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Mechanisms underlying intensity-dependent changes in cortical selectivity for frequency-modulated sweeps

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Razak KA. Mechanisms underlying intensity-dependent changes in cortical selectivity for frequency-modulated sweeps. J Neurophysiol 107: 2202-2211, 2012. First published January 25, 2012; doi:10.1152/jn.00922.2011.-Frequency-modulated (FM) sweeps are common components of species-specific vocalizations. The intensity of FM sweeps can cover a wide range in the natural environment, but whether intensity affects neural selectivity for FM sweeps is unclear. Bats, such as the pallid bat, which use FM sweeps for echolocation, are suited to address this issue, because the intensity of echoes will vary with target distance. In this study, FM sweep rate selectivity of pallid bat auditory cortex neurons was measured using downward sweeps at different intensities. Neurons became more selective for FM sweep rates present in the bat's echolocation calls as intensity increased. Increased selectivity resulted from stronger inhibition of responses to slower sweep rates. The timing and bandwidth of inhibition generated by frequencies on the high side of the excitatory tuning curve [sideband high-frequency inhibition (HFI)] shape rate selectivity in cortical neurons in the pallid bat. To determine whether intensity-dependent changes in FM rate selectivity were due to altered inhibition, the timing and bandwidth of HFI were quantified at multiple intensities using the two-tone inhibition paradigm. HFI arrived faster relative to excitation as sound intensity increased. The bandwidth of HFI also increased with intensity. The changes in HFI predicted intensity-dependent changes in FM rate selectivity. These data suggest that neural selectivity for a sweep parameter is not static but shifts with intensity due to changes in properties of sideband inhibition.

vocal processing; auditory cortex; sideband inhibition

SENSORY SYSTEMS OPERATE OVER a wide range of stimulus intensities. Perceptual constancy for stimulus features with intensity may arise, because neural response selectivity is intensity tolerant or through interpretations of intensity-dependent changes in population activity patterns. In the auditory system, intensity-tolerant neuronal responses have been suggested to underlie perceptual invariance for complex sounds (e.g., vocalizations) with intensity (Sadagopan and Wang 2008; Suga 1977). However, spectral and temporal response properties, which may contribute to recognition of vocalizations, change with intensity (Krishna and Semple 2000; Lesica and Grothe 2008). Thus the extent to which single neuron selectivity for vocalizations is invariant to sound intensity remains unclear.

Frequency-modulated (FM) sweeps are important components of many species-specific vocalizations, including human speech. The presence of FM sweep components is important in speech recognition (Zeng et al. 2005). Cortical neural selectivity for the rate and direction of FM sweeps is present in every species examined (Atencio et al. 2007; Brown and Harrison 2009; Hart et al. 2002; Heil et al. 1992b; Mendelson et al. 1993; Nelken and Versnel 2000; Razak and Fuzessery 2002; Shamma et al. 1993; Suga 1965; Tian and Rauscheker 1994; Trujillo et al. 2011; Washington and Kanwal 2008). The impact of sound intensity on FM sweep selectivity has not received much attention.

The first objective of this study was to determine whether FM sweep rate selectivity is influenced by intensity in the auditory cortex of the pallid bat, which produces a downward FM sweep to echolocate obstacles along its flight path. The sweep traverses frequencies between 20 and 70 kHz with durations between 2 and 5 ms. Approximately 70% of the neurons in the auditory cortex, tuned to the echolocation call frequencies, are sweep direction and rate selective (Razak and Fuzessery 2006). Whether this selectivity changes with intensity is not known. The effect of intensity on FM sweep selectivity in bats is of behavioral relevance, because bats actively change the intensity of echolocation calls (Boonman and Jones 2002) or receive different intensity echoes because of changes in target distance.

FM sweep selectivity, at least in part, arises through interactions between the excitatory and inhibitory receptive field components (Fuzessery et al. 2006; Gittelman and Pollak 2011; Gordon and O'Neill 1998; Razak and Fuzessery 2006, 2009). In the pallid bat, the timing and bandwidth of sideband inhibition, relative to excitatory frequencies, predict rate selectivity for the downward sweeps used in echolocation (Razak and Fuzessery 2006, 2009). Inhibition generated by frequencies on the high side of the excitatory tuning curve [high-frequency inhibition (HFI)] arrives later than excitation generated by the characteristic frequency (CF) tone. Therefore, downward sweeps with fast sweep rates can reach the excitatory frequencies before inhibition arrives and elicit a neural response. For slow downward sweeps, the delayed inhibition has sufficient time to arrive at the neuron with or before the excitation and reduces responses. Thus the delayed HFI shapes rate selectivity for downward FM sweeps. The second objective of this study was to determine whether intensity-dependent changes in interactions between excitatory and inhibitory inputs predict FM rate-selectivity changes.

