Title

Distinguishing sensitivity to direction and velocity of fast frequency chirps from periodicity tuning in the inferior colliculus

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Abstract

Neurons in the rabbit inferior colliculus (IC) are selective for the direction of fast frequency chirps (frequency sweeps) contained in Schroeder-Harmonic Complexes (SCHR). This selectivity may point to a feature sensitivity to fast chirp direction and velocity in the IC shared by all mammals. Here, we introduce a novel stimulus consisting of Aperiodic Random Chirps (ARC) to distinguish neurons' sensitivity to chirp direction and velocity from the confounding periodicity of SCHR stimuli. Extracellular, single-unit recordings were made in the IC of five Dutch-belted rabbits. SCHR stimuli were presented at a range of velocities and periodicities. Corresponding ARC stimuli and sinsuoidally amplitude-modulated (SAM) noise were also presented. The prevalence of direction selectivity and direction bias of selectivity to chirps in SCHR and ARC responses was quantified, revealing that the majority of IC neurons (90.3%) are selective to chirp direction. Additionally, although this selectivity was biased for negative SCHR chirp directions, neurons with both positive and negative biases for every SCHR and ARC condition were observed. Modulation-transfer-function type was not significantly related to chirp selectivity, but characteristic frequency was related to both the prevalence of direction selectivity and direction bias of chirp responses. Responses to ARC and SAM noise stimuli were used as input to a generalized linear model (GLM) to predict SCHR responses rates, based on the premise that ARC and SAM noise responses correspond to neurons' velocity and periodicity response profiles, respectively. The results of the GLM analysis reveal that velocity-terms represented a substantial percentage of the total explainable variance, over 50% for some neurons. We discuss how chirp direction and velocity sensitivity may have implications for midbrain-level encoding of speech.

Introduction

The inferior colliculus (IC) is a major convergence point of excitatory and inhibitory inputs from several auditory brainstem nuclei. The convergence of these inputs results in a variety of complex response properties, such as different types of frequency tuning (reviewed in Davis, 2005) and sensitivities to interaural differences, duration, amplitude modulation (AM), and frequency modulation (FM) (Casseday et al., 1994; Koch and Grothe,1998; Caspary et al., 2002; Pollak et al., 2011; Yin et al., 2019). Here, we examine the sensitivity of IC neurons to the direction and velocity of fast FM sweeps. This study is distinct from previous IC FM literature for two reasons. First, we use an awake rabbit animal model, an auditory generalist. Second, we use particularly fast FM stimuli, primarily Schroeder-phase (SCHR) harmonic complexes, comprised of fast frequency sweeps within each fundamental period. Additionally, we apply novel stimuli to disentangle sensitivity to FM from sensitivity to periodicity. The results of this study suggest a sensitivity to fast FM in the mammalian IC that has implications for the neural processing of complex sounds such as speech and music.

Sensitivity of IC neurons to FM sweep direction and velocity has been extensively studied. Starting with Suga (1965), bats have frequently been used in physiological studies of FM sweeps. These studies showed that the bat IC contains neurons that respond preferentially, or even exclusively, to a particular FM direction, a response property thought to be an adaptation for echolocation (Suga, 1965; Fuzesery, 1994; Fuzessery and Hall, 1996; Gordon and O'Neill, 1998; Pollak et al., 2011). Other studies have reported neurons selective for sweep direction in mice (Hage and Erhet, 2003; Li et al., 2010; Geis and Borst, 2013) and rats (Poon et al., 1992), which have communication calls containing FM components. Additionally, many studies have used sounds with long-term statistics based on natural sounds to characterize IC neuron responses with spectrotemporal receptive fields (STRFs) (Escabi and Schreiner, 2002; Escabi et al., 2003; Andoni et al., 2007; Wooley et al., 2009). These sound stimuli contain time and frequency modulations with statistics comparable to those of spectral-peak, or formant-frequency, transitions observed in real-world sounds, such as animal

vocalizations or speech. Finally, one study demonstrated that IC neurons in anesthetized gerbil are sensitive to sweeps in SCHR stimuli differing in direction and velocity (Steenken et al., 2022).

Most prior physiological studies of IC FM sensitivity have largely been carried out in species in which FM sensitivity was thought to be behaviorally advantageous, based on the properties of conspecific vocalizations. Here we report sensitivity to fast FM rates in rabbits, an animal not usually thought to produce vocalizations containing fast FM sweeps, such as those produced by bats, mice, or rats, and therefore presumably lacking special adaptations for processing such sounds (Awan et al., 2014). Instead, rabbits have representative mammalian hearing, with about average high-frequency hearing among similarly-sized mammals, and low-frequency hearing similar to that of humans (Heffner and Masterson, 1980).

In this study, we demonstrate that rabbit IC neurons are sensitive to the direction and rate of FM sweeps contained in SCHR stimuli. These stimuli have interesting properties that make them useful for studying FM sensitivity. The harmonic components of SCHR stimuli have flat magnitude spectra and curved phase spectra that create a stimulus with a maximally flat envelope (Schroeder, 1970). The phase differences across harmonic components result in a linear frequency sweep within each fundamental period, extending from the fundamental frequency (F0) to the highest harmonic component. Here, IC neurons are shown to be selective for direction and velocity (sweep rate) of SCHR stimuli. SCHR sensitivity is notable for the sheer speed of the sweeps involved: the sweep velocities in SCHR stimuli with F0s ranging from 50-400 Hz and harmonics up to 16 kHz are about 800-6000 Hz/ms, which overlaps the range of sweep velocities in the bat echolocation pulse (300-23000 Hz/ms). Additionally, the sweeps used here occurred over a relatively low-frequency region (here from F0 to 16 kHz), further differentiating SCHR sweeps from many other FM stimuli (the bat echolocation pulse occurs at 30-60 kHz). Finally, FM sweeps in SCHR stimuli are distinct in that each sweep occurs within a fundamental period, as a result of the phase differences between harmonics. We refer to these fast SCHR sweeps as SCHR chirps.

SCHR chirp sensitivity in the IC suggests a mechanism that may influence midbrain-level encoding of speech. When speech sounds, or any animal vocalizations, are produced, resonances within the vocal tract act as second-order filters, introducing phase shifts between components of harmonic sounds (Klatt, 1980). In the same way that SCHR chirps are produced by the relative phases of harmonic components, vocalizations will contain chirp-like features to which IC neurons may be similarly sensitive. IC neurons in this study were sensitive to SCHR chirps over a frequency ranges relevant to human speech. Close examination of IC responses to SCHR chirps may reveal a novel influence on midbrain response to speech and other harmonic sounds.

IC selectivity to fast FM chirps (Steenken et al., 2022) suggests a novel feature sensitivity that could be shared among all mammals, including humans. However, work must be done to fully understand this new sensitivity. The IC displays a well-documented tuning for AM frequency (e.g., Krishna and Semple, 2000; Nelson and Carney, 2007; Kim et al., 2020). Because SCHR stimuli are periodic, periodicity tuning in the IC may confound estimates of neural sensitivity to chirps. Here, we design new stimuli with the aim of eliminating or manipulating the effects of periodicity sensitivity in neural responses to chirps. Using the Aperiodic Random Chirp (ARC) stimulus, we analyze responses to aperiodic SCHR chirps, allowing the quantification of velocity sensitivity in stimuli without periodicity. We introduce a strategy using the generalized linear model (GLM) to predict SCHR chirp responses based on responses to ARC stimuli and SAM noise, with GLM coefficients providing a metric to measure the relative contributions of velocity or periodicity sensitivity to SCHR responses. By analyzing single-unit responses with this strategy, we characterized each neuron by the dominant influence on its SCHR responses. Although some neurons' SCHR responses were best reflected by AM tuning, periodicity tuning cannot explain differences in responses SCHR stimuli with different sweep directions. Many neurons' SCHR responses were dominated by velocity sensitivity. For most neurons, the variance explained by the model was increased when including both periodicity and velocity terms. Sensitivity to the direction of FM chirps was present in nearly all IC neurons.

Methods

Subjects

Extracellular, single-unit recordings were made in the central nucleus of the IC of six female Dutch-belted rabbits. The age of the animals during experiments ranged from 1 – 5 years.

Distortion-product otoacoustic emissions (DPOAE) were periodically monitored to assess the hearing of the subjects (frequency range and level of stimulus). Animals were euthanized when their DPOAE magnitudes decreased or neural thresholds increased

IC Recordings

IC recordings were made in daily two-hour sessions. To prevent head movement of the awake rabbits, a headbar (metal or 3D-printed plastic) was surgically affixed to the skull prior to recordings. Additionally, rabbits were wrapped in a towel and secured in a custom-made chair to limit movement during data collection. Recordings took place in sound-attenuated booths (Acoustic Systems, Austin, TX, USA). Recordings were made using four sets of platinum iridium tetrodes, consisting of four 18-μm wires (California Fine Wire Co., Grover Beach, CA, USA), coated in epoxy, and twisted together, with impedances of approximately (ask Jo or check blue notebook for recent impedances). Tetrodes were surgically implanted in the ICC under anesthesia, via a small (~2mm diameter) craniotomy in the top of the skull. Tetrdoes were advanced before or after recording sessions using a manual microdrive (Neuralynx 5-drive). Neural recordings were made using an RHD recording system (Intan Technologies, LLC., Los Angeles, CA, USA), with Intan software running on a Windows PC.

