FM Velocity Selectivity in the Inferior Colliculus is Inherited from Velocity Selective Inputs and Enhanced by Spike Threshold

Running Head: FM Velocity Selectivity in the IC

Joshua X Gittelman*, Na Li
Section of Neurobiology, Institute for Neuroscience
339 Patterson Laboratories
The University of Texas at Austin
2401 Speedway
Austin, Texas 78712, USA

*Correspondence to: Joshua Gittel at the above address or
E-mail: jxg@mail.utexas.edu
Telephone: 512-471-4849
Facsimile: 512-471-9651

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Abstract

Frequency modulation (FM) is computed from the temporal sequence of activated auditory nerve fibers representing different frequencies. Most studies in the inferior colliculus (IC) inferred from extracellular recordings that the precise timing of non-selective inputs creates selectivity for FM direction and velocity (Andoni et al. 2007; Fuzessery et al. 2006; Gordon and O'Neill 1998). We recently reported that two additional mechanisms were more important than input timing for directional selectivity in some IC cells: spike threshold, and inputs that were already selective (Gittelman et al. 2009). Here, we show that these same mechanisms, selective inputs and spike threshold, underlie selectivity for FM velocity and intensity. From whole-cell recordings in awake bats, we recorded spikes and postsynaptic potentials (PSPs) evoked by downward and upward FMs that swept identical frequencies at different velocities and intensities. To determine the synaptic mechanisms underlying PSP selectivity (relative PSP height), we derived sweep-evoked synaptic conductances. Changing FM velocity or intensity changed conductance timing and size. Modeling indicated that excitatory conductance size contributed more to PSP selectivity than conductance timing, indicating that the number of afferent spikes carried more FM information to the IC than precise spike timing. However, excitation alone produced mostly suprathreshold PSPs. Inhibition reduced absolute PSP heights, without necessarily altering PSP selectivity, thereby rendering some PSPs subthreshold. Spike threshold then sharpened selectivity in the spikes by rectifying the smaller PSPs. This indicates the importance of spike threshold, and that inhibition enhances selectivity via a different mechanism than previously proposed.

keywords: in-vivo whole-cell, response selectivity, spike timing, awake mammal, intensity
The brain encodes various parameters of the same stimulus differently. Furthermore, the same parameter can be encoded differently, depending on location in the brain. In the auditory nerve (AN), frequency cues are tonotopic, whereas intensity cues include firing rate and the number of fibers recruited (Heinz and Young 2004; Meyer and Moser 2010). Frequency modulations (FMs) are critical speech components (Bregman 1990), and in this bat are used for both communication and echolocation (Bohn et al. 2008). In terms of neural coding, there are different cues available to distinguish FM direction and velocity starting in the cochlear nucleus (Britt and Starr 1976). Downward and upward FMs that sweep the same frequency range at the same intensity evoke equal spike counts, and are distinguishable only by spike temporal pattern.

In contrast, slow and fast FMs that sweep the same frequency range evoke different spike counts as well as different timing. Thus, spike timing is the only cue for FM direction, whereas for velocity there are two cues, spike timing and spike count.

The cues to distinguish amongst FMs change as the signal ascends to the inferior colliculus (IC), where essentially all auditory brainstem output converges before reaching the forebrain (Morest and Oliver 1984). Beginning in the cochlear nucleus (CN), some excitatory signals are converted to inhibitory (Wickesberg and Oertel 1990), and some cells fire different numbers of spikes to downward and upward FMs (Britt and Starr 1976; Erulkar et al. 1968; Fernald and Gerstein 1972). Thus, after the AN, selectivity for FM direction or velocity could rely on differences in spike timing or spike rate of either excitation or inhibition.

Although different cues exist to distinguish between FM velocities as opposed to direction, their processing is almost certainly intertwined. Some extracellular studies have inferred that IC cells distinguish FM direction by the same mechanisms that distinguish velocity (Andoni et al. 2007; Gordon and O'Neill 1998; Suga 1968), while others have inferred that velocity and direction are processed differently, at least in some cells (Fuzessery et al. 2006). However, nearly every proposed mechanism in the IC relies on the timing of non-selective inputs, but see: (Fuzessery and Hall 1996). One ‘timing’ hypothesis posits that the relative arrival time of excitation compared to inhibition underlies selectivity. Cells fire when excitation leads inhibition,
but not when inhibition leads or coincides with excitation. A second ‘timing’ model posits that the degree of coincidence in the arrival of the presynaptic spikes generates selectivity. More coincident arrival generates stronger input, whereas less coincident arrival generates weaker input. For example, FMs evoking coincident arrival of the excitatory afferents would increase firing probability, whereas different FMs evoking staggered arrival of excitation would not. For inhibition, the same temporal pattern would have the opposite effect on firing. A substantial body of evidence supports these mechanisms, including that blocking inhibition reduces or eliminates selectivity (Andoni et al. 2007; Fuzessery and Hall 1996; Koch and Grothe 1998), indicating that inhibition is especially important.

Both models assume differences in input timing, but do not require differences in the amount of excitation or inhibition activated (input size). Using whole-cell recordings, we recently showed that in many IC cells, input size and spike threshold were more important than input timing for generating directional selectivity (Gittelman et al. 2009). Here we ask which parameters are most important for distinguishing FM velocity and intensity.
Materials and Methods

All of the cells presented here were presented previously (Gittelman et al. 2009; Gittelman and Pollak 2011). Here we include additional data from the same cells. We recorded from the central nucleus of the inferior colliculus in awake Mexican free-tailed bats (*Tadarida brasiliensis*) using patch electrodes in whole-cell current-clamp mode. All animal procedures were in accordance with a protocol approved by the University of Texas Institutional Animal Care Committee. Detailed experimental methods were reported previously (Gittelman et al. 2009).

**Surgery.** Bats were sedated with Isoflurane inhalation and then anesthetized with an intraperitoneal injection of ketamine/xylazine (75 - 100 mg/kg Ketamine, 11 - 15 mg/kg Xylazine, Henry Schein, Inc. Melville, NY). Recordings began after recovery from anesthesia so data were obtained from awake animals.

**Internal solution** (in mM): K-gluconate (115), HEPES (10), KCl (7), MgATP (4), Na₂GTP (0.3), EGTA (0.5), Na₂Phosphocreatine (10). Membrane potentials were not corrected for liquid junction potential. To improve the conductance estimates in 3 cells, we substituted cesium for potassium to minimize the effects of voltage-gated potassium currents, and included 1 mM QX-314 to block sodium channels. Results of the conductance estimates were similar to those obtained with standard internal solution, and so the data were combined.

