates extend the time between the best FM’s onset and the FM sweep traversing a neuron’s excitatory response area. Invoking the “leaky integrate-and-fire” model (Jolivet et al. 2004), a mismatch between the FM rate and the integration time constant in AC neurons may prevent the excitatory post-synaptic potential (EPSP) from reaching spiking threshold. Similarly, a narrow range of frequency inputs as in narrowband/slow FM sweeps may lead to a subthreshold EPSP after summation of EPSPs arriving from different dendritic compartments, each of which receives a different frequency input. The amount of spatial summation may also be constrained by inhibitory sidebands skirting and/or overlapping its excitatory/facilitatory tuning (Kanwal et al. 1999) so that even broadband FMs fail to trigger spiking activity in narrowly tuned DSCF neurons in the RH. Hence, auditory hemispheric asymmetry may originate from a difference either in the bandwidth of excitatory frequency tuning and/or the time-scale at which single neurons in the right versus the left hemisphere integrate auditory inputs (Telkemeyer et al. 2009). Our data on correlations between FM parameters and response latency suggest that long-duration FMs are likely to take longer to reach a neuron’s effective excitatory response area and therefore elicit responses later compared to shorter FMs preferred by left DSCF neurons. Interhemispheric interactions can play an important role in determining response characteristics and/or stimulus selectivity in cortical neurons. In the asymmetry observed here, activity in the LH may directly influence the CF and/or FM responses of DSCF neurons on the opposite side, but this remains to be investigated. The presence of inter-hemispheric connectivity was reported previously for the ventral portion of the DSCF area on the two sides in mustached bats (Liu and Suga 1997), and is consistent with a report of laminar organization.
of excitatory-excitatory, E-E, neurons in the A1 of cats (Imig and Reale 1980).

With respect to cellular morphology, one histological study of Nissl-stained cell-bodies in the DSCF area found no hemispheric differences in neuronal densities (Sherwood et al. 2005) supporting the possibility of a physiological difference originating from differences in synaptic connectivity within the dendritic arbor as explained above. Since Nissl stains provide only faint staining of dendritic segments proximal to the cell body, this method also misses differences in anatomical characteristics of cortical neurons (e.g. dendritic morphology, symmetry of synaptic densities, or whether neurons are GABAergic or glutamatergic) that are fundamentally important in auditory processing. Alternately, an absence of cytoarchitectural differences within A1 across the two hemispheres may also suggest that hemispheric differences either originate at subcortical levels or result from inter-hemispheric interactions.

Temporal summation and duration selectivity

The role of FM duration as a parameter that determines FM selectivity in DSCF neurons is not obvious in the cortex, although it does appear to play a role in the inferior colliculus (Fuzessery et al. 2006). Our data show that neurons do not show a systematic preference for duration in either hemisphere of either sex (see figures 9C and 9D) and neurons in the right hemisphere in both sexes prefer relatively long duration (slow rate) FMs (see figure 10C). A principal components analysis of the acoustic structure of social calls yielded duration as an acoustic parameter that varies independently of other parameters (Kanwal et al. 1994). Therefore call duration plays an important role in a classification of calls based on multiple acoustic parameters in different call types. Duration-tuned neurons are known to exist both in the inferior colliculus (Brand et al. 2000; Ehrlich et al. 1997; Fuzessery et al., 2006) and in the cortex of bats (Galazyuk and Feng 1997; Razak and Fuzessery 2006) and
other species (He et al. 1997), but this does not necessarily mean that duration tuning
represents an important underlying mechanism for FM selectivity in the cortex. In the AC of
pallid bats as well, FM rate selectivity, at least for rates in the echolocation range, does not
depend on duration tuning to tones, as it does in the inferior colliculus (Razak and Fuzessery
2006). More recently, Gittelman and Pollak (2011) and Gittelman and Li (2011), using whole
cell patch clamp recordings in the inferior colliculus have established that spike threshold and
the timing of excitatory and inhibitory postsynaptic potentials enhance FM velocity (rate)
selectivity. Our findings are generally consistent with previous reports of FM selectivity within
the AC, in that cortical neurons do not exhibit a systematic preference for FM duration.