Stimuli generated using custom MATLAB (Mathworks, Natick, MA, USA) code were presented using a MOTU audio interface (16A, Mark of the Unicorn, Cambridge, MA, USA) and a Benchmark digital-to-analog converter (DAC3 HGC, Benchmark Media Systems, Inc., Syracuse, NY, USA). Etymotic ER2 earphones (Etymotic Research, Inc., Elk Grove Village, IL, USA) were used to present sound stimuli via custom ear molds (Hal Hen ear mold material). Prior to every session, stimuli were calibrated using ER-7C or ER-10B+ probe-tube microphones (Etymotic Research, Inc., USA) and an Etymotic probe tube. The calibration curve (magnitude and phase) were included in a pre-emphasis filter that was used to compensate all stimuli for the acoustic system's frequency response.

Action potential times and waveforms were extracted from neural recordings using custom MATLAB applications. To identify single-unit events, the voltage recording was bandpass-filtered between 300 – 3000 Hz. An action potential was identified when the filtered voltage signal exceeded a threshold, defined as four standard deviations of the voltage signal. Action potentials were sorted into clusters, presumed to represent different neurons, using features of the waveforms, such as the slope of the spike repolarization (Schwarz et al., 2012). Single-units were identified when less than 2% of the interspike intervals were smaller than 1 ms. Data were recorded over the span of X sessions, and included recordings from X neurons.

Stimuli

Recording sessions began by presenting a set of stimuli to define the neurons' basic response properties, including frequency response maps, modulation transfer functions, and SCHR responses. To generate frequency response maps, a randomly-ordered sequence of 0.2-s-duration tones at frequencies ranging from 250 Hz – 16 kHz was presented diotically at 10, 30, 50, and 70 dB SPL. Tones were separated by 0.4 s of silence. Each stimulus condition was presented three times. Response maps, plots of average discharge rate (including the onset response) vs. frequency at each stimulus level, allowed identification of a neuron's characteristic frequency (CF), the frequency for which threshold was lowest.

Modulation transfer functions (MTF) were estimated by computing the average rate in response to 100% modulated sinusoidal-AM noise as a function of modulation frequency, over a range of 2 – 350 Hz. The carrier was wideband noise (100 Hz – 10 kHz), presented diotically. AM noises were 1 s in duration, separated by 0.5 s of silence. Modulation frequencies were presented in random order, with 5 repetitions of each frequency. Stimuli were presented at a spectrum level of 30 dB SPL. Based on Kim et al., (2020), MTF types were categorized as band-enhanced (BE) if 1. the MTF had three consecutive points greater than the product of the unmodulated rate (r_{um}) and a criterion K, and 2. the MTF did not have more than one point below the product of r_{um} and $1/K$. Similarly, MTFs were categorized as band-suppressed (BS) if 1. the MTF had three

consecutive points less than r_{um}/K, and 2. the MTF did not have more than one point above $r_{um}K$. For this study, K was set to 1.2. In the population of neurons studied, X% were BS, X% were BE, X% were hybrid, and X% did not fall into any of these categories. For BE neurons, a best modulation frequency (BMF) was determined. For BS neurons, a worst modulation frequency (WMF) was determined, and for hybrid neurons both a BMF and WMF were found.

SCHR stimuli were produced using the phase spectrum introduced in Schroeder (1970). For a harmonic complex with n components, the phase of the nth component was described by $\theta_n = C\pi n(n+1)/N$, where N is the total number of harmonics, and C is a scalar that describes the phase-direction and duty cycle of the stimulus. A random sequence of SCHR stimuli were presented, with F0 equal to 50, 100, 200, or 400 Hz and C equal to -1, -0.5, 0, 0.5, or 1. For all cases, the frequency of the highest harmonic component was 16 kHz. Thirty repetitions of each F0 and C combination were presented. Stimuli were presented diotically at 60 dB SPL, with a duration of 0.4 seconds followed by 0.6 seconds of silence. Average discharge rates over the stimulus duration were calculated for each F0 and C combination. The relative response to pairs of SCHR stimuli with opposite signed C values (e.g., ±1, ±0.5) were quantified using a bias metric, defined as $(R_{neg} - R_{pos})/(R_{neg} + R_{pos})$, where *R* is average rate over the entire stimulus. Within-repetition statistics were performed using receiver-operating-characteristic (ROC) analysis (Egan, 1975). Neurons were deemed significantly biased if percent-correct identification of chirp direction exceeded 70.7% for either direction.

ARC stimuli were randomly spaced (40 – 60 ms) sets of isolated SCHR chirps (i.e., single fundamental periods extracted from a SCHR stimulus). Each set of ARC chirps consisted of 1 or 3 isolated chirps, constructed by using the SCHR algorithm to produce 1 or 3 periods, respectively. A random sequence of chirp velocities was generated, corresponding to SCHR F0s of 25, 50, 100, 200, 400, and 600 Hz. Chirp direction (upward or downward) was also randomly varied across stimuli. Each combination of velocity and direction was presented a total of 840 times. Stimuli were presented diotically at 65 dB SPL. Response rates were calculated by summing spikes over a time window beginning at chirp onset, and spanning a duration equal to the duration of the chirp set plus the minimum spacing between chirps (40 ms).

Modeling

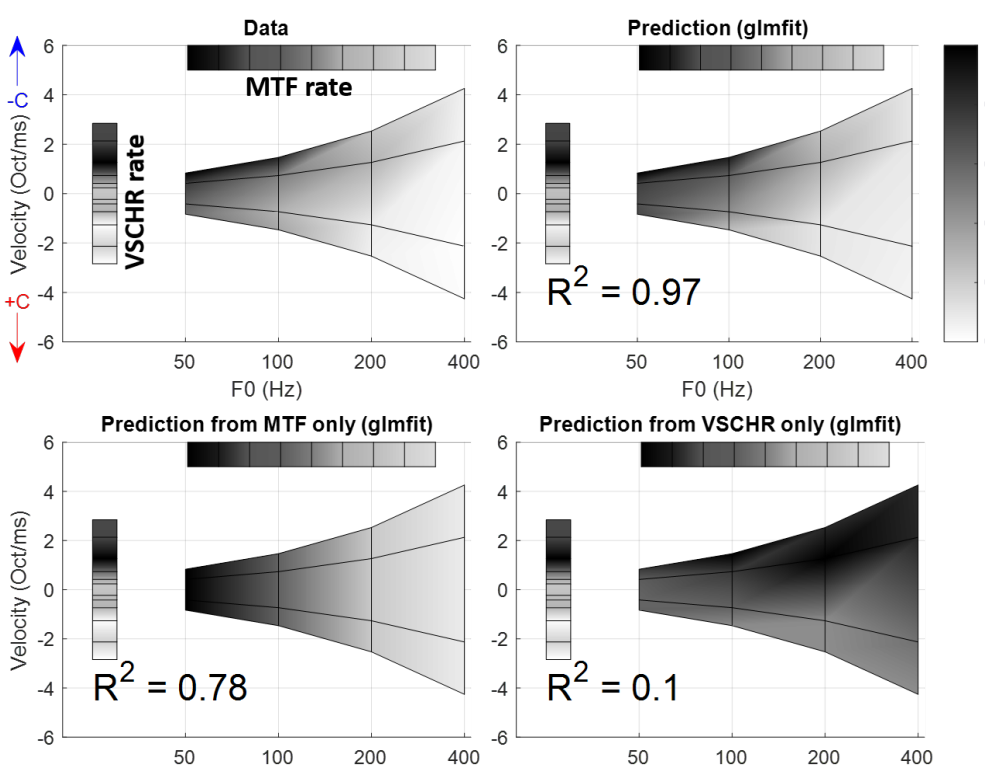

Figure A – Example of SCHR rate GLM predictions

We made predictions of each neuron's SCHR response based on MTFNs and ARC response rates using the generalized linear model (GLM). First, SCHR average-rate responses were visualized as a surface on an F0/velocity axis (Figure A), which reveals the nonlinear relationship between C-value and velocity. Next, SCHR rates were predicted based on MTFN and ARC responses at corresponding periodicities and velocities (data was not interpolated). For the two periodic stimuli (MTFN and SCHR), cycle rate was used to quantify responses, defined as the average rate per period of F0. Cycle rate (sp/cycle of F0) was selected instead of overall average rate (sp/sec) to take into account the strong phase-locking of many neurons to F0, which resulted in average rates that linearly increased with F0. For the aperiodic stimulus (ARC), which had no fundamental period, rate was calculated using the previously mentioned method of summing spikes over a window equal to chirp duration plus the minimum spacing duration (40 ms), to accommodate the latency of IC responses. The relationship between dependent and independent variables in the GLM was defined by

$R_{SCHR} = b_1 R_{MTFN} + b_2 R_{ARC} + b_3 (R_{MTFN} * R_{ARC}) + b_0$, (Eqn. 1)

where R_{MTFN} and R_{VSCHR} refer to corresponding rates from a neuron MTF and chirp responses. R_{SCHR} is the predicted response to SCHR stimuli in cycle rate, and b_n are the GLM coefficients. GLM coefficients were estimated using the MATLAB function "glmfit" (2021a, MathWorks), with the distribution argument set to 'normal' and constant argument set to 'on'. After the SCHR rate prediction was calculated using estimated coefficients, the coefficient of determination (R^2) of the predicted and recorded SCHR cycle rates was determined to assess prediction quality. This process was repeated for two additional, simpler GLMs:

$R_{SCHR} = b_1 R_{MTFN} + b_0$ (Eqn. 2)

$R_{SCHR} = b_1 R_{ARC} + b_0$ (Eqn. 3)

These simpler GLMs depended only on one response feature and thus could be used to assess the importance of MTFN or ARC for predicting SCHR responses. The R^2 of SCHR responses predicted by these two models were obtained (${R_{MTFN}}^2$ and ${R_{ARC}}^2$) and used to quantify the influence of AM sensitivity and chirp velocity sensitivity on each neuron's SCHR responses.