METHODS

Pallid bats were netted in Arizona, California, and Texas and held in a 11 \times 14-ft room at the University of California, Riverside. The bats were able to fly in this room and were provided crickets/ mealworms and water ad libitum. The room was maintained on a reversed 12:12 light cycle. All animal-use protocols of this study were approved by the Institutional Animal Use and Care Committee, and all

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procedures followed the animal welfare guidelines required by the National Institutes of Health and the Institutional Animal Care and Use Committee.

Surgical procedures. Recordings were obtained from the right auditory cortex of bats (n = 12, both males and females), anesthetized with isoflurane inhalation, followed by an intraperitoneal injection of pentobarbital sodium (30 μ g/g body wt) and acepromazine (2 μ g/g body wt). To expose the auditory cortex, the head was held in a bite bar, a midline incision was made in the scalp, and the muscles over the dorsal surface of the skull were reflected to the sides. The front of the skull was scraped clean and a layer of glass microbeads applied, followed by a layer of dental cement. The bat was then placed in a Plexiglas holder. A cylindrical aluminum head pin was inserted through a cross-bar over the bat's head and cemented to the previously prepared region of the skull. This pin served to hold the head secure during the recording session. The cross-bar holding the head pin was secured behind the bat, leaving no interference between the speaker and the ear. The location of A1 was determined relative to the rostrocaudal extent of the midsagittal sinus, the distance laterally from the midsagittal sinus, and the location of a prominent lateral blood vessel, which lies parallel to the midsagittal sinus. The size of the exposure was usually $\sim 2 \text{ mm}^2$. Exposed muscle was covered with petroleum jelly, and exposed brain surface was covered with silicone oil to prevent desiccation.

Recording procedures. Experiments were conducted in a warm (~80°F), sound-proof chamber, lined with anechoic foam (Gretch-Ken Industries, Lakeview, OR). Bats were kept anesthetized throughout the course of the experiments with additional pentobarbital sodium (one-third of a presurgical dose) injections. Acoustic stimulation and data acquisition were driven by custom software (Batlab; written by Dr. Donald Gans, Kent State University, Kent, OH) and a digital signal processing (DSP) board (Microstar Laboratories, Bellevue, WA). Programmable attenuators (PA5; Tucker-Davis Technologies, Alachua, FL) allowed control of sound intensities before amplification by an integrated amplifier (Yamaha AX-430). Stimuli were delivered using an LCY-K100 ribbon tweeter (Ying Tai Trading, Hong Kong; distributed by Madisound, Middleton, WI), placed 8 inches from the left ear at 45° to the long axis of the bat's body. The amplifier-speaker frequency response curve, measured with a one-fourth-in microphone (Brüel and Kjaer, Denmark), was flat within \pm 3 dB for frequencies from 7 to 40 kHz. The roll-off from 40 to 70 kHz was 20 dB/octave.

All data shown are from extracellular single-unit recordings, identified based on window discriminator threshold-crossing and consistency of action potential amplitude and waveform displayed on an oscilloscope. Recordings were obtained using glass electrodes (1M NaCl, 2–10 M Ω impedance) at depths between 200 and 600 μ m. Penetrations were made orthogonal to the surface of the cortex. Action potentials were amplified by an extracellular preamplifier (2400A; Dagan, Minneapolis, MN) and a spike signal enhancer (FHC, Bowdoin, Maine) and band-pass filtered (0.3-3 kHz, Krohn-Hite, Brockton, MA). Waveforms and peri-stimulus time histograms were stored using the Microstar Laboratories DSP board and Batlab software. Responses were quantified as the total number (20 stimulus repetitions; 1 Hz repetition rate) of action potentials occurring within 200 ms of stimulus onset. Adjustments for spontaneous activity were not necessary, because there was no spontaneous activity in these recordings.

Data acquisition. The focus of this study was on the high-frequency FM sweep-selective region of the pallid bat A1. This region is likely to be involved in echolocation behavior. The FM sweepselective region contains neurons tuned between 25 and 70 kHz and is located rostral and medial to the lower frequency neurons (tuning 5–35 kHz), which are noise selective (Razak and Fuzessery 2002, 2006). The FM sweep-selective neurons respond better to downward sweeps than to noise and upward sweeps that contain the same spectral energy (Razak and Fuzessery 2002). With the use of tones, noise, and upward and downward sweeps as search stimuli, neurons with CF >25 kHz and a stronger response to downward FM sweeps than noise and upward FM sweeps were isolated. The following response properties were then determined.

Excitatory frequency tuning curve. Pure tones (25–70 kHz, 5 ms duration, 1 ms rise/fall times, 1 Hz repetition rate) were used to determine the CF and minimum threshold (MT) for tones. CF was defined as the frequency that elicited action potentials to at least five successive stimulus repetitions at the lowest intensity. The intensity was then increased in 5 or 10 dB steps to record the frequency-intensity combinations that produced excitatory responses (tuning curve).