**Sound presentation.** The sweep frequency range (one octave, fixed) and velocity range (5 – 320 oct/ s) were chosen to approximate the range this bat uses (Andoni et al. 2007; Bohn et al. 2008). The start and stop frequencies were adjusted to maximize the depolarizing response. We then obtained responses to downward and upward FMs that logarithmically swept this fixed range while varying the sweep velocity and intensity. Velocities were incremented in factors of 2 (i.e. the sweep duration was halved) to cover a 4-fold to 64-fold velocity range. In recordings that were sufficiently stable, we estimated the excitatory and inhibitory conductance pairs evoked by downward and upward FMs at 2 - 3 velocities (7 cells) or 2 - 3 intensities (3 cells). The conductance set for each cell consisted of 4 or 6 conductance pairs, each pair consisting of an excitatory and an inhibitory conductance evoked by a unique FM.
Quantifying responses. A sweep was counted as evoking spikes if firing probability ≥ 25%. Spikes were filtered from the PSP responses by detecting spike threshold (dV/dt > 10 V/s), interpolating a straight line across the spike waveform and then smoothing. We found that PSP height correlated well with spiking, and generally better than dV/ dt (the rising slope of the depolarization). We therefore quantified PSPs in terms of height, defined as depolarizing, hyperpolarizing or no response. No response meant the change in membrane potential (V_m) was < 5X membrane noise (SD of V_m prior to sound presentation). We measured EPSP height as the maximum depolarization – rest potential, and IPSP height as the maximum hyperpolarization – rest. Responses were classified as depolarizing if the EPSP height exceeded the IPSP height, or if it was ≥ 2 mV. Failing these criteria, the responses were classified as hyperpolarizing.

Estimating access resistance, membrane resistance and membrane capacitance: Electrode capacitance was minimized by capacity compensation, and then membrane properties (resistance, time constant and capacitance), access resistance and pipette time constant were estimated by fitting voltage responses to small hyperpolarizing current steps (25 – 50 pA, 200 ms duration) with a double exponential. The fast and slow time constants were attributed to the pipette and membrane respectively. In recordings judged acceptable for conductance derivation, the fast time constant < 1 ms and the associated access resistance (50 – 150 MΩ) was less than the estimated membrane resistance (170 – 340 MΩ), see (Gittelman et al. 2009) for more detail.

Deriving synaptic conductances. Access resistance is relatively high and unstable in vivo compared to slice. These conditions are especially problematic in voltage clamp, and potentially introduce errors when employing series resistance compensation; with each trial, any change in access resistance would alter the error without concurrent adjustment of the compensation. In contrast, the errors introduced in current clamp will be relatively consistent, so that all the PSPs measured in the same cell would be subject to similar error, as would the derived synaptic conductances. We therefore estimated synaptic conductances in current clamp mode according to (Gittelman et al. 2009; Priebe and Ferster 2005):

\[ C_m \cdot \frac{dV_m}{dt} = - \Sigma I_m + I_{inj} \] (1)
where $C_m$ is the membrane capacitance, $dV_m/dt$ is the membrane potential slope, $I_m$ is the current across the cell membrane, and $I_{inj}$ is the current injected through the electrode. We assumed 3 membrane currents: excitatory, inhibitory and leak. Equation 1 can be expanded to include the conductance and driving force terms:

$$C_m \frac{dV_m}{dt} = - (ge (V_m-V_e) + gi (V_m-V_i) + g_{leak} (V_m-V_{leak})) + I_{inj} \quad (2)$$

The conductances ($g$) are: excitatory, $ge$; inhibitory, $gi$; leak, $g_{Leak}$. $V_m$ is the measured membrane potential, and the reversal potentials for $g_{leak}$, $ge$ and $gi$ are (respectively) $V_{Leak}$, $V_e$, and $V_i$. Most of these terms can be measured or estimated. $V_m$ and $dV_m/dt$ were measured directly. Membrane capacitance and resistance ($1/g_{leak}$) were measured as described above. $V_e$ was assumed to be 0 mV, and $V_i$ was estimated to be $-63$ mV from the changes in the PSP polarity while different amounts of constant current were being injected. $V_{Leak}$ was calculated from the steady state $V_m$, membrane resistance measured at the steady state $V_m$, and the $I_{inj}$.

Using the above values, there are only two unknowns in equation 2, $ge$ and $gi$.

Consequently, $ge$ and $gi$ can be estimated from FM-evoked responses while hyperpolarizing the cell to only 2 different steady state potentials. In practice, we required FM-evoked responses recorded at a minimum of 3 different steady-state potentials over a voltage range of $\geq 40$ mV.

Cells were hyperpolarized to minimize the effects of voltage-gated channels, except when Cs$^{2+}$ and QX-314 were in the pipette, in which cases cells were both depolarized and hyperpolarized.

We note in the results (Fig. 4) that voltage-gated channels contributed little to the sound-evoked conductance estimates. This does not mean that voltage-gated channels make no contribution to the estimates, only that the contribution is relatively small compared to the sound-evoked ligand-gated channels. We strengthen that argument with a second line of evidence here, namely that computed PSPs from the derived conductances did not match the measured PSPs under conditions where we would expect voltage-gated channels to contribute substantially to the membrane response. First, computed PSPs did not match measured PSPs when the membrane was depolarized (and sometimes at rest). For these reasons, we did not use PSPs measured under depolarizing conditions to estimate the conductances, unless channel blockers were used.
Second, we have never successfully derived synaptic conductances in cells that exhibit evidence for rapid, powerful voltage-gated channels below rest. Specifically, some IC cells show evidence for large, rapid hyperpolarization activated cation currents (IH), and low-threshold potassium currents (LVK) (Koch and Grothe 2003; Sivaramakrishnan and Oliver 2001; Tan et al. 2007; Xie et al. 2008). We have never obtained good predictions of membrane responses from derived conductances in these cells, which we estimate represent between a quarter and a third of the cell population; they have been excluded from the conductance analyses presented here.

Modeling: Model cells consisted of excitatory, inhibitory and leak conductances with corresponding reversal potentials and a capacitance. We made a unique model cell for each neuron used in the conductance estimates based on responses to current steps in each cell. The validity of the conductance estimates was determined by two criteria. As shown in Fig. 4, we used the estimated values for $g_e$ and $g_i$ to predict the voltage responses in the models. Correlation analysis between the predicted PSPs and measured PSPs indicates that the conductance estimates account $\geq 94\%$ of the variance ($R^2 \geq 0.94$ for all conductance sets). Predictions were accurate across a broad range of membrane potentials ($\geq 40$ mV), indicating that the state of voltage-gated channels changed little during the time course of the PSPs and thus had a relatively small effect on the conductance estimates. See (Gittelman et al. 2009) for more detail.