Results

A. Individual IC neurons are strongly selective for SCHR direction (and velocity)

Single-unit neurons were initially characterized using RMs, MTFs, and SCHR dot rasters (Figure X). As described in Methods, RMs were used to determine CF as well as reveal off-CF inhibitory regions. MTFs were used to assign an MTF-shape to characterize response to modulation as well as to identify BMF or WMF. For initial analysis of SCHR responses, dot rasters were constructed to identify salient trends within the response of each neuron. Dot rasters provided a useful starting point because they illustrate both detailed timing and rate differences between SCHR conditions and begin to characterize neurons' direction and velocity response preferences. SCHR dot rasters are organized into rows by F0 and then by C-value. Responses to negative C-values (-SCHR) are plotted with blue dots, and positive C-values (+SCHR) with red dots. Black is used for C = 0. Combined with RMs and MTFs, the SCHR dot raster provided an initial response profile from which underlying mechanisms could be hypothesized. Subsequent analyses were informed by this initial characterization, with the intent of testing the hypothesis that SCHR responses were the result of some combination of modulation tuning, chirp velocity tuning, and off-CF inhibition in the RM, with individual neurons weighting the relative importance of these influences differently.

At this point, it is worthwhile to walk the reader through an example neuron's initial characterization, to demonstrate how the SCHR response profile of a given neuron may be informed by a combination of modulation tuning, chirp velocity tuning, and off-CF inhibition.

[NEURON WALKTHROUGH GOES HERE]

B. The majority of IC neurons are selective for SCHR direction

F0	C-Value	Overall (n = 154)	BE (n = 30)	BS (n = 58)	Hybrid (n = 40)	Low CF (n = 36)	Mid CF (n = 37)	High CF (n = 81)
50 Hz	±1	54.6	76.7	46.6	52.5	58.3	64.9	48.2
	±0.5	59.2	81.0	47.1	60.0	56.7	73.9	55.6
100 Hz	±1	51.3	76.7	44.8	37.5	63.9	62.2	40.7
	±0.5	51.2	66.7	49.0	34.3	50.0	73.9	44.4
200 Hz	±1	49.4	66.7	46.6	35.0	63.9	54.1	40.7
	±0.5	35.2	71.4	23.5	22.9	33.3	60.9	27.8
400 Hz	±1	48.1	46.7	46.6	50.0	47.2	46.0	49.4
	±0.5	48.8	66.7	41.2	45.7	46.7	56.5	47.2
Any		90.3	93.3	93.1	82.5	97.2	91.9	86.4

Table 1 - Percent of direction selective neurons by subgroup (left-most column) as determined by ROC analysis. Neurons are sorted into subgroups by MTF shape and CF. Neurons with CF less than 6 kHz were labeled "Low CF", and neurons with CF greater than 6 kHz were labeled "High CF". Direction selectivity was quantified for each F0/C condition. The percentage of neurons selective for any F0/C condition was also determined (right-most column).

After establishing that individual IC neurons displayed sensitivity for SCHR chirp direction, this property was quantified over the full population of recorded neurons. SCHR direction sensitivity was quantified within a given F0 and C-value by performing ROC analysis on response rates for SCHR sign-pairs. SCHR sign-pairs for which direction could be discriminated via ROC test were labeled direction selective. Table 1 shows the percentage of neurons (151 in total) that were direction selective for each F0 and C condition. Direction selectivity is more common for lower F0s. Neurons were most direction selective for the F0 = 50 Hz, C = +/- 1 condition, notably the lowest-velocity SCHR chirp presented. Neurons were least direction selective for the F0 = 200 Hz, C = +/- 0.5 condition.

For a given F0, the C = +/- 0.5 condition has a chirp velocity equivalent to the 2F0 and C = +/- 1 condition. However, these equivalent-velocity pairs differ in prevalence of direction selectivity. This suggests that prevalence of direction selectivity does not depend solely on chirp velocity, and is possibly influenced by SCHR periodicity or duty cycle as well.

Finally, 90.3% of neurons were direction selective for at least one F0 and C condition, demonstrating that SCHR chirp direction selectivity is near ubiquitous.

C. The majority of IC neurons respond more strongly to –SCHR chirps than +SCHR

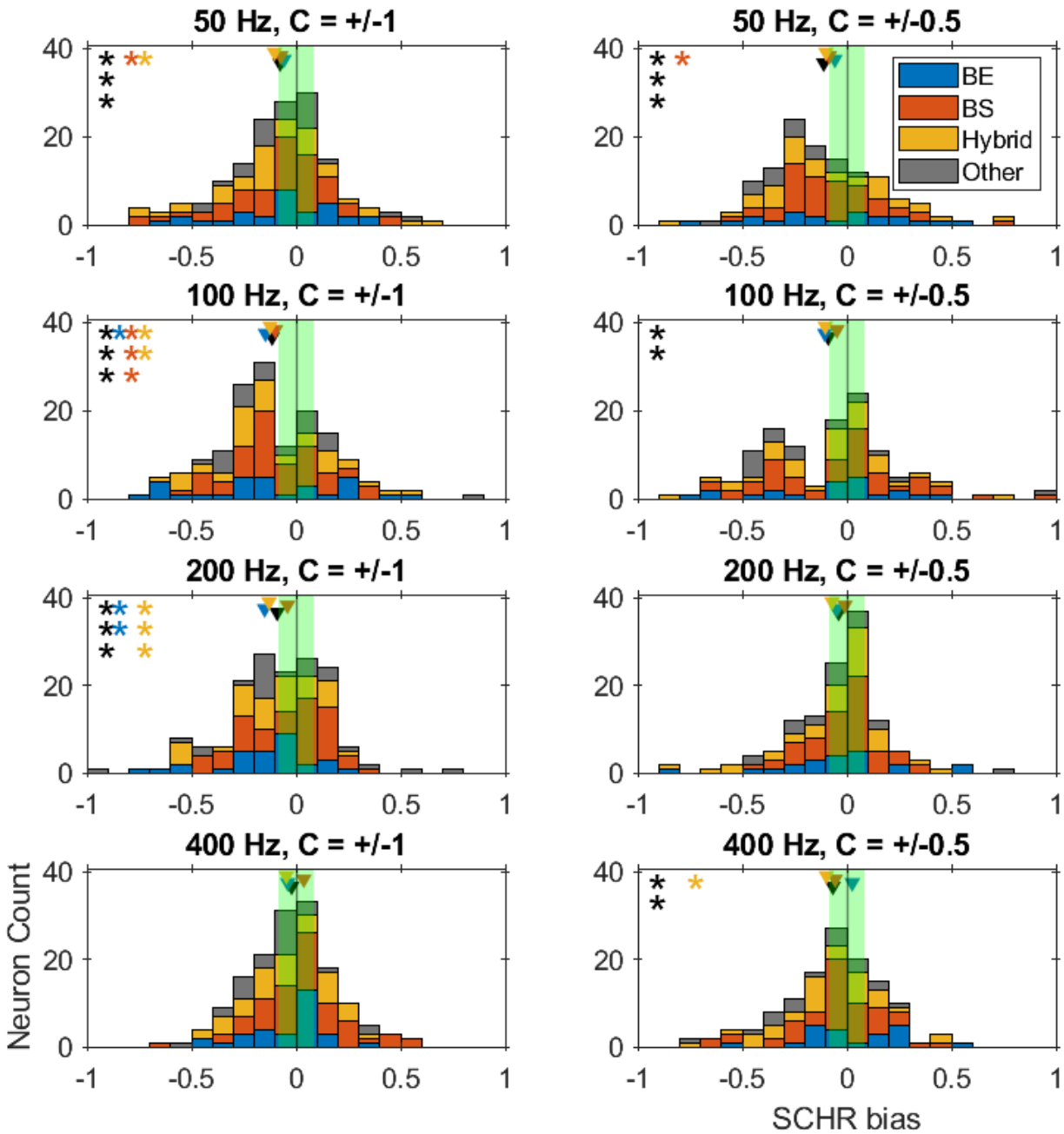

*Figure 1 – Histograms of direction bias separated by MTF shape for SCHR responses (bin width = 0.05). Panels are organized by F0 (rows) and C (columns). 154 neurons are represented for C = +/- 1 conditions (left column), and 125 neurons are represented for C = +/- 0.5 conditions (right column). Stacked color bars denote MTF shapes: BE (blue), BS (orange), Hybrid (yellow). Other MTF shapes are colored gray. Population mean direction bias is marked with a black triangle. Mean direction biases for BE, BS, and Hybrid neuron subpopulations are marked by triangles of their respective colors. Asterisks in the top left corner of each panel indicate that the means are significantly different than zero (one-sample t-test, two-tailed, * p < 0.05, ** p < 0.01, *** p < 0.001). Transparent rectangle indicates standard deviation of the direction bias distribution after within-SCHR-condition shuffling, analogous to the noise floor of the initial distribution. Neurons with biases outside of this range are tallied by direction.*

For each neuron, SCHR chirp direction bias was quantified using the direction bias metric (eq. 1). Figure 1 shows the distribution of direction bias scores for each SCHR condition. Distributions are unimodal, except for conditions 100 Hz, C = +/- 1, 100 Hz, C = +/- 0.5, and 200 Hz, C = +/- 1, which are bimodal. The bimodality of these conditions suggests two classes of IC neurons exist with different direction bias scores.