FM rate selectivity. FM rate-selectivity functions were obtained at three different intensity ranges above MT for FM sweep responses: 5-9 dB (level I), 10-14 dB (level II), and 15-30 dB (level III). Preliminary studies indicated that neurons, which were nonmonotonic for FM sweep intensities, typically start to show reduced responses for intensities >15-20 dB above MT. Therefore, the maximum intensity range chosen was in the 15- to 30-dB above MT. The minimum range was within 5-9 dB of MT to test the selectivity close to threshold. The actual intensity used within each level range varied across neurons. Rate selectivity was determined by recording responses to a linear downward sweep with 20-30 kHz bandwidth centered near the CF and presented with durations of 2-200 ms (sweep rates between 0.1 and 15 kHz/ms). The sweep parameters chosen encompassed those found in the echolocation call of the pallid bat. It should be noted that even at the fastest rates used, FM sweep-selective neurons respond selectively to sweep parameters (e.g., better response to downward than upward sweeps) and can distinguish between fast sweep rates and noise (Razak and Fuzessery 2002, 2006). It has also been shown previously that most neurons tuned to the echolocation frequencies are selective for sweep rate and not simply sweep duration or bandwidth (Razak and Fuzessery 2006). The sweep duration-response function was then converted to rate-selectivity functions by dividing the sweep bandwidth by the sweep duration (kHz/ms). The sweep bandwidths, durations, and intensities were presented in a randomized order. A neuron was considered FM rate selective if responses declined by >50% of maximum response with changing sweep rate.

To quantify the degree of rate selectivity, 50% cut-off rate and the rate-tuning index (RTI) were calculated for each neuron. The 50% cut-off rate is the rate at which the neuronal response decreases to 50% of maximum as sweep rate is slowed. For neurons with a peak rate-selectivity function (responses declined on both side of a maximum), only the 50% cut-off for slow rates was quantified. This was because preliminary data showed that many neurons did not have an upper 50% cut-off rate when the intensity was increased (e.g., see Figs. 1 and 2).

The RTI is a measure of rate selectivity. This measure is called "speed tuning" in the literature (Atencio et al. 2007; Brown and Harris 2009).

The RTI was calculated as follows: $RTI = (n/n - 1) \times [1 - (mean/max)]$, where "n" is the number of FM sweep rates assessed, "mean" is the average response across all rates tested, and "max" is the maximum response. RTI values lie between 0 and 1 with higher values indicating more selectivity.

Two-tone inhibition over time-tuning curves. To determine the arrival time and bandwidth of inhibition, a "two-tone inhibition over time" method was used (Brosch and Schreiner 1997; Calford and Semple 1995; Fuzessery et al. 2006; Gordon and O'Neill 1998; Razak and Fuzessery 2006). Two tones, one excitatory (at CF, 5 ms duration) and the other inhibitory (10 ms duration), were presented with different delays between them. The intensity of both tones was the same. The frequency of the inhibitory tone was varied between 25 and 70 kHz (1 kHz steps), and its onset time varied with respect to that of the excitatory tone. The delay-frequency combinations that resulted in inhibition of response to the excitatory tone for at least four out of five (80% inhibition) consecutive presentations served to map out the bandwidth and arrival times of HFI. Inhibition, which occurred only

when the excitatory tone was delayed, denotes inhibition that arrived after excitation ("delayed" or "slow" inhibition). Inhibition, which occurred even when the excitatory tone was advanced, denotes inhibition that arrived before excitation ("early" or "fast" inhibition).

To obtain more accurate quantification of the arrival times of HFI, the two-tone procedure was repeated with the best inhibitory frequency (center frequency of the HFI bandwidth) (K. A. Razak and Z. M. Fuzessery, unpublished observations) and the CF tone to determine the delay at which response magnitude decreased to 50% of the excitatory tone-alone (control) response. This value is referred to as the arrival time of inhibition. It is important to note that the phrase "arrival time" is used to describe the relative delay between the two tones, at which the response to the CF tone is inhibited. Terms such as "faster" or "slower" arrival times are used to describe the observation that inhibition occurred at shorter or longer delays between the two tones, respectively. These terms are not intended to suggest absolute changes in latency of inhibitory or excitatory inputs. The absolute latency of inhibitory inputs cannot be determined with the methods used in this study.

The two-tone inhibition procedure was repeated at three different intensities (*levels I*, *II*, *III*), based on MT for the CF tone. The intensity of both tones was increased or decreased in tandem. Because of the frequency response characteristics of the speaker, the intensities of the two tones may not be identical, and this was not adjusted for. This is unlikely to affect the results, because in almost all neurons, the HFI was within 7 kHz of the CF. This small difference in frequencies will not result in a large difference in speaker output for the two tones (20 dB/octave roll-off for frequencies between 40 and 70 kHz). The comparison of two-tone inhibition across intensity will not be affected, because both tones were changed by the same intensity. The absolute tone and sweep intensities tested were typically similar, because the MTs for these sounds were within 5 dB of each other.