When testing the importance of conductance timing and integral, predictions were done at rest, except when the internal solution included Cs$^{2+}$ and QX-314, which depolarized resting potential to $\sim -30$ mV. In these 3 cells, we made predictions with the model resting potential set to -50 mV, close to the mean resting potential measured in our IC cells without channel blockers (-47 ± 6 mV). Because the excitatory and inhibitory conductance temporal envelopes (shapes) were different, we measured conductance latency three ways: latency to peak; latency to 20% of peak height; and latency to 50% of peak height. Overall results were similar for all tests (latency to peak shown).
Results

FMs in this bat's vocalizations typically sweep approximately one octave at velocities from 5 to over 300 octaves/second (oct/ s), and the velocity preferences of IC neurons match that range (Andoni et al. 2007; Bohn et al. 2008). To assess the mechanisms underlying FM velocity selectivity, we recorded responses from 24 IC cells to downward and upward FMs that swept a one-octave frequency range at physiologically relevant velocities and intensities. In 10 of these cells, we derived the synaptic conductances evoked by 4 - 6 different sweeps per cell.

IC cell sensitivity to FM direction, velocity and intensity

Most IC cells (21/24) were selective for FM direction, velocity and intensity in that changing any one of those parameters affected the number of evoked spikes and the PSPs. The preferred FM was the direction/velocity/intensity combination that evoked the most spikes. PSP selectivity was quantified in terms of peak height, with the preferred FM evoking the largest PSP (see quantifying responses in methods).

We illustrate FM selectivity with three cells, each preferring different FMs (Fig. 1). For each cell, we show membrane potential responses (left) and render those responses graphically (right). The first cell was tested with 6 velocities, from 5-160 octaves/second (oct/ s, incremented in factors of 2 for a 32-fold range), and at 2 intensities, 25 and 65 dB SPL. In terms of spikes, this cell did not distinguish direction, but was selective for velocity and intensity, firing best to slow velocity, lower intensity FM sweeps (Fig. 1a1. Preferred sweep: 5 oct/ s, 25 dB SPL). The cell also exhibited PSP selectivity, in that changing velocity or intensity changed the PSP height (Fig. 1a2). Note that every sweep presented evoked PSPs, but only one velocity/intensity combination evoked spikes. Thus, the presynaptic cells fired to sweeps that failed to evoke postsynaptic spikes. Interestingly, with sweep velocity and intensity held constant, most of the PSPs evoked by downward sweeps were very similar to the PSPs evoked by upward sweeps of the same velocity/intensity (no or weak directional selectivity in the PSPs), and no downward and upward pairs evoked large differences in the PSP heights (strong PSP selectivity). In the graphic display, the
downward and upward plots are nearly mirror images, consistent with presynaptic cells that were
non- or weakly selective for FM direction.

The second cell preferred downward, medium velocity sweeps at medium intensity (Fig. 1b. Preferred sweep: downward, 120 oct/ s, 45 dB SPL) and the third cell preferred downward, fast velocities at high intensity (Fig. 1c. Preferred sweep: downward, 320 oct/ s, 75 dB SPL).

Once again, every sweep presented evoked PSPs but only a subset evoked spikes. Both cells were sensitive to velocity, direction and intensity in that changing any parameter changed spike probability, and also changed the PSPs. However, like the first cell, changing velocity had a bigger impact on spike probability and PSPs than changing direction. This is particularly clear in the PSPs, where the downward and upward graphs are again approximately symmetrical.

Of the three cells that were ‘non-selective’ (not shown), two fired with nearly equal probability to downward and upward sweeps at all the velocities and intensities tested (velocity ranges, 8- and 16-fold; intensity ranges, 40 and 60 dB SPL). In the third cell, changing direction and intensity did not change the number of evoked spikes. This cell fired to every velocity presented (32-fold range), but it fired more to slow velocities than to fast velocities.

Previous studies on FM selectivity in the IC using extracellular electrodes focused primarily on the synaptic mechanisms underlying response selectivity in the spike counts, or the mathematical relationship between sound and spikes (Andoni et al. 2007; Fuzessery and Hall 1996; Fuzessery et al. 2006; Gordon and O’Neill 1998; Poon et al. 1991; Suga 1968). Intracellular recordings allowed us to examine PSP selectivity, and how spike threshold affects the transition from PSP selectivity to spike selectivity. Importantly, the distribution of preferred velocities in terms of spikes that we measured intracellularly was similar to that shown previously for this species (Fig. 2a, (Andoni et al. 2007)).

**Spike threshold sharpens selectivity**

Fewer stimuli evoked spikes than PSPs in most cells, indicating that spike threshold sharpened response selectivity for FM velocity and, to a lesser extent, intensity. To summarize the extent to which spike threshold sharpened velocity selectivity, we plotted the number of
velocities that evoked PSPs against the number that evoked spikes (Fig. 2b). We included only
the 22 cells where we tested at least a 4-fold velocity range (at least three velocities, incremented
in factors of 2). A velocity was counted as evoking spikes if it did so in at least one direction/
intensity combination. The three non-selective cells and one cell that was FM selective in terms of
spike counts showed no evidence that spike threshold sharpened selectivity; every sweep
velocity that evoked PSPs also evoked spikes. However, in 18/22 cells fewer velocities evoked
spikes than the number of velocities that evoked PSPs. Further, in 16/22 cells we failed to test
the full range of sweep velocities that evoked PSPs, either due to not having implemented the
software for rapid screening yet, or because the large (up to 32-fold) range of velocities evoked
PSPs. Hence, we have likely underestimated the extent to which spike threshold sharpens
selectivity. Clearly, spike threshold plays a major role in shaping velocity selectivity.

Similarly, spike threshold sharpened intensity selectivity in some cells. We plotted the
number of intensities that evoked spikes against the number that evoked PSPs in the 18 cells
where at least a 30 dB SPL range of intensities was tested (2 - 5 intensities tested, up to 60 dB
SPL range). An intensity was counted as evoking spikes if it did so in at least one direction/
velocity combination. In 8/18 cells, more intensities evoked PSPs than the number of intensities
that evoked spikes, indicating that spike threshold sharpened intensity selectivity. In 10/18 cells,
all the intensities that evoked PSPs also evoked spikes, including 4 cells where a 60 dB SPL
intensity range was tested. However, in 15/18 cells we failed to capture the full range of sweep
intensities that evoked PSPs, so we may have underestimated the extent to which spike
threshold sharpens intensity selectivity.

PSPs were more sensitive to sweep velocity than to direction or intensity

In order for spike threshold to enhance spike selectivity by rectifying subthreshold inputs,
velocity (i.e., same direction and intensity). This provided a distribution of PSP height differences as a function of velocity for each cell. We then quantified the effect of velocity on PSP selectivity as the average and the maximum value of that distribution. We did the same for direction and intensity, and then compared the velocity effect on PSP selectivity to effect of direction on selectivity, and so on.