The inverted black triangle indicates the mean of each distribution. Distributions with means significantly different from zero are marked with a black asterisks (top left corner). As a whole, the mean direction bias is generally negative. Mean direction bias is significantly negative for both 50 Hz and 100 Hz conditions, as well as for 200 Hz, C = +/- 1 and 400 Hz, C = +/- 0.5 conditions. This suggests that negative direction bias is more common at lower F0s.

To quantify the inherent variation in direction bias scores, a distribution was generated of shuffled responses to the two chirp directions, within each SCHR condition and for each neuron. This is analogous to the noise floor of the initial direction bias distribution. The standard deviation of this shuffled distribution is plotted for each SCHR condition (green rectangle). The percentage of neurons outside this region are tallied in Table 2.

F0	C-Value	Negative	Positive	Total
50 Hz	±1	46.8	24.7	71.4
	±0.5	57.6	23.2	80.8
100 Hz	±1	59.1	23.4	82.5
	±0.5	43.2	25.6	68.8
200 Hz	±1	49.4	26.0	75.3
	±0.5	34.4	25.6	60.0
400 Hz	±1	35.7	27.9	63.6
	±0.5	42.4	24.0	66.4

Table 2 – Percent of neurons with direction bias scores that fall outside of the standard deviation of the shuffled distributions, by SCHR condition. The "below" column corresponds to neurons that fall outside of this region with negative bias, and the "above" column corresponds to neurons with positive bias.

In Table 2, the percent of neurons with direction bias scores that fall outside one standard deviation of the shuffled distribution is reported. For each SCHR condition, the majority of neurons have direction biases which can be distinguished from the measurement noise. Neurons' responses to lower F0 SCHR chirps are generally more discriminable than higher F0s, but there is not a clear relationship between +/-1 and +/- 0.5 C-conditions. For all SCHR conditions, more discriminable neurons have negative direction bias than positive. Table 2 demonstrates that individual neurons' direction bias scores are most commonly negative, but even within SCHR conditions with overall negative mean direction bias (i.e. 50 Hz, C = +/- 1), there are many neurons with positive direction bias scores.

D. MTF shape is not strongly related to prevalence of direction selectivity nor the direction of direction bias

The prevalence of SCHR chirp direction selectivity depends on F0, hence it is relevant to determine whether prevalence of direction selectivity is related to MTF type. Table 1 shows ROC analysis results for neurons separated by MTF shape. BE neurons display a greater prevalence of direction selectivity for individual SCHR conditions compared to other MTF-type groups (BS and Hybrid) as well as the overall group. BS neurons display lower prevalence of direction selectivity for individual SCHR conditions compared to the overall group. Direction selectivity prevalence of hybrid neurons appears comparable to the overall group. Interestingly, despite the large differences between BE and BS groups within individual SCHR conditions, the percentage of neurons in each group that are direction selective at any SCHR condition (bottom row) are similar. This could mean that BS neurons are more frequently selective only at a few SCHR conditions compared to BE neurons. Direction selectivity prevalence among BE, BS, and Hybrid neurons is high, with Hybrid neurons displaying the lowest amount.

In Figure 1, histogram bins are divided into stacked color bars indicating MTF shapes. Mean direction biases for these MTF shapes are marked with a downward triangle of their corresponding color. Groups with means significantly different from zero are marked with an asterisk in the upper left corners. This figure shows that neurons of each MTF shape—BE, BS and Hybrid—are generally biased for negative SCHR chirps. This negative bias is significant generally for lower F0s, although 200 Hz, C = +/- 1 and 400 Hz, C = +/- 0.5 are also significant (for BE and Hybrid, and for Hybrid, respectively). Additionally, the MTF group direction biases differ from the overall biases by showing less/no significant bias for C = +/- 0.5 SCHR conditions. Within MTF subgroups, there are differences between all three with respect to SCHR condition, but not in a way that

appears systematic. Notably, the Hybrid group is significantly negatively biased at 400 Hz, C = +/- 0.5 while the BE and BS groups are not. Also, BS neurons are significantly negatively biased for 50 Hz F0 SCHR groups, while BE and Hybrid neurons are not.

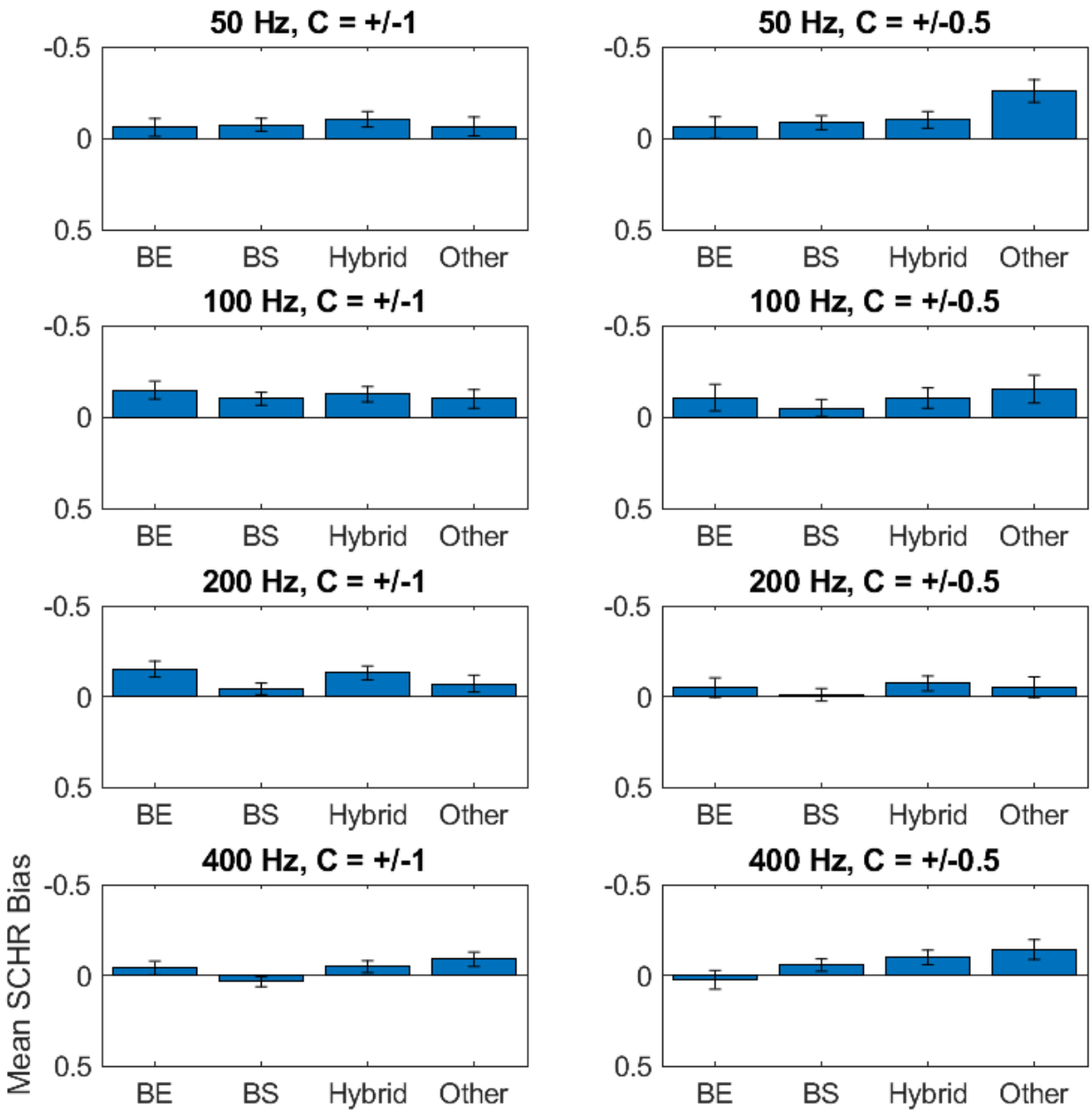

Figure 2 – ANOVAs show no difference in means between MTF groups for any SCHR condition ($p < 0.05$). Reported error bars are standard error of the means.

Figure 2 shows the MTF group means for each SCHR condition. ANOVAs performed on each condition show no statistical difference between MTF group means for any condition. This result indicates that MTF shape does not have a strong influence on the direction of direction bias scores.

E. CF range is related to direction of direction bias

Table 1 shows the results of ROC analysis for neurons separated by CF range—low (<3 kHz), mid (>3 kHz, <6 kHz), and high (>6 kHz). Low and high CF neurons show similar prevalence of direction selectivity for individual SCHR conditions to the overall group, but mid CF neurons generally have greater prevalence for individual SCHR conditions compared to the overall group. Despite this, the mid CF group has a comparable percentage of neurons that are selective for any condition, compared to the overall group. Conversely, the low CF group has the highest direction selectivity prevalence for any group tested, at 97.2%. This suggests that low CF neurons are selective at fewer SCHR conditions simultaneously compared to mid CF neurons. Finally, the high CF group has a lower percentage of neurons selective for any condition compared to the overall group.