Data representation and analysis. Response magnitudes are displayed as the number of action potentials elicited by 20 stimulus repetitions at 1 Hz repetition rate. Error bars show SE. One-way ANOVA with a Tukey test for post hoc pair-wise comparisons was used for statistical comparisons of mean (\pm SE) response selectivity measures at the three intensity levels. Significant differences were inferred at a P < 0.05 level.

RESULTS

Neurons become selective for faster sweep rates with increasing intensity. The effect of intensity on FM sweep rate selectivity was studied in 53 neurons from the high-frequency (CF between 25 and 70 kHz) region of the pallid bat auditory cortex. Figure 1 shows FM sweep rate-selectivity functions and poststimulus time histograms (PSTHs) at three different intensities from a neuron. The maximum response magnitudes were similar across the intensities tested. For an intensity close to MT (level I: MT + 7 dB), the neuron responded best to a sweep rate of ~ 1 kHz/ms. When the intensity was increased by 5 dB (level II: MT + 12 dB), rate-selectivity function shifted to the right, and the peak response occurred for rates $\sim 5-6$ kHz/ms. At MT + 17 dB (level III), the peak shifted further to the right to rates ~ 10 kHz/ms. The 50% cut-off rate (Fig. 1A) shifted to faster rates when sound intensity was increased. The RTI increased from 0.41 to 0.65 with a 10-dB increase in intensity, indicating sharper rate selectivity at higher intensities. The PSTHs also show changes in response latencies with FM sweep rate. This is to be expected, because the time at which a sweep reaches excitatory frequencies depends on the sweep rate, causing response latencies to decrease for faster sweep rates (Heil et al. 1992a).

Figure 2, A–D, shows data from four additional neurons, in which FM rate selectivity was altered by intensity. As in Fig. 1, each neuron exhibited similar maximum response magnitudes at the different intensities tested. Qualitatively similar changes can be seen in each example in Fig. 2, A–D, and the neuron in Fig. 1. The rate-selectivity functions shifted progressively to the right (toward faster rates) as intensity increased. Figure 2, E and F, shows data from two neurons, in which the response magnitude declined with a 10-dB increase in sound intensity (nonmonotonic neurons). In these neurons as well, rate-selectivity functions shifted to the right with intensity before the reduction in overall response magnitudes took effect.

Figure 3A shows the population distribution and mean \pm SE of the 50% cut-off rate at the three different intensity levels tested. The distribution and mean shifted toward faster rates with intensity, and a one-way ANOVA showed that the means were significantly different (P < 0.001, Tukey pair-wise comparisons; P < 0.05 for all three intensity pairs). Figure 3B shows the distribution of neurons with different 50% cut-off rates at the three intensity ranges tested. Within 5–9 dB of threshold (*level I*), most neurons had a cut-off rate <1 kHz/ms. At the middle sound level (*level II*), most neurons had a cut-off rate between 1.1 and 3 kHz/ms. Many neurons (~50%) exhibited a cut-off rate >3 kHz/ms, only at the highest intensity (*level III*).

Figure 3*C* shows the distribution and mean \pm SE of the RTI values at the three different intensity levels tested. The distribution and mean shifted toward higher RTI at higher intensities. A one-way ANOVA shows that the mean values were significantly different (*P* < 0.001, Tukey pair-wise comparisons; *P* < 0.05 for all three intensity pairs). Figure 3*D* shows that the percentage of neurons with RTI <0.5 decreased, and the proportion with RTI >0.6 increased progressively with intensity. These data show that neurons became more sharply tuned for FM sweep rate with increasing intensity.

Mechanisms underlying intensity-dependent changes in 50% cut-off rate. Rate selectivity for downward sweeps in the majority of neurons in the pallid bat auditory cortex is shaped by the arrival time and bandwidth of HFI (Razak and Fuzessery 2006, 2009). Faster arrival time and/or larger bandwidth of HFI predict a higher 50% cut-off rate (Razak and Fuzessery 2006). Therefore, the higher 50% cut-off rates seen with increasing intensities may occur because of faster arrival and/or increasing bandwidth of HFI. The two-tone inhibition paradigm was used to determine the arrival time and bandwidth of HFI at multiple intensities from 32 neurons. The same three intensity ranges used for rate selectivity (levels I, II, and III) were used with the two-tone paradigm. The intensity of both excitatory and inhibitory tones was the same but changed in tandem. In 22/32 neurons, both rate-selectivity and two-tone inhibition functions were obtained at multiple intensities.