Our results suggest that FM duration is not a primary determinant of the response
strength to a particular FM. Right DSCF neurons in males do not integrate over the entire
duration or bandwidth of a slow FM because peak responses are often reached well before
the end of the FM (see figure 11A), and sometimes even during the first half of the stimulus.
Furthermore, if neurons were selective for or tuned to a particular duration, then a
comparison of a neuron’s preference for FM rate and bandwidth would yield a consistent
preference for that (same) duration. This was not the case at the level of single units as
shown in figure 9. Finally, our selection of the peak response magnitude within a narrow (5
or 10 ms) time window minimizes the influence, if any, of stimulus duration on FM
preference. If one adopted the total number of spikes as a response measure, stimulus
duration could become a confounding factor, but even so this is unlikely since DSCF
responses are phasic in nature and are greatly dependent on FM direction and rate as well
as the value of the central frequency in an FM.

With the exception of the long quasi CF (QCFl) call type, unidirectional FMs with a
duration longer than 50 ms are not commonly present in species-specific social calls (Kanwal
et al. 1994), suggesting once again that FM rate may be the main determinant of FM duration
selectivity of DSCF neurons. The duration of linear FMs cannot be completely ruled out, however, as an important factor in generating parametric FM selectivity across the two sides, particularly in females. In a few neurons in females, the preferred duration can be longer than 200 ms. These neurons could play an important role in responding to the whistle-like, QCFl call type with a small (3.6%) modulation in frequency and a duration that ranges from 105 to 500 ms (Kanwal et al. 1994). This call is frequently produced by this species during affiliative interactions (Clement et al. 2006).

Right-left differences in response latencies

The data presented in figure 12 show that in both males and females, the response-onset latency to a neuron’s preferred FM is frequently shifted from that to its preferred CF. In roughly half the neurons in males, and in several neurons in females, where response-onset latencies to the preferred FM are shorter than to a preferred CF, an upsweep or downsweep of frequencies leads to a rapid build-up of excitatory post-synaptic potential, possibly via rebound excitation (Gittelman and Li 2011; Gittelman and Pollak 2011; Zhang et al., 2003). This suggests the existence of synaptic mechanisms for processing FMs that are different from those for processing the simpler CF stimuli. In other words, response-onset latency is not a fixed, intrinsic property that is determined by synaptic delays within ascending auditory inputs. Rather, this temporal response parameter, as recorded in DSCF neurons is strongly influenced by the acoustic parameters of sounds (see figures 12 and 13). Our data showing that left hemispheric neurons frequently have shorter latencies suggests that complex sounds, such as those used for communication, may activate the LH before the RH. Given the presence of multiple frequencies within FMs and communication sounds (Kanwal et al., 1994), the neural circuit activated by complex sounds may be quite different from that activated by a tone. In fact, many cortical neurons in mustached bats, e.g. those in the FM-FM area are specialized to respond to FMs (Ohlemiller et al. 1996).
The presence of FM specialization, as in the FM-FM and other cortical areas in mustached bats, suggests that the mechanisms determining responses to these two types of stimuli in the DSCF area may also be different. Given that the neurons recorded here respond to both tones and FMs, a comparison between excitatory/inhibitory frequency-response areas and the preferred FM bandwidth for the same neuron could illustrate a possible causal relationship between FM rate/duration and response latency. Because of time constraints (both regulatory and technical) in holding and recording from a single neuron in awake animals, we did not obtain these data. From studies in the squirrel monkey (Godey et al. 2005), and owl monkey (Atencio et al. 2007), however, response latency to different speeds or rates of wideband FMs can be used to predict the bandwidth of excitatory-response areas in the AC. However, FM-specialized, combination-sensitive neurons, as exist in the mustached bat's FM-FM area (Ohlemiller et al., 1996), exhibit tuning to a different frequency range (compared to that present in their best FM) when combinations of pure tones are presented simultaneously. This makes it difficult to assume a linear relationship between latency and FM rate/duration since the neuron treats the two as somewhat independent and qualitatively different stimuli.