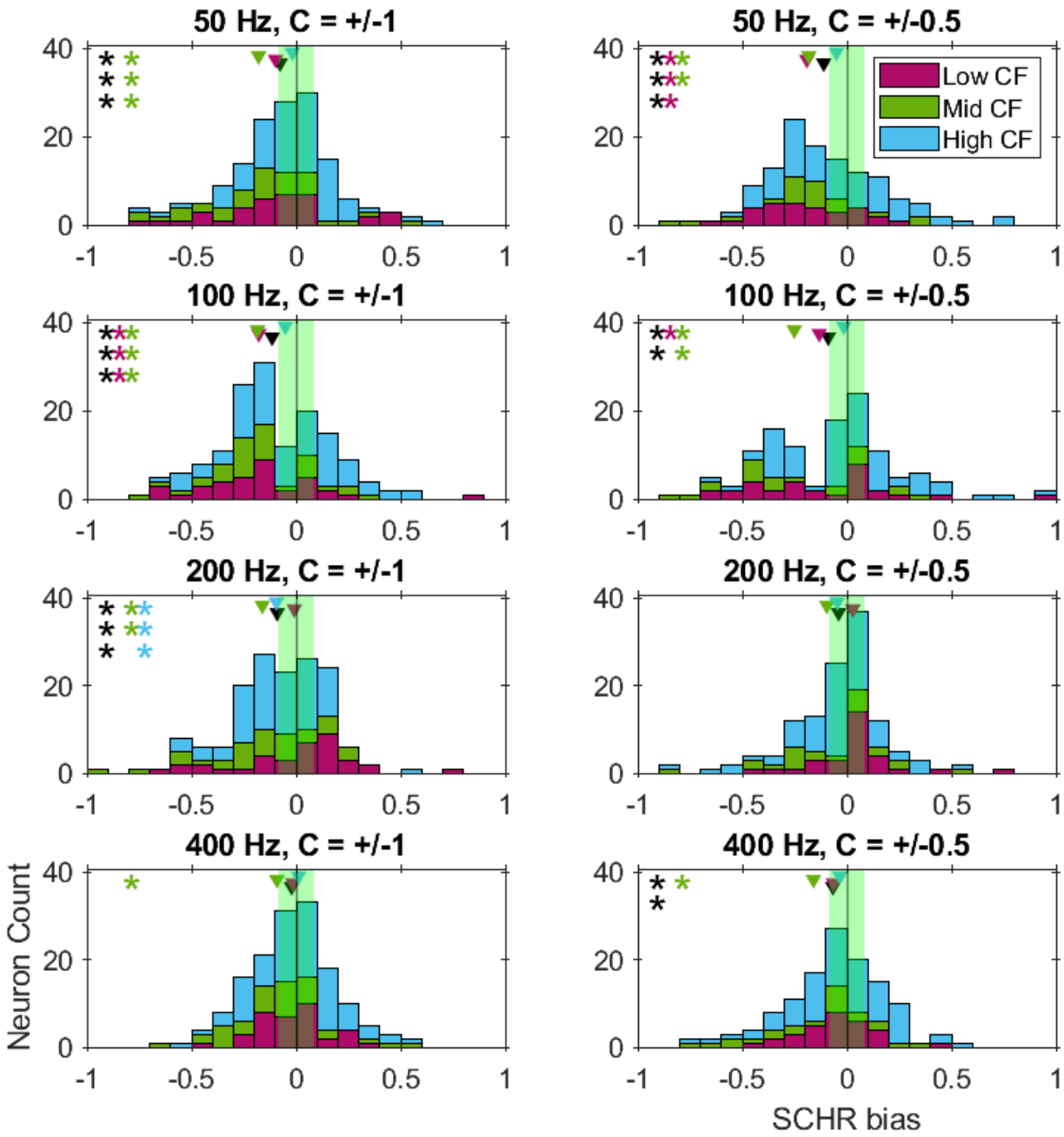

Figure 3 - Histograms of direction bias separated by MTF shape for SCHR responses (bin width = 0.05). Panels are organized by F0 (rows) and C (columns). 154 neurons are represented for C = +/- 1 conditions (left column), and 125 neurons are represented for C = +/- 0.5 conditions (right column). Stacked color bars denote CF ranges: low (<3 kHz, magenta), mid (>3 kHz and <6 kHz, green), and high (>6 kHz, cyan). Population mean

*direction bias is marked with a black triangle. Mean direction biases for low, mid, and high CF neuron subpopulations are marked by triangles of their respective colors. Asterisks in the top left corner of each panel indicate that the means are significantly different than zero (one-sample t-test, two-tailed, * p < 0.05, ** p < 0.01, *** p < 0.001). Transparent rectangle indicates standard deviation of the direction bias distribution after within-SCHR-condition shuffling, analogous to the noise floor of the initial distribution.*

In Figure 3, histogram bins are divided into stacked color bars indicating CF ranges. As in the previous histogram, groups with means significantly different from zero are marked with an asterisk in the upper left corners. All CF groups are generally biased towards negative chirps. Low CF neurons appear to be significantly negatively biased at lower F0s, but show no significant bias at higher F0s. Mid CF neurons are negatively biased robust to F0, with all but one SCHR condition (200 Hz, C = +/- 0.5) significantly selective. In contrast, high CF neurons are not significantly biased for either direction for any SCHR condition, except for 200 Hz, C = +/- 1. Unlike the MTF groups, the three CF groups have distinct direction bias profiles with respect to F0 and C.

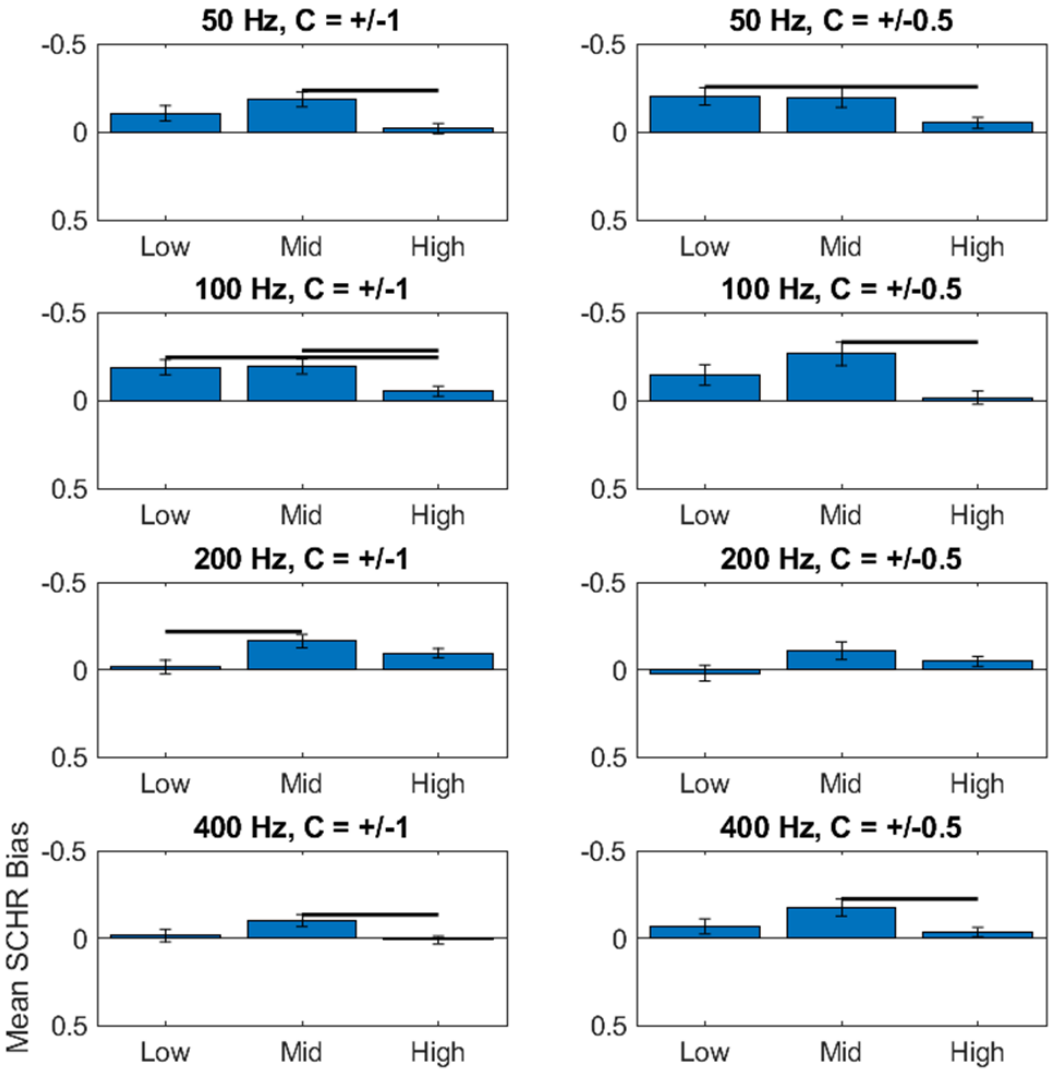

Figure 4 – Comparison of CF group direction bias means of SCHR responses, with bars indicating statistical significance (Tukey-Kramer test, p < 0.05). Reported error bars are standard error of the means.