Figure 4A shows data from a neuron tested at four different intensities (the highest two were in *level III*). Also shown is the response to the excitatory tone, presented alone (termed control response) at the four intensities. The neuron responded similarly to the excitatory tone at the four intensities tested. The plots show the effects of presenting the excitatory tone after a HFI tone at various delays. Positive delays indicate that the excitatory tone was presented after the inhibitory tone. Close to threshold (MT + 7 dB), the excitatory tone had to be delayed \sim 9.5 ms for the response to decline to 50% of control re-



Fig. 1. A: frequency-modulated (FM) rate-selectivity functions of a neuron obtained at 3 different intensities above minimum threshold (MT). The *y*-axis shows the number of spikes in response to 20 stimulus repetitions. The arrows mark the 50% cut-off rate, the rate at which response declines below 50% of maximum response. DFM: downward FM sweep; CF: characteristic frequency. *B*: example poststimulus time histograms of the neuron's response. The *left*, *middle*, and *right* columns show data for the MT + 7-dB, MT + 12-dB, and MT + 17-dB presentations, respectively. The sweep rate and number of spikes are shown in each panel. Binwidth: 1 ms. Sound onset was at 0 ms.

sponse. When tone intensities were further raised by 5, 10, and 15 dB, the response declined to 50% of control at delays of 8, 3.4, and 2.2 ms, respectively. As the two-tone intensity increased, inhibition took effect at shorter delays. This suggests that the arrival time of inhibition became faster, relative to CF-driven excitation, when the intensity of both tones was raised.

The bandwidth of HFI at each intensity was measured at the delay that produced 50% inhibition. The range of high frequencies that caused inhibition for the neuron in Fig. 4A was 38-40 kHz, 38-40 kHz, 36-41 kHz, and 36-42 kHz at the four intensities, respectively. Thus the bandwidth of HFI also increased with intensity in this neuron. It has been shown previously (Razak and Fuzessery 2006) that the ratio of the

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Fig. 2. A–F: 6 representative neurons in which FM rate selectivity was affected by sound intensity. A–D: neurons in which maximum response magnitude was similar across the intensity range tested (monotonic neurons). The 50% cut-off rate shifted to faster rates, and the neurons became more selective to FM sweep rates with increasing intensity. The CF and the sweep bandwidth used are shown within each panel. *E* and *F*: neurons in which maximum response magnitude decreased with increasing intensity (nonmonotonic neurons). In these neurons, as well, rate-selectivity functions shifted right with increasing intensity.



bandwidth of inhibition (in kHz) and the 50% arrival time (in ms) predict the 50% cut-off rate (in kHz/ms). For the neuron in Fig. 4A, this ratio was 0.32, 0.38, 1.5, and 3.2 kHz/ms at the four different intensities tested. Figure 4B shows the rateselectivity functions from the same neuron at similar intensities used in the two-tone procedure (only three intensities were tested). The 50% cut-off rates were 0.36, 1.31, and 4.1 kHz/ms, similar to the predicted values. The neuron in Fig. 4C also showed a faster arrival time of HFI with intensity. The frequency range of inhibition, however, remained constant at 41-44 kHz. The predicted 50% cut-off rates were 0.98 and 2.7 kHz/ms at the two intensities, for which rate-selectivity functions were obtained. The actual 50% cut-off rates were 1.2 and 3 kHz/ms (Fig. 4D). Thus for these two neurons, changes in one or both properties of HFI predicted an intensity-dependent, rightward shift of rate selectivity.

Figure 5 shows additional examples of intensity-dependent changes in arrival time of HFI. The neuron in Fig. 5A did not meet the 50% criterion for inhibition when the two tones were presented at 5 dB above CF threshold. However, when the intensity was increased by another 2 dB, the neuron showed two-tone inhibition. With increasing intensity, 50% inhibition occurred at progressively shorter delays, suggesting faster arrival of inhibition. The neurons in Fig. 5, *B* and *C*, also

showed similar trends. The neuron in Fig. 5D exhibited a reduced control response at the highest intensity tested. This is the same nonmonotonic neuron shown in Fig. 2E. HFI arrived faster with increasing intensity in this neuron as well. Reduced responses to CF may underlie the nonmonotonic responses to FM sweeps with increasing intensity (Fig. 2E).

Data from one neuron suggested a second mechanism for nonmonotonic responses to FM sweeps. The maximum response of this neuron to FM sweeps decreased by $\sim 65\%$ with a 5-dB increase in intensity (Fig. 6A). However, the control response to tones was similar across the intensities tested (Fig. 6B). The nonmonotonic responses to sweeps cannot be explained by responses to CF tones. The two-tone inhibition plots suggest that the response to FM sweeps decreased with increasing intensity, because the excitatory responses were squelched by progressively faster HFI. At the highest intensity tested, HFI arrived faster (indicated by the negative arrival time) than CF excitation. Any downward sweep at this intensity will generate inhibition before excitation and elicit only a weak response if any. Although only one such neuron was found in this study, these data show that nonmonotonic responses to FM sweeps can occur without nonmonotonic responses to the CF tone.