Echolocation versus communication

Finally, we consider the behavioral advantage that a R-L asymmetry might bestow on the life of a bat. A R-L difference in FM response properties of neurons may emerge from a behavioral dichotomy in the usage of left versus right DSCF neurons. Given their poor visual acuity, mustached bats, like most microchiropterans (Suthers and Wallis 1970) must echolocate to track and capture insects on a daily basis to satisfy their high metabolic demand. During echolocation, DSCF neurons track relative target velocity, which is essentially a pitch-perception task requiring high frequency resolution for detecting minute differences between CFs and/or very low rate (<0.05 kHz/ms) upward FMs in echoes.
returning from an approaching target (Mueller and Kanwal, unpublished). Response magnitude curves obtained here (see figure 8b) show that RH neurons in males are biased to detect approaching targets since their peak response magnitudes increase to CFs and CF-like (very low rate FM) stimuli. More so than those on the right, left hemispheric DSCF neurons in males are highly sensitive to and respond well to stimuli that contain a relatively steep FM, such as the “bent-upward FM” emitted in fear, or traverse a relatively broad bandwidth, like the “fixed sinusoidal FM” (fSFM) and the “rectangular broadband noise burst” (rBNB) emitted during aggression between males (Clement et al. 2006; Clement and Kanwal 2012; Kanwal 2012). Male bats also emit rBNB calls to achieve or maintain dominance within a colony of thousands of individuals (Clement and Kanwal 2012). Differentiating fast from very slow FMs and CFs associated with echolocation can be critical for survival. Females are not observed to engage in such aggression and thus their A1 may not be similarly lateralized as in males. Although sex-specific differences in vocalization frequencies do exist at least for echolocation (Suga et al. 1987), differential frequency tuning and call selectivity of neurons in males versus females has not been carefully analyzed.

In summary, while RH neurons may be dedicated to processing echolocation signals carrying information about the environment, many LH neurons in males appear to be designed to process FM rates and bandwidths frequently encountered in processing social calls. In this respect, hemispheric lateralization may serve a similar function as reported in macaques (Poremba et al. 2004; Poremba and Mishkin 2007). In bats, activity in the left hemispheric DSCF neurons may alert the animal to social exigencies, particularly aggression, that require immediate attention. Rapid activation of LH neurons by broadband calls such as rBNB may in fact modify the sensitivity of the RH to respond to similar calls and/or particular acoustic features or FM parameters within a social call. Further studies are required to delineate inter-hemispheric differences (Kanwal 2012) and interactions for call
processing that may result from differences in the processing of spectrotemporal parameters within FMs.

Acknowledgements:

The Ministry of Agriculture, Land and Marine Resources in Trinidad who kindly permitted us to export mustached bats, and F. Muradali assisted with collection and exportation procedures. This work was supported in part by Grants DC02054 and DC008822 to J.S.K. and DC75763 to S.D.W. from the National Institute for Deafness and Other Communication Disorders, and by HD046388 from National Institute for Child Health and Human Development to Vittorio Gallo. The authors thank Dr. Ru San Chen for advice with the statistical analysis, Mr. Robert T. Naumann for assistance with data analyses and Dr. Gerd Schüller and Mr. Leon Der for the design and fabrication of the stereotaxic recording setup. The funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
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### TABLES

Table 1. Table of predicted outcome values and their significance (p-values) separated by percentiles of FM rate corresponding to Figures 7D-7E. $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$.

<table>
<thead>
<tr>
<th>Males+ Females</th>
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<th>33rd%</th>
<th>67th%</th>
<th>99th%</th>
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<td>0.04-1.26</td>
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<td>-1.42</td>
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<tr>
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<td>(-7.41)</td>
<td>(-0.80)</td>
<td>(-0.92)</td>
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<td>-1.10</td>
<td>8.90</td>
<td>-8.81</td>
</tr>
<tr>
<td></td>
<td>(2.42)</td>
<td>(-0.28)</td>
<td>(0.85)</td>
<td>(-1.36)</td>
</tr>
<tr>
<td>Sex</td>
<td>5.47**</td>
<td>6.10*</td>
<td>4.54</td>
<td>4.31</td>
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<td>(2.93)</td>
<td>(2.28)</td>
<td>(1.30)</td>
<td>(1.47)</td>
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<td>Hemi*Rate</td>
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<td>12.10*</td>
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<td>4.17</td>
</tr>
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<td></td>
<td>(0.13)</td>
<td>(2.43)</td>
<td>(-0.43)</td>
<td>(1.94)</td>
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<tr>
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<td>65.15***</td>
<td>40.30***</td>
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<td>(20.64)</td>
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<th>67th%</th>
<th>99th%</th>
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<tbody>
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Table 2. Table of predicted outcome values and their significance ($p$-values) separated by percentiles of FM bandwidth corresponding to Figures 8D-8E. ($p < 0.05^*, p < 0.01^{**}, p < 0.001^{***}$).