Figure 4 shows the CF group means for each SCHR condition. Following a one-way ANOVA, a Tukey-Kramer multiple comparison test was applied for each SCHR condition to determine statistically significant differences between bias means. For two SCHR conditions, low CF means are significantly more negatively

biased than high CF means (50 Hz, C = +/- 0.5 and 100 Hz, C = +/-1). Both of these SCHR conditions are for lower F0s. For higher F0s, low CF means tend towards zero, with one SCHR condition (200 Hz, C = +/- 1) significantly different that the relatively more biased mid CF neurons. Taken together, this suggests that low CF neurons are more strongly negatively biased at low F0s, and not biased for higher F0s. Mid CF neurons bias means are significantly more negative than those of high CF neurons for five SCHR conditions (50 Hz, C = +/- 1, both 100 Hz and both 400 Hz conditions). As seen in Figure 3, the negative bias of the mid CF group is robust to F0 changes. Similarly, the high CF group appears to be relatively unbiased, regardless of F0. Overall, there are strong differences in direction bias score across CF groups.

F. Direction bias of ARC responses reflect SCHR chirp responses

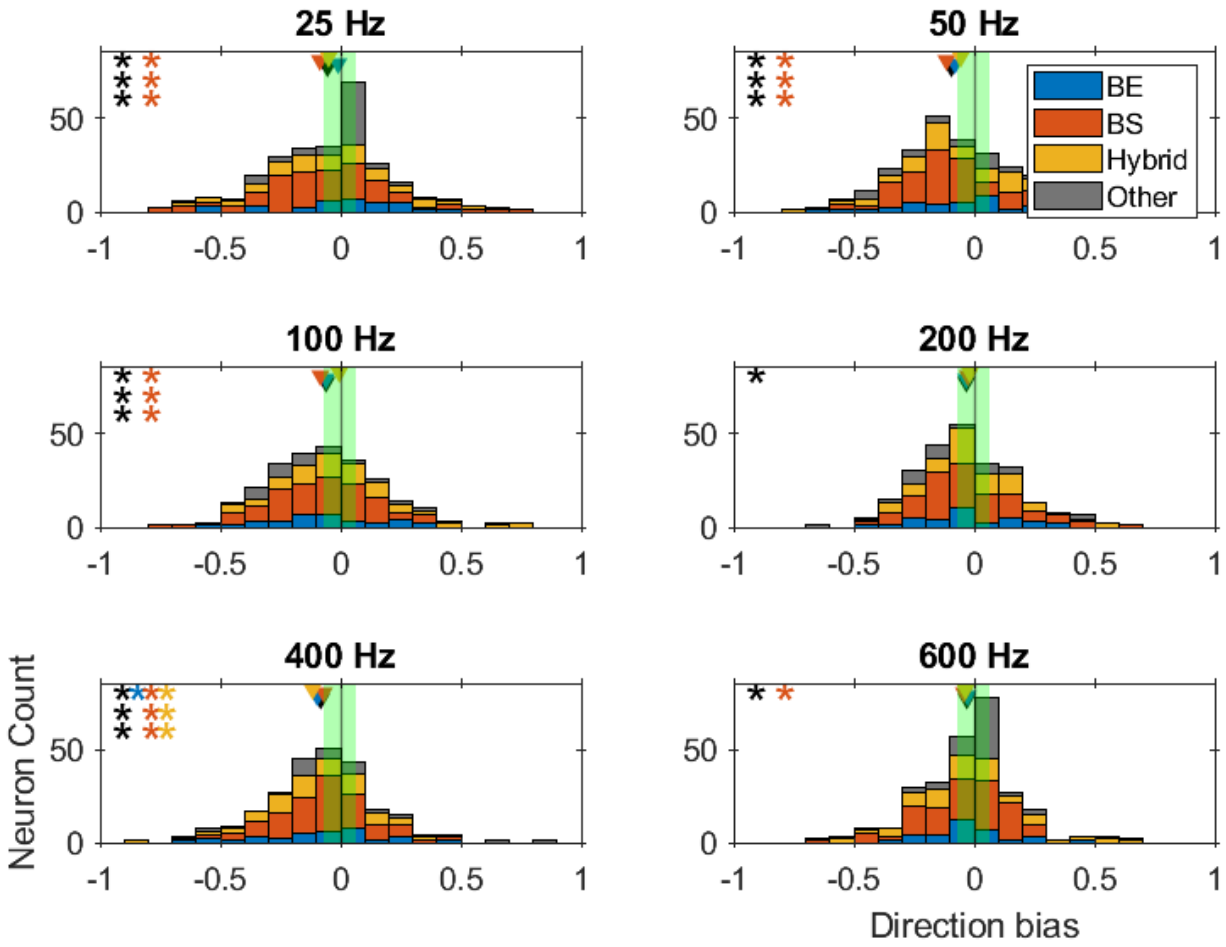

Figure 5 - Histograms of direction bias separated by MTF shape of ARC responses (bin width = 0.05). 272 neurons are represented for each panel, which are organized by equivalent-SCHR-F0 (increasing chirp velocity, left-to-right, top-to-bottom). Otherwise, histograms are identically arranged to Figure 1.

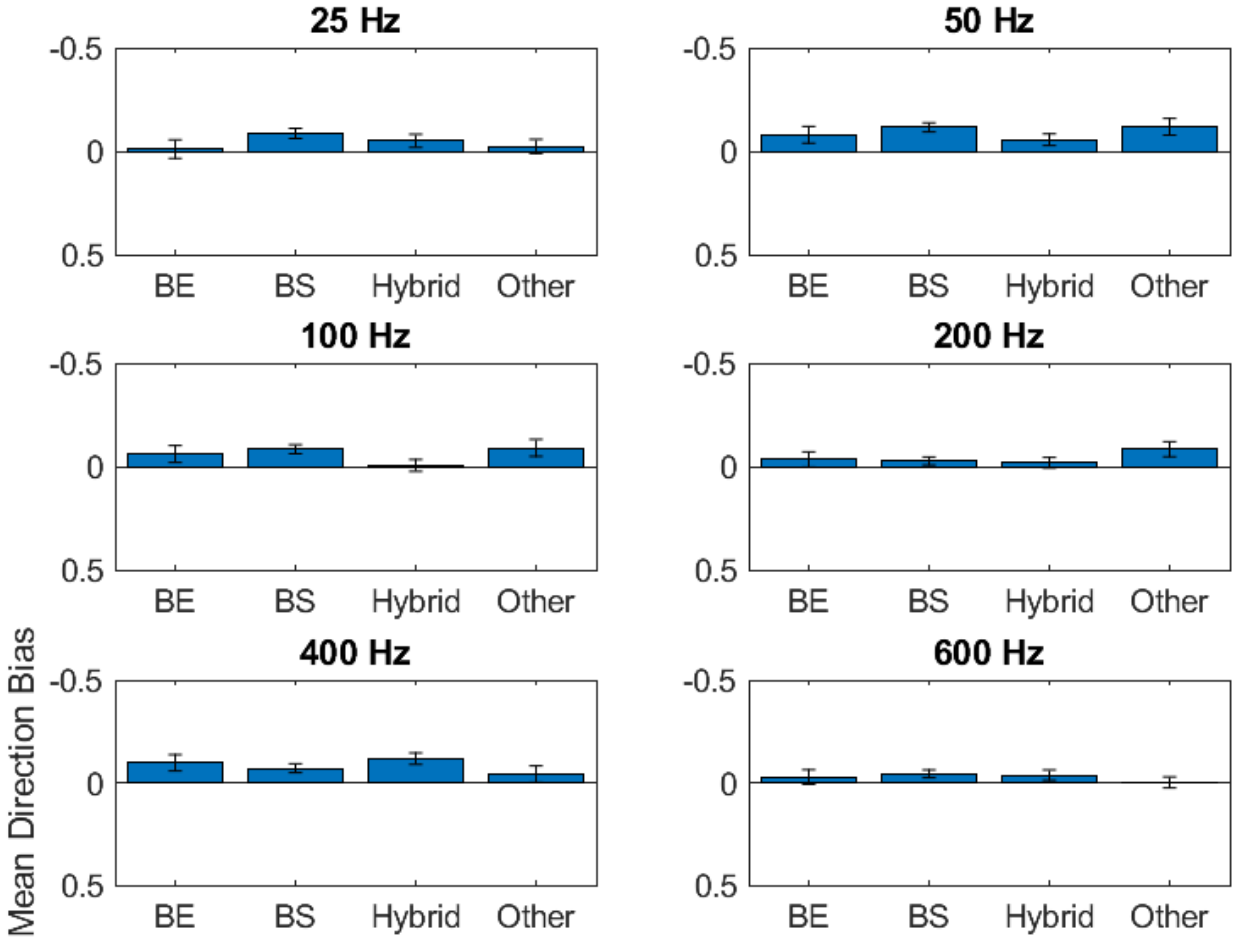

Figure 6 - ANOVAs show no difference in means between MTF groups for any ARC condition ($p < 0.05$). Reported error bars are standard error of the means.