Sweep velocity generated bigger differences in PSP height than either intensity or direction. For each cell, we plotted the average and maximum height differences generated by changing sweep velocity against the differences generated by changing direction or intensity, with the diagonal lines showing unity on each plot (Fig. 3a-d). In the majority of cells, changing velocity generated equal or larger PSP differences than changing either direction or intensity (most points near or above the unity line). On average, velocity generated significantly more PSP height variance compared to sweep direction or intensity (p < 0.01 for both, paired T test). The three non-FM-selective cells fell near the unity lines (open circles). When we compared PSP differences generated by changing direction to changing intensity, the cells were scattered around the unity line, indicating that direction and intensity generated about the same amount of PSP selectivity (Fig. 3e, f, p = 0.4, paired T test).

Generating PSP selectivity

To understand how the information to discriminate between FMs is conveyed to the IC, we need to know what differences in the synaptic inputs were evoked by the different FMs and how those input differences translated into PSP selectivity. In the next sections, we first show that we can derive synaptic inputs evoked by different FMs in the same cell, and that the derived inputs predict the PSP responses. We then examine two examples in some detail to see how changing sweeps changed the synaptic inputs, and how those input differences relate to differences in PSP height. Finally, we used modeling to show which input differences predicted PSP selectivity best.

Derived synaptic conductances predict PSPs
We derived synaptic conductance pairs (excitatory (ge) and inhibitory (gi)) corresponding to individual FM sweeps in 10 cells. In each cell, we presented 4 to 6 FMs, both downward and upward, that swept the same frequency range while varying velocity (7 cells) or intensity (3 cells). Thus, for each cell we had a conductance set consisting of 4 or 6 conductance pairs, each pair evoked by a unique FM sweep.

We show measured responses to 6 FMs (Fig. 4a, 3 different velocities, upward and downward, same one-octave frequency range, same cell as Fig. 1b), the conductances derived from the measured responses (Fig. 4b), and the PSPs computed from the derived conductances (Fig. 4a, dashed red). We derived each conductance pair from PSPs evoked by an FM presented while the membrane potential was hyperpolarized to different steady states with different amounts of constant current (Gittelman et al. 2009; Priebe and Ferster 2005). We make four points. First, the cell distinguished between the different FMs with a range of membrane responses from predominantly hyperpolarizing to spiking (Fig. 4a, top row at resting potential). Second, changing sweep velocity or direction changed the waveforms of the conductance pairs (ge and gi) in terms of size (integral), shape (temporal envelope), and the relative latency (delay) between ge and gi. (Fig. 4b, c - see Fig. 5 for details of the downward responses). Third, shifting the steady state membrane potential with constant current changed the PSP waveforms substantially; the hyperpolarizations changed to depolarizations, indicating that the membrane was hyperpolarized below the reversal potential for chloride. (Hyperpolarization also eliminated spikes.) In cells where voltage-gated channel blockers were included in the internal solution, we could depolarize the membrane sufficiently to reverse the sound-evoked depolarizations (not shown). Finally, predicted PSPs (dashed red, Fig. 4a) computed by stimulating a model cell with the derived conductance pairs closely matched the measured PSPs (solid black, Fig. 4a). The same model cell, based on current step responses measured in this cell, was used for all 6 conductance pairs. Correlation analysis between the measured and the predicted PSPs suggests that the conductance estimates accounted for 98% of the variance in the example cell (Fig. 4d), and ≥ 94% for the population (not shown).
These last two points indicate that voltage-gated channels did not contribute substantially to the conductance estimates, at least in these cells. That the size of the PSPs changed with hyperpolarization indicates that the membrane potential near the synapses was changed, which should alter how voltage-gated channels shape the PSPs. If those voltage-gated channels shaped the PSPs substantially, their effects would depend on the level of hyperpolarization. However, in the cells presented here, a single set of derived conductance waveforms with no voltage sensitivity predicts measured PSPs over a broad range (> 40 mV) of hyperpolarizing potentials. This is consistent with a relatively small contribution from voltage-gated channels when the membrane is hyperpolarized (see methods, Deriving synaptic conductances for additional discussion).

Defining the conductance parameters

Given that changing sweeps changed multiple aspects of the conductance waveforms, we now ask which aspects generated selectivity in the PSPs. We examined five conductance parameters, three intended as proxies for afferent spike timing, and two for the total number of afferent spikes, i.e. population spike rate (see discussion). The first ‘timing’ parameter was the relative latency between excitation and inhibition (delay between ge and gi). Leading excitation would increase firing probability of the postsynaptic response, whereas leading or coincident inhibition would decrease firing probability. We tested latency to peak, 20% of peak height, or 50% of peak height (peak shown). Changing the definition of latency did not alter the overall results. The second ‘timing’ parameter was the temporal envelope (shape) of ge, representing the relative arrival time of all of the excitatory afferent spikes; more coincident arrival would generate a relatively tall and narrow ge, whereas less coincidence would generate a shorter, wider ge. All other parameters being equal, a tall, narrow ge would excite the postsynaptic cell more than a short, wide ge. Similar arguments apply to inhibition, and thus the third ‘timing’ parameter was gi shape.
The first 'rate' parameter was the integral of the derived ge waveform. Assuming that each FM activated the same afferents (see discussion), then more afferent spikes would evoke a larger ge, and fewer afferent spikes would evoke a smaller ge. All other parameters being equal, a larger ge would excite the postsynaptic cell more than a smaller ge. Similar arguments apply to inhibition, and thus gi integral is the second rate parameter.

Differences in any one of the 5 parameters could generate PSP selectivity. However, when the parameters are combined, one (or more) will dominate. We examine these parameters in some detail for two neurons, one that preferred medium velocities to slow or fast velocities, and another that was non-monotonic, firing preferentially to lower intensities over high intensities (Fig. 5). These cells were chosen because their selectivity is different than that found in the auditory nerve, where slower velocities and higher intensities evoke more spikes. In other words, the output of both cells required neural computation beyond peripheral coding, and there should be evidence of that computation in the synaptic conductance waveforms. Second, they illustrate that although changing sweeps can change all of the 5 conductance parameters, it is not always obvious which parameters affect PSP selectivity most.