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Table 1. The numbers in each row represent the relationship between a given variable (rate, hemisphere, sex, hemisphere x rate, or variable intercept) using the coefficient of regression (top of row) and the corresponding t-score (bottom of row, parentheses). Each column corresponds to a different range of FM rates. The first column lists the effects of each variable and the total range of FM rates (0.04-4.0 kHz/ms) on peak response magnitude. The second, third, and fourth columns, respectively, correspond to the lower (0.04-1.26 kHz/ms or 33%), middle (1.26-2.48 kHz/ms or 67%), and highest (2.48-4.0 kHz/ms or 99.9%) third of the total range of FM rates. *p*-values show how significantly each variable affected the magnitudes of neural responses. Sex is excluded as a variable for predicted outcomes for male and female bats alone.

Table 2. The numbers in each row represent the relationship between a given variable (bandwidth, hemisphere, sex, hemisphere x rate, or variable intercept) using the coefficient of regression (top of row) and the corresponding t-score (bottom of row, parentheses). Each column corresponds to a different range of FM bandwidths. The first column lists the effects of each variable and the total range of FM bandwidths (0.4-7.9 kHz) on peak response magnitude. The second, third, fourth, and fifth columns, respectively, correspond to the first (0.4-1.8 kHz or 25%), second (1.8-3.5 kHz or 50%), third (3.5-4.8 kHz or 75%), and fourth (4.8-7.9 kHz or 99.9%) quartiles of the total range of FM bandwidths. *p*-values show how significantly each variable affected the magnitudes of neural responses. Sex is excluded as a variable for predicted outcomes for male and female bats alone.
**FIGURE LEGENDS**

**Figure 1.** Combination-sensitive CF/CF and CF/FM responses in the Doppler-Shifted Constant Frequency processing (DSCF) area. Idealized spectrograms of auditory stimuli (above) and peristimulus time histograms (below) to show the role of response facilitation by stimulus combinations in 3 representative neurons. Responses in 2 ms bins for three DSCF neurons to their BF\textsubscript{low}, BF\textsubscript{high}, BF\textsubscript{low} + BF\textsubscript{high}, BF\textsubscript{low} + Best FM (optimized for rate, bandwidth, and central frequency), and Best FM alone. Each stimulus was presented at its best amplitude of excitation (BAE). Each neuron demonstrates the synergistic effects of pairing acoustic stimuli (tones or FMs) in the BF\textsubscript{high} range with a tone in the BF\textsubscript{low} range. Spectrograms of CFs at BF\textsubscript{low} are depicted as unfilled rectangles, and stimuli in the BF\textsubscript{high} range are shown in black. Each stimulus was repeated 200 times.

**Figure 2.** Schematic depicting the experimental design to determine the parameters for the best FM stimulus at each recording location. (A) Flow chart on left shows the sequence of presentation of different arrays in which a single stimulus parameter was systematically modified. Each FM array was composed of 15 stimuli: a no-stimulus control and 14 FMs, (or 13 FMs and a CF in the BF\textsubscript{high} range). Each FM-array was repeated 100 times. (B), (C) and (D) on the right are samples of spectrograms representing each FM array. B. Spectrograms of 5 upward FMs with equal bandwidths (10 kHz) and center frequencies (60 kHz) but different rates (R1-R5). C Spectrograms of 5 upward FMs with equal rates (1 kHz/ms) and center frequencies (60 kHz) but different bandwidths (B1-B5). (D) Spectrograms of 5 upward FMs with equal rates (2 kHz/ms),
bandwidths (10 kHz), and durations (5 ms), but different center frequencies (C1-C5).