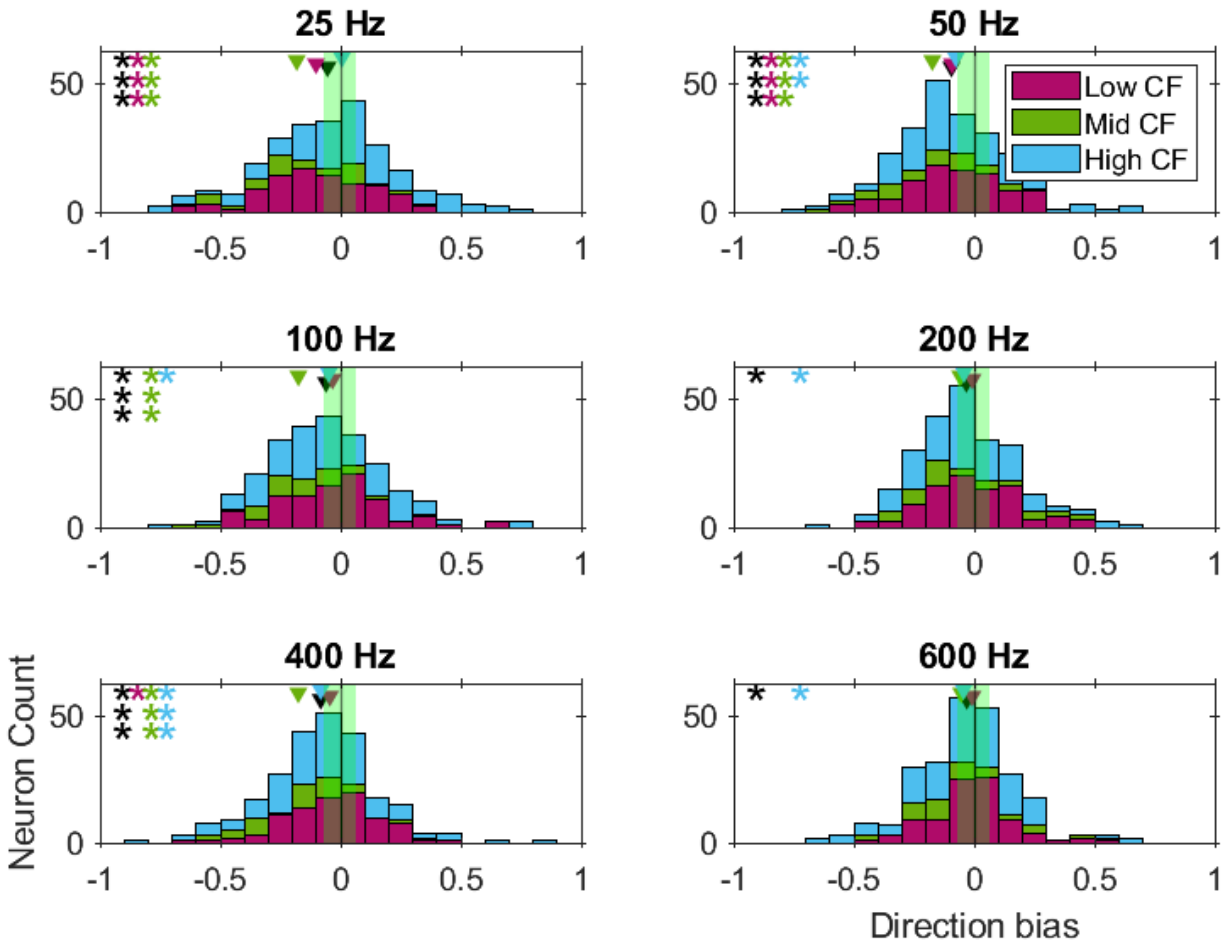

Figure 7 - Histograms of direction bias separated by CF range of ARC responses (bin width = 0.05). 272 neurons are represented for each panel, which are organized by equivalent-SCHR-F0 (increasing chirp velocity, left-to-right, top-to-bottom). Otherwise, histograms are identically arranged to Figure 1.

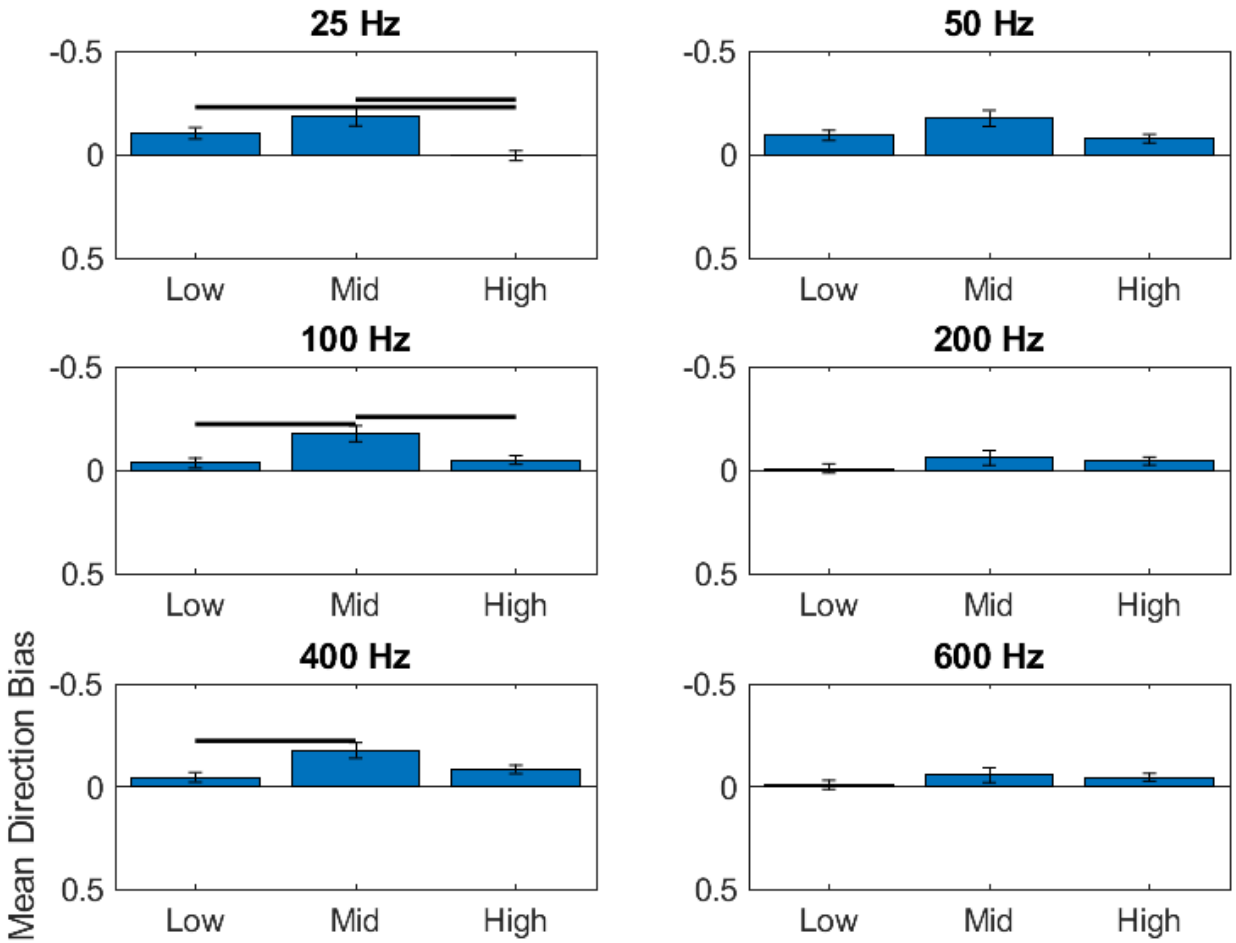

Figure 8 - Comparison of CF group direction bias means of SCHR responses, with bars indicating statistical significance (Tukey-Kramer test, $p < 0.05$). Reported error bars are standard error of the means.

Many of the same analyses ran for SCHR responses were ran for ARC stimuli responses. The direction bias profiles seen in SCHR responses were generally preserved for ARC responses. Figure 5 shows that the means for the overall group at all chirp conditions are significantly lower than zero, mirroring the result seen for SCHR responses. Also like the SCHR results, each MTF group is negatively biased for at least one chirp condition. However, for ARC response biases, BS neurons appear to be strongly negatively biased for all chirp conditions except for 200 Hz. Conversely, BE and Hybrid neurons are not significantly biased for any chirp condition except for 100 Hz. This may suggest that BS neurons are more selective for negative chirp directions when those chirps are aperiodically presented. Likewise, BE and Hybrid neurons may be less selective for negative aperiodic chirps. Despite these differences from SCHR response biases however, Figure 6 shows that there are no significant differences between the means of MTF group bias scores for any chirp condition.

Figure 7 shows that low CF neurons are significantly negatively biased for lower equivalent-F0s (and therefore lower chirp velocities), but this bias recedes for higher chirp velocities. Mid CF neurons appear to be more robust to this change in chirp velocity/equivalent-F0, with significant negative bias scores at 25 Hz, 50 Hz, 100 Hz, and 400 Hz chirp conditions. Breaking from the pattern observed in SCHR responses, high CF neuron also have a robust negative direction bias that spans all chirp conditions except for 25 Hz. Figure 8 depicts relatively fewer significantly different pairs compared to the SCHR response biases in Figure 4. Low CF neurons at 25 Hz equivalent-F0 are significantly more negatively biased than high CF neurons, but by 100 Hz and 400 Hz, low CF neuron bias scores are closer to zero, and are significantly lower than mid CF neurons. Mid CF neurons are significantly more negatively biased than high CF neurons for 100 Hz equivalent-F0, but

for no other condition due to the relatively increased bias for high CF neurons (compared to SCHR response biases).