Changing sweep velocity changes the conductance parameters

Using the downward evoked responses from the cell in Fig. 4, we show how the 5 conductances parameters changed with changes in FM velocity (Fig. 5a-d). Medium sweeps (60 oct/ s) were preferred, fast sweeps (240 oct/ s) were 'less-preferred', evoking fewer spikes and smaller PSPs, whereas slow sweeps (15 oct/ s) were 'null', evoking no spikes and hyperpolarizing PSPs (Fig. 5a, top). Examination of the conductance waveforms shows that changing velocity affected all 5 of the conductance parameters (Fig. 5a-d). Although small, the delays between excitation and inhibition were consistent with the dominant hypothesis for velocity selectivity, where leading excitation promotes spiking, and leading or coincident inhibition reduces spiking (Fig. 5b). The preferred sweep evoked excitation that led inhibition by 1.2 ms, whereas the less-preferred sweep evoked excitation that led by only 0.7 ms (Fig. 5b, measured at the
peak). The null sweep evoked essentially coincident excitation and inhibition. However, further examination of the conductance waveforms shows that there are other differences that could promote velocity selectivity in this cell. The preferred sweep evoked the largest excitation (biggest integral), and the null sweep evoked the largest inhibition (Fig. 5c), both consistent with the measured PSP selectivity. The null sweep also evoked the broadest excitation (Fig. 5d), which as explained above should be the least efficacious, and is therefore consistent with PSP selectivity. However, other conductance parameters were not consistent with PSP selectivity. The preferred sweep evoked a larger inhibition than the less-preferred sweep (Fig. 5c), and the null sweep evoked the broadest inhibition (Fig. 5d). In other words, there are numerous differences in the conductances, some which should enhance PSP selectivity, but also some which should reduce selectivity. What is not clear from this analysis is which of these parameters most determined the PSP response. We return to this question after examining a cell in which we varied intensity.

Changing sweep intensity changes the conductance parameters

Changing sweep intensity also altered the conductance waveforms, although not as much as changing velocity. Once again, some of the differences were consistent with enhancing the measured PSP selectivity, while others should reduce selectivity (Fig. 5e-h). This cell was non-monotonic, firing with 100% probability to downward, 150 oct/s sweeps at low and medium intensity sweeps (15 and 45 dB SPL), but firing with only 60% probability to the same sweep at high intensity (75 dB SPL). In terms of integrals, the high intensity (less-preferred) signal evoked the smallest excitation, consistent with the PSP selectivity (Fig. 5g). Although the peak amplitude of the less-preferred inhibition was lower, it was so much broader than the other two inhibitory traces that it had the largest integral, again consistent with the PSP selectivity. Furthermore, the shape of the less-preferred excitation was broader than that of other two signals, also consistent with PSP selectivity (Fig. 5h). However, the less-preferred signal evoked the broadest inhibition (Fig. 5h), and the biggest delay between excitation and inhibition, with excitation leading (Fig. 5f). These differences should reduce selectivity. To summarize, changing the sound changed many
conductance parameters, and it is not clear which changes were the most important, or how they combined to generate the differences in the PSPs.

Determining which conductance parameters dominated the PSP response

We used modeling to determine which conductance parameters most affected PSP selectivity. We first asked which parameter, when tested in isolation from the other parameters, best predicted the PSP height differences. We then compared the rate parameters to the timing parameters.

To illustrate the model, we made a hypothetical conductance set consisting of three conductance pairs that differed in 3 parameters only: the delay between ge and gi; the ge integral; and the ge shape (Fig. 6a). For simplicity, there were no differences in gi integral or shape. We computed PSPs from each conductance pair in a model cell. The PSPs differed in height, representing PSP selectivity. These are the 'control' PSP and conductance waveforms.

We assessed each parameter's contribution to PSP selectivity by making 'experimental' conductance sets to test each parameter's ability to predict control PSP heights. The experimental sets consisted of three conductance pairs each, one corresponding to each of the three control conductance pairs (Fig. 6c-e). Each set had the differences in a single parameter preserved but differences in the other parameters eliminated. We computed 'experimental' PSPs from the conductance sets and then compared the experimental PSP height to the corresponding control PSPs. The individual parameter that best predicted the relative differences in the control PSP heights was taken as the dominant parameter for generating PSP selectivity.

To make the experimental conductance set with the ge integrals preserved, we first eliminated all the parameter differences by averaging the control excitatory waveforms, and aligning excitation and inhibition at their peaks (Fig. 6b). We then scaled the average ge waveform so that the integral of each experimental ge was equal to the integral of the corresponding control ge (Fig. 6c). We computed experimental PSPs, plotted the PSP heights against the heights of the corresponding control PSPs, and fit the points using linear regression.
(Fig. 6c, right). The computed experimental PSPs from this conductance set correlated strongly with the control PSPs ($R^2 = 0.95$, slope = 1.7), showing that the differences in the ge integrals alone predicted relative control PSP height. Therefore the ge integrals were important for generating PSP selectivity.

To test the delay parameter, we used the average conductance waveforms, and then shifted inhibition in time so that the delays between ge and gi matched the corresponding control delays (Fig. 6d, compare to delays in (a)). The computed experimental PSP heights did not correlate with the controls ($R^2 = 0.14$, slope = 0.1). Thus, with no differences in the other conductance parameters, the differences in the delays between ge and gi could not predict the control PSP differences, indicating that the delay parameter was relatively unimportant for generating PSP selectivity. Note that the delay parameter did affect the PSP heights, but it did not dominate the response when combined with the other parameters.

We tested ge shape by using the control ge waveforms in each experimental conductance pair, aligning them with the average gi waveforms, and scaling them so that integral of each experimental ge was equal to the average control ge integral (Fig. 6e). The computed experimental PSPs were inversely correlated with the controls ($R^2 = 0.88$, slope = -1), suggesting that ge shape reduced the differences in the control PSP heights, and therefore reduced PSP selectivity.

In this hypothetical example, the ge integral was the dominant parameter for determining PSP selectivity. The ge shape was of secondary importance, with the effect of reducing selectivity. The delay between ge and gi was relatively unimportant; although delay did affect the PSPs, the effect was not systematically correlated with the control PSP height. This example had no differences in the gi integral or shape, so these parameters did not affect PSP height (slope = 0). For actual data presented in the following sections, we also tested the influences of gi integral and shape.

ge integrals are the major determinants of response selectivity
We show a cell with 6 conductance pairs and their corresponding measured and computed (control) PSPs (Fig. 7a). The 6 conductance pairs were derived from 6 FMs (downward and upward at 3 different velocities). Using the strategy described in the preceding section, we tested each parameter individually for its ability to predict the relative heights of the control PSPs. The ge integral was the dominant parameter for determining PSP selectivity in this cell (Fig. 7b). The experimental conductance set with the differences in ge integral preserved predicted experimental PSP heights that were strongly correlated with the control PSP heights ($R^2 = 0.96$, $p < 0.05$). Thus, relative PSP selectivity of this cell could be predicted knowing only the relative ge integrals, with no information about the timing of the inputs or about inhibition. However, the slope was less than 1 (0.56), showing that other parameters enhanced the differences in PSP height.