Duration covaries with rate and bandwidth in B and C, respectively.

Figure 3. **Generation of a normalized FM-rate response curve.** (A) Top: Raster plot of a single left DSCF neuron’s responses to 14 upward FMs varying in rate from 0.04 to 4.0kHz/ms. FM bandwidth (5.25 kHz) was fixed and duration covaried with rate. FM sweeps were centered on the BF_{high} (58.95 kHz) and paired at onset with a 30-ms CF at BF_{low} (25.50 kHz) as shown in figure 1. The CF at BF_{low} was presented at BA (81 dB SPL) throughout each of the 100 trials. The amplitude of each FM (shown on the right ordinate) was attenuated by 10 dB-SPL every 10 trials. This methodology provided the best amplitude (BA) of the best FM for each neuron. It also allowed amplitude tuning to impact the final response curve so that comparisons across neurons were not biased by selecton of a particular amplitude. Bottom: Peristimulus time histograms summing responses at each FM rate. Peaks (unfilled circles) connected by grey lines show an FM rate response-curve for a single neuron. Each response is based on 100 stimulus trials (bin width = 10 ms) for each of the 14 FMs at several different intensities ranging from -9 to 91 dB SPL. (B) Same as ‘A’ for a second DSCF neuron tested with same FM parameters. BF_{high} (also central frequency of FM) = 59.26 kHz, and BF_{low} = 25.35 kHz, and BA at BF_{low} = 91 dB SPL. (C) Average of the two response curves taking the mean of responses at each FM rate. Note that the y-axis changes from “Spikes/100 trials” to “Percent Peak Response” due to normalization. Blue color here and in successive figures signifies left hemisphere. Error-bars are big given that the plot represents average of only two neurons.

Figure 4. **Response profile of left (blue) and right (red) hemimpsheric DSCF neurons to CF tone pairs in males and females.** (A). Amplitude envelopes (top panels) and
idealized spectrograms (middle panels) for CF pairs (BF_{low}+BF_{high}) presented to awake mustached bats. Filled rectangles represent parametrically optimized CFs and unfilled rectangles represent a 30 ms CF at BF_{low} (~25 kHz). Peri-stimulus time histograms (bottom panels) representing responses from DSCF neurons in the LH and RH for each sex. (B). Bar graphs representing means and standard error of means for each population for peak response latencies on each side in males and females for CF stimuli. Latencies were significantly different (p < 0.01**) in males. (C). Kernel plots showing the variability in peak response latencies for each population shown above. Tones were presented as 200 repetitions at a rate of 4/s.

Figure 5. Raw data demonstrating differences in preferences for FM rate and effects of FM duration on the right versus the left side. Data in panels A through F include a spectrographic plot of the FM stimulus (top), peri-stimulus raster plot (middle), and a peristimulus time histogram (bottom) of each neuron’s response to 100 repetitions of the FM. Panels A-C represent the firing patterns of a single left DSCF neuron in response to FM rates of 0.04, 0.34, and 4.0 kHz/ms. Panels D-F represent the firing patterns of a single right DSCF neuron to the same rates. Each of the FMs described here had a bandwidth of 5.25 kHz. Responses from both the left and right DSCF neurons were obtained from male bats. Responses are shown for a bin width = 10 ms, and collapsed over 10 different amplitude levels (91 to -9 dB-SPL). All FM stimuli were paired with each neuron’s best CFs present at its best amplitude (not shown).

Figure 6. Classification and occurrence of FM rate and bandwidth preference in DSCF neurons by side and sex. (A). Two examples of neuronal response curves showing peak responses expressed in percent of the highest peak value, as a function of FM rate ranging from 0.04 to 4.0 kHz/ms. Response curve on the left shows a peak ("tuning") to
an FM rate (1.87 kHz/ms) > the mean preferred rate, whereas the one on the right has a
peak at an FM rate (0.34 kHz/ms) that is < the mean preferred rate for the population.
(B). Pie charts showing the percentage of neurons with best FM rates below and above
the mean preferred rate in the DSCF area on the left versus the right in males versus
females. (C). Pie charts showing the percentage of best FM bandwidths below and
above the mean preferred bandwidth. Light and dark shades of blue and red
respectively, represent the proportion of DSCF neurons with best FM parameters that
are < and > the average for rate and bandwidth for all neurons (0.59 kHz/ms for FM rate
and 4.5 kHz for FM bandwidth).