The population summary of properties of HFI is shown in Fig. 7. The mean $(\pm SE)$ arrival time was faster with in-

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Fig. 3. Population summary of effects of increasing intensity on FM rate selectivity. A: on average, the 50% cut-off rate increased to progressively faster rates with increasing intensity. B: most neurons exhibited 50% cut-off rates <1 kHz/ms at the lowest intensity tested. With increasing intensity, the percentage of neurons showing faster 50% cut-off rates increased progressively. C: on average, the rate-tuning index (RTI), a measure of rate selectivity, increased with intensity. D: the percentage of neurons with RTI >0.6 also increased with intensity, indicating that neurons became more rate selective.

creasing intensity (Fig. 7*A*, one-way ANOVA, P < 0.001; Tukey pair-wise comparisons, P < 0.05, for all three intensity pairs). The mean (±SE) bandwidth of HFI increased with intensity (Fig. 7*B*, one-way ANOVA, P < 0.001; Tukey pair-wise comparisons, P < 0.05, for all three intensity pairs). In the 22 neurons, from which both two-tone inhibition and rate-selectivity functions were obtained at

different intensities, there was a significant correlation between predicted (based on 50% arrival time and bandwidth of HFI) and actual 50% cut-off rate at each of the three intensity ranges. This suggests that the intensity-dependent changes in FM rate selectivity occurred through changes in the spectrotemporal interactions between excitatory and inhibitory components of the receptive field.



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Fig. 4. A and C: 2-tone inhibition plots obtained at various intensities in 2 example neurons. Both tones had the same intensity but were raised or lowered by the same value. Positive (negative) delays on the x-axis indicate that the inhibitory tone was presented before (after) the excitatory tone. The excitatory and inhibitory tone frequencies used are indicated in the top-middle part of the panel. Arrows (50% arrival time of inhibition) indicate the delay of excitatory tone relative to the inhibitory tone, at which response declined to 50% of control response (excitatory alone, orphan symbols on top-left part of the panels). The 50% arrival time became progressively faster with increasing intensity. B and D: FM rate-selectivity functions of the neurons whose 2-tone inhibition plots are shown in A and C, respectively. As in the examples shown in Figs. 1 and 2, these neurons showed a rightward shift of rate-selectivity functions with increasing intensity. The arrows indicate 50% cut-off rate.

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Fig. 5. A-D: 4 example neurons in which 2-tone inhibition plots were obtained at multiple intensities. The excitatory and inhibitory tone used for each neuron is shown in the bottom-left part of the panel. Only high-frequency inhibition (HFI) was tested in this study, because it shapes selectivity for downward FM sweeps. The orphan symbols on the top left of each panel show the response to the excitatory CF tone presented alone. Each plot in a panel shows the response of the neuron to a 2-tone pair, in which the tones had the same intensity but were presented with different delays between them. The different plots in each panel were obtained by repeating the 2-tone experiment at different intensities relative to MT tone response. Note that both tones' intensities were raised by the same value. Positive delays on the abscissa indicate that the onset of the excitatory CF tone followed the onset of the inhibitory tone. Negative delays denote that the excitatory tone started before the inhibitory tone. The arrows indicate the 50% arrival time of inhibition defined as the delay at which the response to 2 tones declined below 50% of the response to the excitatory CF tone presented alone. In each of these neurons, the 50% arrival time of HFI became faster with increasing intensity.

DISCUSSION

The objective of this study was to determine the effects of sound intensity on FM sweep rate selectivity in the pallid bat auditory cortex. The first main result was that rate selectivity increased with increasing intensity [quantified using the rate-selectivity index (RTI); Fig. 3C]. Neurons became more rate selective at higher intensities, due to stronger inhibition of responses to slower rates. The sweep rate at which the responses declined below 50% of maximum (50% cut-off rate; Fig. 3A) shifted to faster rates with intensity. The second main result was that the timing and bandwidth of HFI changed with intensity (Fig. 7) and predicted intensity-dependent changes in FM rate selectivity (Fig. 8). Thus FM sweep selectivity can be predicted by interactions between inputs driven by two component tones at multiple intensities. The influence of other mechanisms proposed for FM rate selectivity in the pallid bat auditory system, such as nonlinear facilitation (Razak and Fuzessery 2008; reviewed in Fuzessery et al. 2011) and duration tuning (Fuzessery et al. 2006), may explain some of the variance between predicted and actual rate-selectivity measures (Fig. 8).



The pallid bat belongs to a small group of bats called "gleaners", which use echolocation primarily for obstacle avoidance and listen for prey-generated noise (e.g., rustling) to localize terrestrial prev (Barber et al. 2003; Bell 1982). The echolocation calls consist of FM sweeps with frequencies between 20 and 70 kHz, sweep bandwidths $\sim 20-40$ kHz, and duration between 2 and 5 ms. The sweep rates (bandwidth/duration) are therefore between 4 and 20 kHz/ms. In the present study, the average 50% cut-off rate of neurons increased from 0.8 kHz/ms to 3 kHz/ms, as sound intensity increased from 5-10 dB above threshold to 15-20 dB above threshold (Fig. 3). This suggests that as echoes increase in intensity, cortical neurons, tuned in the echolocation frequency range, become more selective for sweep rates present in the echolocation calls. The 50% cut-off rate is typically the middle of the range of sweep rates, over which a neuron's response shows maximum change and therefore, is the stimulus value around which the neuron provides maximum information (Harper and McAlpine 2004). Thus the sweep rates at which pallid bat cortical neurons provide maximal information are not static but increase with intensity toward rates present in its echolocation calls.