G. Velocity cues facilitate maximal predictions of SCHR rate using the GLM

Starting from a premise that responses to SCHR stimuli are dependent on AM (periodicity) tuning and/or velocity tuning, we used MTFs and ARC responses to predict SCHR responses using the GLM. Figure A shows a visualization of this strategy on an example neuron (NAME HERE). Here, the neuron's SCHR response is shown as a surface plot on F0-Velocity axes; darker colors correspond to higher response rates. This neuron responded more strongly to lower F0s and to –SCHR (Figure A, top left). The color bars above and to the left of the SCHR surface are the MTFN and ARC responses of the neuron, respectively, and describe how the neuron's response rate depends on periodicity or velocity alone. Using a weighted combination of these two responses, we can use the GLM to predict the SCHR response (Figure A, top right). By fitting an equation that uses both periodicity and velocity terms (Eqn. 2), a highly accurate prediction of the recorded SCHR data was made ($R^2 = 0.97$). Removing either the periodicity or velocity terms reduced the quality of the prediction. Predicting the SCHR response using the MTFN-only model (Eqn. 2) results in the disappearance of the distinct direction selectivity at lower F0s (Figure A, bottom left). Notably, there is no way a model using periodicity tuning alone can explain the observed direction selectivity. Conversely, a prediction of SCHR response using the ARC -only model (Eqn. 3) only explained a small amount of variance in the data ($R^2 = 0.10$). This neuron is somewhat typical in that more variance in the data can be explained by the MTFN-only model than ARC -only model. However, this neuron is also not unusual in that the ARC terms contribute a large percentage of the variance explained by the combined model, as compared to the MTFN-only model.

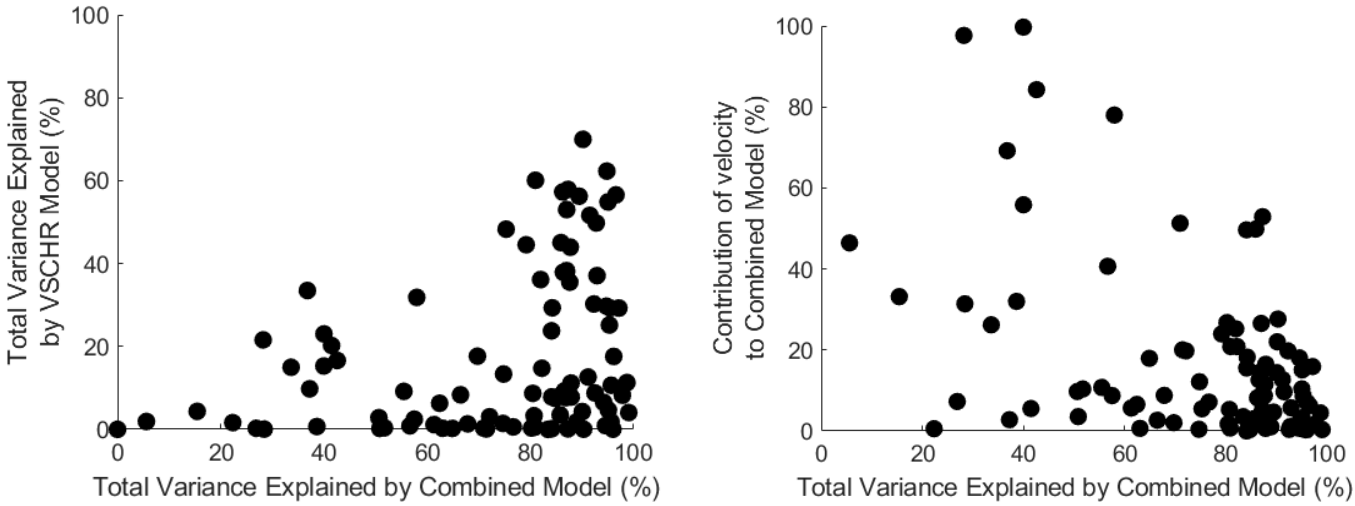

Figure 9 – A. (left) Percent variance explained in the SCHR response using the ARC model alone versus percent variance explained by the combined model. B. (right) Percent contribution of the ARC terms to the total explainable variance by the combined model versus.variance explained by the combined model.

Using this method, an R^2 value was obtained for each of these three models by comparing predictions to neural responses. Figure 9a is a scatterplot that shows the R^2 values from the ARC-only model versus the R^2 from the combined model. Many neurons are clustered around the x-axis, indicating that the total variance explained by the ARC-only model is low. This conversely suggests that periodicity tuning is the more important feature in predicting a neuron's SCHR response. However, for some neurons with a high total explainable variance by the combined model (60-100%), the ARC-only model explains between 10-70% of variance in the data, suggesting a substantial contribution by velocity tuning.

Figure 9b shows the contribution of the ARC terms to the total explainable variance versus variance explained by the combined model. This figure makes apparent that for many neurons with relatively low (20-60%) total explainable variance, the contribution of the velocity terms can explain over 50% of the explained variance. This suggests that velocity response properties are important predictors of these neurons' SCHR response, perhaps more so than the relatively weaker MTF predictor.

Finally, as demonstrated by the example neuron (Figure A), direction selectivity between SCHR pairs cannot be explained with a model using periodicity alone. Because SCHR direction selectivity is common (92.0%, Table 1a), most IC neurons responses are necessarily influenced by velocity sensitivity to some extent.

Discussion

SCHR direction selectivity exists on an individual level. The vast majority of neurons are sensitive for SCHR direction for at least one condition. Most neurons are significantly biased towards the –SCHR direction. There is not a significant effect of MTF type on prevalence of direction selectivity, with BE and BS neurons having essentially the same percentage of sensitive neurons. There was also not an effect of MTF type on direction of SCHR selectivity. Neurons of low to mid CF were more often selective for SCHR chirp direction when compared to high CF neurons. Low and mid CF neurons were also more significantly biased for negative SCHR chirp directions than high CF neurons. For low CF neurons this bias was stronger for lower F0s, but for mid CF neurons this bias was robust to F0. ARC responses portray the same stories, with MTF not having a large effect on prevalence of direction selectivity or direction of chirp bias, and CF having an effect on direction bias (which is greater for lower velocities). Overall, based on regression lines, ARC bias is a weak predictor of SCHR bias. However, SCHR velocity selectivity is an underlying influence on SCHR response rate, but it is not as big of an influence of SCHR response rate as AM tuning.

Many possible mechanisms for chirp sensitivity have been suggested in the literature. IC neurons receive excitatory and inhibitory inputs with varying frequencies and latencies that may interact to produce chirp selectivity (Pollak et al., 2011). The interaction between excitatory and inhibitory inputs varying in latency has long been proposed as a mechanism for frequency sweep selectivity (Gordon and O'Neill, 1998). Work in bats have outlined a mechanism that imagines a chirp passing through an excitatory field with inhibitory sidebands (Gordon and Oneill, Fuzessery). Additionally, travelling wave latency is another way to generate direction differences. EE interactions through coincidence detection is a way to produce velocity sensitivity (Krips and Furst). A combination of EE vs EI mechanisms may be at play.

From Recio et al., there is a "peakiness" difference in envelope between SCHR directions. Filter asymmetry leads to differences in amplitude at output of cochlear filter, which is later compressed, leading to lower amplitude +SCHR than –SCHR. However, this filter asymmetry changes with frequency. We already know that this can't explain the full story, or else every neuron would be selective for -SCHR. Neurons exist with same CF but different direction selectivities, and provide evidence against this model.

Steenken et al. discusses a TFS-based coding scheme for low CF AN neurons and ENV-based scheme for high CF neurons – which gets converted into rate at the IC. This seems to be related to the fact that they saw higher rate differences at higher CFs.

One possible reaction to this work is to ask whether the fast chirp sensitivity observed is unique to Schroeder-like stimuli, and whether an alternative stimulus could be proposed to reflect the same sensitivity. Similar stimuli exist in the literature to illustrate neurons' tuning properties in response to high-velocity spectrotemporal fluctuations. One example is the Dynamic Moving Ripple (DMR) stimulus described in Escabi and Schreiner (2002). Designed to mimic spectrotemporal modulations present in natural sounds such as speech or animal vocalizations, the stimulus is composed of a bank of sinusoidal carriers with an imposed spectrotemporal envelope. This stimulus is used to generate a spectrotemporal receptive field (STRF), which describes how a neuron's response properties vary over frequency and time. The stimulus works well to identify neurons' sensitivity to slower frequency modulations, but is not able to generate chirps of equivalent velocity to those found in SCHR stimuli. The ripple velocity of the DMR stimulus is roughly controlled by an instantaneous modulation frequency random variable Fm (i.e. frequency of the imposed envelope). In the original study, this variable has an upper bound of 350 Hz; meanwhile, in order to achieve an equivalent velocity to the SCHR chirps used in this study, this upper bound would have to increase to beyond 5000 Hz. This far outstrips the limit of phase locking in the inferior colliculus (Kuwada, 1987), rendering the DMR stimulus ineffective at describing response properties to chirps of this speed. This further highlights the SCHR

stimulus' uniqueness – SCHR chirps are both "fast" in the velocity sense, and "slow" in the phase-locking sense, with F0s low enough for IC neurons to consistently and periodically respond to.

STRFs are an insufficient way of portraying this kind of chirp sensitivity. There is a timing discrepancy between STRF and SCHR velocities. Additionally, SCHR selectivity may be non-linear, and noise may not contain salient-enough chirps.

Recent work in the lab on the role of efferents raise interesting questions surrounding a perceived "build-up" effect observed in SCHR responses.

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