Figure 7. **Population response curves and predicted outcomes of multi-level modeling**
for FM rate. All FMs were presented in the direction preferred by each neuron; FM
bandwidth remained constant with changes in FM rate. Response curves were
normalized to their absolute maxima before averaging. Response curves are shown for
LH (blue) and RH (red) in males and females averaged together (A), in males (B) and in
females (C). Dashed vertical lines indicate the mean best FM rate of all 187 neurons).
Each response is based on 100 stimulus trials (bin width = 10 ms) for each of the 14
FMs at intensities ranging between -9 to 91 dB SPL. (D –F) Line graphs of predicted
outcomes of multi-level modeling for measuring the effects of FM rate, sex, and
hemisphere (see also Table 1) on the peak responses of DSCF neurons from 4 male (E)
and 2 female (F) mustached bats (number of spikes in 10 ms bin). Ordinate is
percentage of peak response in a 10 ms bin and abscissa is FM rate from 0.04 to 4.0
kHz/ms.

Figure 8. **Population response curves and predicted outcomes of multi-level modeling**
for FM bandwidth. All FMs were presented in the direction preferred by each neuron;
FM rate remained constant and duration co-varied with changes in FM bandwidth. Response curves were normalized to their absolute maxima before averaging. Response curves are shown for LH (blue) and RH (red) in males and females averaged together (A), in males (B) and in females (C). Dashed vertical lines indicate the mean best FM bandwidth of all 256 neurons. (D –F) Line graphs of predicted outcomes of multi-level modeling measuring the effects of FM bandwidth, sex, and hemisphere (see also Table 2) on the peak responses of DSCF neurons from 6 male (E) and 4 female (F) mustached bats (number of spikes in 10 ms bin). Ordinate is percentage of peak response in a 10 ms bin and abscissa is FM bandwidth from 0.4-7.9 kHz.

Figure 9. Effect of FM duration on response magnitude. FM rate (A) and FM bandwidth (B) response curves from two single DSCF neurons (one from each hemisphere) of a male mustached bat. Duration scale is computed from rate (bandwidth was fixed at 5.25 kHz in A and 3.5 kHz in B) and bandwidth (rate was fixed at 0.04 kHz/ms) in A and B, respectively. X-axis tics are sparsely labeled to improve legibility. (C) Population response data for shifts in best FM duration computed from best FM duration in FM bandwidth – best FM duration in FM rate curves for each side in males and in females. (D). Density histograms for the left and right side are superimposed on the respective side on top of each scatter plot. Duration shifts in neurons on the left and right side that are tuned to fast FMs (≥ 0.6 kHz/ms) are indicated by unfilled circles and those to slow (< 0.6 kHz/ms) by filled circles.

Figure 10. Distribution of different FM parameters between each hemisphere of male and female bats. Bar graphs comparing the mean and S.E.M. (above) and kernel density plots (below) to show the distribution of best FM parameters for neurons in the left and right DSCF area in males (124 neurons) and females (123 neurons). Plots are
shown for FM rate (A), FM bandwidth (B), FM duration (C) and FM central frequency (D). Neurons in B, C and D are from the same sample as in “A.” Significance levels for differences in the RH versus LH for mean of each FM parameter are as follows: FM bandwidth \(p = 0.02^*\), FM rate \(p < 0.01^{**}\), and FM duration \(p < 0.01^{**}\).

Figure 11. Response profile of DSCF neurons to FM/CF pairs in males and females.

(A). Amplitude envelopes (top panels) and idealized spectrograms (middle panels) for optimal FM/CF pairs presented to awake mustached bats. Filled rectangles represent parametrically optimized FMs and unfilled rectangles represent a 30 ms CF at BF_{low} \((\sim25\ kHz)\). Peri-stimulus time histograms (bottom panels) representing neural responses to stimuli illustrated in the corresponding top panels. (B). Bar graphs comparing peak response latencies of DSCF neurons in males \(p < 0.01^{**}\). (C). Bar graphs comparing response-onset latencies of the same neurons in the right versus left hemisphere from male bats \(p < 0.01^{**}\). Stimuli were presented as 200 repetitions at a rate of 4/s.