The other 4 parameters affected the PSPs, but no other single parameter accurately predicted PSP selectivity. Although the experimental PSPs computed from the ge shape conductance set correlated well with the control PSPs ($R^2 = 0.57$), the slope was small and negative (-0.19), suggesting that ge shape slightly reduced PSP selectivity in this cell. In other words, conductance size was more important for determining PSP selectivity than conductance shape. Preserving any of the other parameters generated experimental PSPs that correlated poorly with the control PSPs ($R^2$ values for: gi integral = 0.03; the delay between ge and gi = 0.01; gi shape = 0.06.). Except for ge integral, the effects of the other parameters were small (gentle slope) and/or not systematic in that the correlation coefficient was low.

We summarize these modeling experiments for the 10 cells by plotting the correlation coefficients ($R^2$) values against the slopes for each manipulation of each cell (Fig. 7g). A high $R^2$ value and positive slope indicate that the parameter was important for generating PSP selectivity; a low $R^2$ value or slope close to 0 indicates that the parameter was relatively unimportant for generating selectivity; a high $R^2$ value and negative slope indicate that the parameter tended to reduce selectivity.

In this analysis, ge integral was the single best predictor of PSP selectivity (Fig. 7g). In 8/10 cells, the fits using ge integral had the highest $R^2$ values. In one cell, using ge shape resulted
in the highest $R^2$, and in one cell ge shape and ge integral gave equal $R^2$ values. Further, ge integral was the only parameter to systematically enhance selectivity in that it always resulted in a positive correlation between the control and experimental PSP heights ($slope > 0$). The $R^2$ values and slopes were not equal to 1, showing that other parameters affected the relative PSP heights in every cell. However, the mean slope from the linear regressions using ge integral was close to 1 ($0.95 \pm 0.15$) suggesting that although the other parameters affected selectivity, their effects were not systematic; sometimes the other parameters enhanced selectivity, and sometimes the other parameters reduced selectivity. In comparison, the mean slopes from the linear regressions were close to 0 for every other parameter we tested (mean slope: gi integral = $0 \pm 0.1$; delay = $-0.01 \pm 0.1$; ge shape = $0.1 \pm 0.1$; gi shape = $-0.1 \pm 0.1$). There were individual cells where these parameters enhanced selectivity, and also cells where they reduced selectivity, but the overall effects of these parameters were not systematic.

Conductance integrals, not timing, determine PSP height.

The preceding analyses show that the ge integral is the best individual predictor of PSP height. However, when the conductance parameters are combined, their effect on PSPs is not the linear sum of their individual effects. It is conceivable that the combined timing parameters would predict PSP heights better than the combined rate parameters. We therefore compared control PSPs to experimental PSPs computed with either the 3 timing parameters preserved, or the 2 rate parameters preserved (Fig. 8). When the rate parameters were preserved (both ge and gi integrals) in the example cell, the experimental PSPs correlated even more closely with the controls with a slightly steeper slope ($R^2 = 0.98$, slope = 0.62, $p < 0.01$, same cell as Fig. 7). In contrast, when the timing parameters were preserved (delay, ge shape and gi shape), the experimental PSPs did not correlate with the controls ($R^2 < 0.01$, slope = 0.02). This cell relied more on the conductance integrals to discriminate amongst the FMs than on the temporal envelopes.

We summarized the comparison between the timing sets and the rate sets by showing the correlation coefficients and slopes for each cell (Fig. 8d). The rate parameters predicted the
control PSPs better than the timing parameters in all 10 cells (higher $R^2$ values). For the rate sets, the mean $R^2$ value was high (0.90 ± 0.05), the mean slope was 1 (1.0 ± 0.14) and 8/10 fits were statistically significant ($p < 0.05$). Remarkably, in 8/10 cells the relative PSP selectivity could be reliably predicted using *no temporal differences whatsoever*. In contrast, the timing sets had lower mean $R^2$ and slope values (0.4 ± 0.09 and 0.02 ± 0.11 respectively), and only one fit was significant ($p < 0.05$, the example cell from Fig. 5a). This indicates that in these cells, the size of the synaptic conductance waveforms were more important for generating PSP selectivity than the temporal envelopes or the delays between excitation and inhibition.

**Eliminating inhibition changes absolute PSP height, but not PSP selectivity**

Having found that the size of the excitatory input is the best predictor of PSP selectivity raises the question of whether blocking inhibition in these cells would have reduced selectivity as shown in previous studies (Andoni et al. 2007; Fuzessery and Hall 1996; Koch and Grothe 1998). To address this question, we compared control PSPs to PSPs computed with excitation only, as if inhibition were completely eliminated (Fig. 9). We show two example cells where we computed control PSPs with both excitation and inhibition, or with excitation alone (Fig. 9a, b). In both examples, we show responses to the preferred sweep direction at different velocities. Control PSPs (gray, dashed) were either below or near-threshold (mean threshold ± 1 SD). The experimental PSPs computed with inhibition eliminated (black) either reached or exceeded threshold, consistent with the result that blocking inhibition would have increased firing rate and either reduced or eliminated velocity selectivity in these cells.

We plotted PSP heights computed with and without inhibition for the 10 cells in which we derived synaptic conductances. In 5/10 cells that fired spikes to sweeps, we compared computed PSP heights to spike threshold measured from actual responses (Fig. 9c-d). For the sweeps that evoked spikes, the control PSPs were within or very close to threshold ± 1 SD. No sweeps evoked spikes in the other 5 cells, so we compared PSP heights to the best (biggest) control PSP (Fig. 9 e, f). We make two points. First, eliminating inhibition always increased the absolute PSP heights, but had limited effects on height order. The height of each individual PSP had a position
relative to the other PSPs in each cell, larger than some, and smaller than others. With inhibition
eliminated, all 50 PSPs in 10 cells increased in height, but only 4 PSPs changed relative position
(circled in Fig. 9c-f). This is consistent with the modeling results indicating that excitation is the
main contributor to relative PSP height, i.e. PSP selectivity. Second, 10/14 subthreshold PSPs
reached or exceeded threshold when inhibition was eliminated, and two of the remaining four
PSPs were very close to threshold (Fig. 9c, d). These results suggest that inhibition shapes
response selectivity by adjusting the gain on PSP height. Blocking inhibition increases the height
of all the PSPs, and many that were subthreshold under control conditions become
suprathreshold, thereby reducing response selectivity.
Discussion

Using modeling and results from in vivo whole-cell recordings, we investigated the mechanisms underlying FM selectivity in IC cells. In the cells we tested, the same mechanisms we previously reported to underlie selectivity for FM direction (Gittelman et al. 2009), namely input size and spike threshold, also underlie selectivity for FM velocity and intensity. Our findings show a more complex view of spike selectivity than proposed in previous IC studies that used extracellular electrodes, and suggest that although FM direction, velocity and intensity are encoded differently in the auditory nerve, the coding strategy for these auditory parameters merge as the signal ascends.