Bats will receive louder echoes as they close in on targets. Therefore, the observed intensity-dependent changes in rate

> 8 10 12





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Fig. 7. A: the mean $(\pm SE)$ 50% arrival time of HFI in the population is plotted for the 3 different intensity ranges tested. On average, the 50% HFI arrival time was faster with increasing intensity. B: the mean $(\pm SE)$ bandwidth of HFI in the population is plotted for the 3 different intensity ranges tested. On average, the bandwidth of HFI broadened with increasing intensity.

selectivity may serve to represent echoes more effectively as the distance to target decreases. However, the relationship among echo intensity, FM sweep rate, and target distance is not straightforward across species of bats. Some species reduce the intensity of the emitted pulse (intensity compensation) with decreasing target distance (Boonman and Jones 2002; Hiryu et al. 2007; Kobler et al. 1985). A combination of intensity compensation, middle ear reflex, emission/pinna directionality, and head direction may also serve to prevent the echoes from growing in intensity as the bat closes in on targets (Kick and Simmons 1984). Whether pallid bats show such intensity stabilization behaviors is not known. The intensity-dependent changes in rate selectivity will be adaptive within the range of target distances in which echo intensity increases. Bats also change echolocation call duration and bandwidth with hunting context. For example, some species broaden the bandwidth and shorten the duration of the sweep (thus increasing sweep rate) as they approach a target (Surlykke and Moss 2000). The increased selectivity of cortical neurons for faster rates may facilitate filtering of echolocation call-related information under these circumstances. Whether the pallid bat changes FM sweep properties while hunting is unknown and is a topic for future investigation.

Analogous changes in neural selectivity have been observed in the visual cortex of macaques. Speed tuning (analogous to FM rate tuning) of primary visual cortex neurons shifts to faster speeds with increasing stimulus contrast (Livingstone and Conway 2007). The change in speed tuning is predicted by contrast-dependent changes in the spatiotemporal organization of the receptive field. In middle temporal area (MT) neurons, suppression of responses to slow-moving stimuli occurs when contrast is high, but not for low-contrast stimuli (Pack et al. 2005). Speed tuning also changes in visual cortical neurons depending on the stimulus context (Li et al. 1999). The presence of a contrasting speed in the receptive field surround of visual cortical neurons shifts speed selectivity of neurons to stimuli in the receptive field center. When motion in the surround is slow, neurons respond better to faster motion in the center and vice versa. These data add to growing evidence that sensory neuron-response selectivity changes with local and global context. Such changes can be explained by the dynamics of interactions between the excitatory and inhibitory components of the receptive field.

The effects of sound intensity on cortical FM sweep processing have been tested previously in cats (Heil et al. 1992b) and humans (Brechmann et al. 2002). In the human study, functional MRI was used to determine how FM sweep-acti-

vated areas of cortex changed with intensity. Changes in measures of selectivity were not addressed. In the cat study, two different intensities (20 dB above threshold and 60 dB SPL) were used to determine intensity effects on FM rate and direction selectivity. Overall, the effect of intensity on rate selectivity was moderate, with $\sim 14\%$ of neurons showing a change in the shape of the rate-selectivity functions. The differences in the data between cat and the pallid bat A1 may arise, because of the different ranges of intensity tested, or may indicate species-specific differences. In the cat study, the intensities tested were between 20 and 50 dB above threshold. In the pallid bat, three different intensities between 5 and 30 dB above threshold were tested. Rate selectivity was different for all three intensity-pair comparisons, indicting that this was not simply a change near the threshold of a neuron. It is possible that the effects on rate selectivity saturate with further increases in intensity (in the range used in the cat study). Gleaning bats do not produce very high-intensity echolocation calls compared with bats that depend primarily on echolocation to hunt aerial prey (Waters and Jones 1995). This will likely allow gleaners to approach prey more closely before being detected. Therefore, very high-intensity FM sweeps may not be relevant for the pallid bat.

Neurons in both the inferior colliculus and the auditory cortex of the pallid bat use sideband inhibition to shape FM sweep selectivity (Fuzessery et al. 2006; Razak and Fuzessery 2006; Williams and Fuzessery 2011). Data presented here show that the delay between HFI and CF tones, which produced inhibition, became shorter with increasing sound intensity. This suggests that the arrival time of inhibition (relative to excitation) became faster with intensity. HFI also had a broader bandwidth when intensity was increased. The fact that changes in timing of inhibition predicted the higher 50% cut-off rate of selectivity functions (this study) and that GABA-A receptor antagonists reduce rate selectivity by affecting timing of inhibition (Razak and Fuzessery 2009) strongly support the notion that timing of sideband inhibition is important for shaping FM rate selectivity in A1. This view is also supported by a correlated reduction in rate selectivity and HFI following manipulation of developmental experience (Razak et al. 2008).