Figure 12. Changes in response latency with CF and FM stimuli. Scatterplots of response-onset latencies for FMs versus CFs within each neuron for the right (red dots) versus the left (blue dots) side in males (A) and females (B). Horizontal dashed lines transect the FM latency axis at values equal to that of the range on the CF latency axis. Diagonal dashed lines represents equivalent response latencies.

Figure 13. Scatter plots showing effect of FM parameters on peak response latencies. Each solid dot represents peak response magnitude of a neuron at its optimal parameter (rate, bandwidth and duration) value for the best FM shown in left (blue) and right (red) DSCF neurons in males (A to C) and females (D to F). Oval lines are centroids (99% confidence intervals) representing centers-of-mass for data points related to left (blue
ovals) and right (red ovals) DSCF neurons. Best FMs were paired at onset with BF_{low}.

Correlation coefficients and significance levels for latency of the response to the bestFM for each parameter in each sex were as follows. Males: bandwidth (left: r = 0.10, p = 0.46, right: r = 0.20, p = 0.244); rate (left: r = 0.30, p = 0.026, right: r = 0.29, p = 0.088); and duration (left: r = 0.03, p = 0.849, right: r = 0.73, p < 0.0001); Females: bandwidth (left: r = 0.03, p = 0.862, right: r = 0.31, p = 0.022), rate (left: r = 0.21, p = 0.157, right: r = 0.28, p = 0.040), and duration (left: r = 0.36, p = 0.016, right: r = 0.56, p < 0.0001).
Table 1. Table of predicted outcome values and their significance ($p$-values) separated by percentiles of FM rate corresponding to Figures 7D-7E. $p < 0.05^*, p < 0.01^{**}, p < 0.001^{***}$.

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<td>65.15***</td>
<td>40.30***</td>
<td>37.16***</td>
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<td>(20.64)</td>
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<td>67th%</td>
<td>99 th%</td>
</tr>
<tr>
<td>Range (kHz/ms)</td>
<td>0.04-4.0</td>
<td>0.04-1.26</td>
<td>1.26-2.48</td>
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<td>-18.57***</td>
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<td>(0.44)</td>
<td>(0.31)</td>
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<td>(0.48)</td>
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<td>67.26***</td>
<td>43.95***</td>
<td>41.16***</td>
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<td>(15.64)</td>
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<td>99 th%</td>
</tr>
<tr>
<td>Range (kHz/ms)</td>
<td>0.04-4.0</td>
<td>0.04-1.26</td>
<td>1.26-2.48</td>
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<td>-34.45***</td>
<td>-6.19</td>
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<td></td>
<td>(1.53)</td>
<td>(3.24)</td>
<td>(-0.04)</td>
<td>(1.76)</td>
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<td>63.27***</td>
<td>41.49***</td>
<td>30.91***</td>
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<td>(27.40)</td>
<td>(19.54)</td>
<td>(5.73)</td>
<td>(6.60)</td>
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Table 2. Table of predicted outcome values and their significance (p-values) separated by percentiles of FM bandwidth corresponding to Figures 8D-8E. (p < 0.05*, p < 0.01**, p < 0.001***).

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<th>Males</th>
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<td>50th%</td>
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<td>1.8-3.5</td>
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<td>-5.91</td>
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<tr>
<td>Sex</td>
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<td>-3.92</td>
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<td>32.59***</td>
<td>52.91***</td>
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Table continued...
Figure 1
Washington and Kanwal
Figure 2
Washington and Kanwal
Figure 3
Washington and Kanwal
Figure 4
Washington and Kanwal
Figure 5
Washington and Kanwal
Figure 6
Washington and Kanwal
Figure 7
Washington and Kanwal
Figure 8
Washington and Kanwal
**Figure 9**

Washington and Kanwal
Figure 10
Washington and Kanwal
Figure 11,
Washington and Kanwal
Figure 12
Washington and Kanwal