Spike threshold sharpens selectivity

Analysis of PSP selectivity compared to spike selectivity shows that spike threshold sharpens velocity and intensity tuning, as we found previously for FM direction (Gittelman et al. 2009). This is the first such demonstration for velocity and intensity in the IC, as previous studies either assumed or did not consider a role for spike threshold (Andoni et al. 2007; Fuzessery et al. 2006; Gordon and O'Neill 1998). However, in vivo whole-cell studies have shown a prominent role for spike threshold in cortical response selectivity in the auditory (Tan et al. 2004; Ye et al. 2010; Zhang et al. 2003), somatosensory (Brecht et al. 2003; Moore and Nelson 1998) and visual systems (Jagadeesh et al. 1993; Priebe and Ferster 2005; 2006). This suggests a common if not ubiquitous role for spike threshold.

We also noted that velocity changes evoked greater PSP variance than changing either direction or intensity, and we speculate on the significance. In terms of differences in spike counts, velocity discrimination begins in the auditory nerve whereas direction discrimination begins in the cochlear nucleus (Britt and Starr 1976). Thus, velocity discrimination has gone through an extra synapse in terms of refinement, and thus we might expect to see a greater difference in the IC afferents compared to direction. In the case of intensity, we speculate that while it is important to distinguish amongst FMs, it is also necessary to compress intensity differences, at least to some extent. In other words, we perceive sounds with the same FM
parameters as the same, even when they occur at different intensities, and thus in some cells
differences in FM might be strengthened, while differences in intensity may be reduced.

**Input size shapes selectivity more than input timing**

Our modeling found that the size of excitation was the single best predictor of PSP
selectivity, and when combined with the size of inhibition, input size captured > 90% of the PSP
height variance in 8/10 cells. This is in contrast with most previous studies, which posited that
input timing, particularly the timing between excitation and inhibition, creates FM selectivity in IC
cells (Andoni et al. 2007; Gordon and O'Neill 1998; Suga 1968), and even in cortical cells (Razak
and Fuzessery 2008; 2006; Ye et al. 2010; Zhang et al. 2003). We found that input timing did
affect PSP selectivity, but the effect was small relative to that of input size. Thus, our modeling
supports a modulatory role for timing, rather than timing as the primary determinant.

**Inhibition reduces PSP gain.**

Perhaps the most striking result was that of blocking inhibition. When inhibition was
removed from the model, PSP selectivity changed very little in that the least-preferred stimulus
that evoked the smallest PSP was still least-preferred, and the most-preferred stimulus that
evoked the largest PSP remained most-preferred. However, all the PSPs increased in size so
that many subthreshold PSPs exceeded spike threshold following inhibition removal. This is
consistent with the finding that blocking inhibition reduces selectivity and increases firing rate, but
the mechanism is different than previously proposed in the auditory system (Andoni et al. 2007;
Fuzessery and Hall 1996; Koch and Grothe 1998; Razak and Fuzessery 2009). Our results here
and in (Gittelman et al. 2009; Gittelman and Pollak 2011) suggest that inhibition enhances
selectivity by keeping null PSPs below threshold, a finding that is consistent with the role recently
proposed for inhibition in spatial motion selectivity in the visual cortex (V1) (Katzner et al. 2011).

**Limitations**
We briefly discuss two important limitations of this study. First, the conductance estimates suffer from space clamp errors, a problem shared with many in vivo studies; whole-cell recordings made from the soma fail to capture inputs from distal synapses (Williams and Mitchell 2008), a situation made worse by the high access resistance common to in vivo whole-cell recordings (Gittelman et al. 2009). The result is that the conductance estimates are effectively low-pass filtered, reduced in size and temporally smeared. We believe that our study minimizes these errors because the cells have relatively high input resistance (Gittelman et al. 2009), and are physically small (unpublished observations), similar in size to the those found in the mustache bat (Zook et al. 1985). Indeed, bat IC cells are much smaller than cortical pyramidal cells (Williams and Mitchell 2008). These characteristics reduce space clamp errors. Furthermore, by presenting only sounds composed of the same frequencies, we are likely activating the same population of afferents in individual cells. Therefore, we believe that the conductance waveforms are subject to similar filtering, validating the within-cell comparison.

Second, the cells with sound-evoked conductance estimates are few (n = 10), and representative of only a subset of IC cells. As we reported previously, we only obtained conductance measurements from cells with relatively slow membrane time constants (slow cells, $\tau = 12.5 \pm 1.0$ ms) (Gittelman et al. 2009). Although there is a population of fast IC cells ($\tau < 5$ ms) (Gittelman et al. 2009; Xie et al. 2008), and slow IC cells have also been reported in mouse, rat and gerbil (Koch and Grothe 2003; Sivaramakrishnan and Oliver 2001; Tan et al. 2007). That the response selectivity of IC cells we recorded from is more affected by input size than timing is consistent with the smearing effect of a slow membrane time constant on timing precision. We note that cortical cells such as pyramidal cells are relatively slow (Oswald and Reyes 2008; Trevelyan and Jack 2002), consistent with less sensitivity to input timing compared to input size. Although whole-cell studies in the auditory cortex have correlated input timing differences with selectivity, these studies did not test the relative contribution of input timing compared to input size (Wehr and Zador 2003; Ye et al. 2010; Zhang et al. 2003).
necessarily affects response selectivity, it is not the dominant determinant in slow cells, independent of species or nucleus.

**Implications for neural coding**

For sounds that are composed of the same frequencies, such as the FMs presented here, the finding that input size is the main predictor of spike selectivity suggests that the information to discriminate among FMs is carried to the IC mainly by the number of afferent spikes within a brief temporal window, rather than by the precise timing of individual spikes. If the FMs we presented likely activated primarily the same afferents, then the differences in conductance size are due to differences in the total number of afferent spikes, rather than differences in synapse location. The alternative, that larger evoked conductance resulted from proximal synapses while smaller conductance resulted from distal synapses, would require that the preferred sweeps activated a different afferent population compared to less-preferred or null sweeps. Given the lack of strongly FM selective cells in the brainstem (Britt and Starr 1976; Erulkar et al. 1968; Huffman et al. 1998), this alternative seems unlikely.