How the arrival time of inhibition relative to excitation changes with intensity remains unclear. One possible mechanism is that the strength of synaptic inhibition grows faster than excitation when intensity is increased. This would result in a steeper slope of the inhibitory postsynaptic potential (IPSP) relative to the excitatory PSP at higher intensities, causing a



Fig. 8. Predicted and actual 50% cut-off rates were significantly correlated at each of the 3 intensity levels tested (*A: intensity I; B: intensity II; C: intensity III)*. Predicted 50% cut-off rate was calculated by dividing the bandwidth (in kHz) of sideband HFI by the 50% arrival time (in ms). The actual rates were obtained from the rate-selectivity functions.

given level of inhibition to be reached earlier. This will manifest as a faster arrival time in the two-tone paradigm. Differential growth in the strength of inhibition and excitation with intensity has been shown in the rat A1 (Tan et al. 2007; Wu et al. 2006). Wu et al. (2006) used in vivo whole cell recordings to isolate inhibitory and excitatory conductance in response to a single tone. Typically, inhibition was slightly delayed relative to excitation. In nonmonotonic neurons, the strength of excitation increases and then decreases with intensity, whereas the strength of inhibition continues to grow. This is correlated with a decreasing delay between excitation and inhibition, ultimately resulting in a reduction of responses with increasing intensity. In monotonic neurons, both excitation and inhibition grew together with intensity, and the delay between excitation and inhibition did not change. Thus differences in how inhibition and excitation grow with intensity predicted response selectivity in the rat A1. A similar mechanism may underlie changes in HFI arrival time with intensity and cause the observed changes in FM rate selectivity.

One data-interpretation caveat in this study is that a barbiturate anesthetic was used during electrophysiology. Pentobarbital increases the duration of GABA-generated IPSP but does not alter the rising slope of IPSP (Scholfield 1978). The rising slope of the IPSP most likely relates to arrival times in the two-tone inhibition paradigm. Thus it is unlikely that arrival time data observed here are an artifact of the anesthetic used. Comparison of FM tuning and two-tone inhibition data obtained from urethane- and pentobarbital-anesthetized pallid bats also showed no differences in arrival time or rate selectivity (Razak and Fuzessery 2009). Urethane affects GABA transmission to a lesser extent than barbiturates (Hara and Harris 2002). Sideband properties and selectivity are unlikely to be artifacts of pentobarbital-enhanced GABA function.

Mechanisms, such as nonlinear facilitation (Razak and Fuzessery 2008; Sadagopan and Wang 2009; Ye et al. 2010), duration tuning (Fuzessery et al. 2006), and interactions between spike threshold and excitatory/inhibitory conductance, generated by sweep-selective inputs (Gittelman and Li 2011; Gittelman and Pollak 2011), also shape FM sweep rate selectivity. How these mechanisms change with sound intensity remains to be studied. Whether different mechanisms dominate at different intensity levels also needs to be evaluated with a broader range of intensities. Results in the pallid bat A1 are consistent with those in rat A1 in that only inhibitory two-tone responses were seen at the different intensity levels (Scholl et al. 2008). There were no intensity-dependent changes in twotone effects from inhibition to facilitation. In the rat A1 study, although the delay between the two tones was varied, the minimum delay was 80 ms. Therefore, it was not possible to determine if arrival times of inhibition, relative to excitation, changed with intensity.

Auditory neurons, like other sensory neurons, exhibit a "centersurround"-type receptive-field organization. Sideband inhibition is typically studied for frequency-intensity or frequency-timing combinations (Brosch and Schreiner 1997; Calford and Semple 1995; Fuzessery et al. 2006; Gordon and O'Neill 1998; Loftus and Sutter 2001; Razak and Fuzessery 2006; Sutter et al. 1999). The major conclusion of this study is that the spectral and temporal interactions between the excitatory and inhibitory frequencies are intensity dependent. A similar conclusion was reached by Sadagopan and Wang (2010), based on recordings from the marmoset cortex, in which the duration of sideband inhibition changed with intensity. Thus properties of sideband inhibition, such as arrival time, bandwidth, and duration, vary with sound intensity in a manner predictive of cortical responses to complex sound responses.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: K.A.R. conception and design of research; K.A.R. performed experiments; K.A.R. analyzed data; K.A.R. interpreted results of

experiments; K.A.R. prepared figures; K.A.R. drafted manuscript; K.A.R. edited and revised manuscript; K.A.R. approved final version of manuscript.

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