In summary, we suggest that FM selectivity in slow IC cells, including direction, velocity and intensity, is generated as follows. Weakly selective excitatory afferents fire more to preferred sweeps, providing larger excitatory inputs compared to less-preferred or null sweeps, largely establishing PSP selectivity in the postsynaptic cell. PSP selectivity is modulated by other input parameters, including input timing and the size of inhibition. However, the dominant role of inhibitory inputs is to reduce PSP amplitudes so that they peak near spike threshold, some above and some below. Spike threshold then sharpens selectivity by rectifying subthreshold inputs, while also amplifying small differences in near-threshold and suprathreshold PSP peaks to produce strong spike selectivity (Gittelman et al. 2009). This would indicate that the different coding strategies noted in the introduction for FM direction and velocity in the auditory nerve are largely eliminated once the signal reaches the IC, where the coding strategy is more uniform.
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Figure legends

Figure 1. FM selectivity for direction, velocity and intensity. Responses to FMs that swept the same one octave frequency range with direction and velocity varied as shown in the PSP plots. (a1, b1, c1) Spike selectivity. Spikes (left) are individual traces labeled with spikes/ trial. For graphic representation of each cell (right), spike probability was normalized to the maximum evoked probability. (a2, b2, c2) PSP selectivity. PSPs (left) are average responses of 10 trials with spikes filtered. For graphic representation of each cell (right), PSPs were quantified in terms of height, and then normalized to the largest PSP. Color-scale applies to spikes and PSPs. Labels in (a) apply to (b, c). (a) Cell 1 preferred slow velocity, low intensity sweeps in either direction (best sweep: 5 oct/ s, 25 dB SPL). (b) Cell 2 preferred downward, medium velocity, medium intensity sweeps (best sweep: downward, 120 oct/ s, 45 dB SPL). (c) Cell 3 preferred downward, fast velocity, high intensity sweeps (best sweep: downward, 320 oct/ s, 75 dB SPL).

Figure 2. Spike threshold sharpens velocity and intensity selectivity. (a) The distribution of preferred velocities (the preferred velocity evoked the most spikes; n = 22; NS, non-selective.) (b) The number of velocities (incremented in factors of 2) tested that evoked spikes compared to the number that evoked PSPs. 18/ 22 cells fell below the unity line, indicating that spike threshold sharpened velocity selectivity in most cells. The three non-selective cells plus one FM selective cell fell on the line. In 16/ 22 cells we did not test the full range of velocities that evoked PSPs, so this is a minimum estimate of how much spike threshold sharpens velocity selectivity. (c) The number of intensities tested that evoked spikes compared to the number that evoked PSPs. (Intensity range 30 - 60 dB SPL, number of intensities tested 2 - 5.) 8 cells fell below the unity line. In 15/ 18 cells we did not test the full range of intensities that evoked PSPs, so this is a minimum estimate of how much spike threshold sharpens intensity selectivity.

Figure 3. PSP height is more sensitive to FM velocity that to direction or intensity. We measured and compared the average (left column) and maximum (right) change in PSP height as a function of changing FM velocity, direction, or intensity. In many cells, changing velocity changed PSP height more than changing either (a, b) direction or (c, d) intensity. (e, f) Changing intensity or direction generated similar PSP height changes in the population of cells. Open circles are the 3 non-selective (NS) cells.

Figure 4. Derived conductance pairs predict membrane responses. (a) Responses (black) from one cell to 6 one-octave FM sweeps at rest and while hyperpolarizing current was injected (16 – 32 kHz sweeps at 45 dB SPL; velocity and direction shown below bottom traces; steady state membrane potential in color on left; spikes (gray, single trials) truncated, firing probability as shown; PSPs are average of 10 trials; same cell as in Fig. 1b; resting membrane potential = -42 mV.) Red traces were computed in a model using the conductance traces shown in (b, c). (b, c) Conductance pairs (excitation (ge) and inhibition (gi)) derived from the membrane responses in (a). (b) ge and gi aligned with the membrane responses and (c) scaled to show differences in integral, shape, and delay between ge and gi. Gray shows 95% confidence interval (bootstrapping). (d) predicted membrane potentials plotted against measured membrane potentials at each time point for all downward and upward FMs at the three velocities shown. Points fit by linear regression, $R^2 \geq 0.98$.

Figure 5. Sweep parameters affect conductance size, shape, and delay between excitation and inhibition. Varying sweep velocity (a-d) or intensity (e-h) altered multiple conductance parameters. (a, e) measured PSPs (black, spikes removed by filtering, firing probability as shown) and PSPs predicted (dashed gray) from derived conductance waveforms (bottom). Sweeps were downward, bars show stimulus times. (a) Velocities varied as shown. Intensity was 45 dB SPL (same cell as Fig. 4). (e) Intensity varied as shown. Velocity was 150 oct/ s. (b, f) Effect on the delay between excitation and inhibition. Traces normalized to peak height. (b) Note the null (15 oct/ s) time scale is longer than the other two. (c, g) Effect on conductance size. Conductances aligned at peaks. (d, h) Effect on conductance shape. Traces aligned at peaks and normalized to maximum peak height.
Figure 6. Determining the conductance parameters underlying PSP selectivity. (a) Control PSPs computed from a hypothetical control conductance set. Conductance pairs differed in: the delay between ge and gi; ge integral; and ge shape. No differences in gi shape or integral. (b) Parameter differences eliminated by: averaging the g waveforms to eliminate size and shape differences; and then aligning the ge and gi peaks to eliminate delay differences. (c-e) Left: experimental PSPs (top) computed from experimental conductance sets (bottom). Right: experimental PSP heights plotted against control PSP heights and fit by linear regression. (c) Testing ge integral. Average g waveforms with ge integrals scaled to be equal to the integrals of the corresponding controls. Control and experimental PSP heights are positively correlated ($R^2 = 0.95$; slope = 1.7), indicating that ge integral was important for PSP selectivity. (d) Testing the delays between ge and gi. Average g waveforms with gi latency shifted to match the control delays (compare delays to (a)). Control and experimental PSP heights are not correlated ($R^2 = 0.14$, slope = 0.1), indicating that the delay was relatively unimportant for PSP selectivity. (e) Testing ge shape. Control ge shapes with the integrals scaled to be equal to the average ge integral. Control and experimental PSP heights are negatively correlated ($R^2 = 0.88$, slope = -1), indicating that ge shape reduced PSP selectivity.

Figure 7. ge integrals predict PSP heights. (a-f) Format same as Fig. 6. (a) Control PSPs (black) computed from the control conductance set derived from the responses of an IC cell to 6 FM sweeps (14 – 28 kHz at 15 dB SPL; velocity and direction as shown). Dashed vertical lines mark excitatory peaks. Rest = -48 mV. Measured PSPs (grey) shown for comparison, spikes removed by filtering, firing probability as shown. (b-f) PSPs computed from experimental conductance sets where the differences in one parameter were preserved as shown. (Right) experimental PSP heights plotted against control PSP heights, fit by linear regression. (g) $R^2$ values plotted against the slopes from each of 10 cells. Symbols indicate which parameter was preserved. For ge integral, we distinguished the cells where FM velocity was varied (closed red circles) from those where intensity was varied (red +).

Figure 8. Rate vs. Time: rate parameters predict PSP height. Format same as Fig. 6. (a) PSPs computed from the control conductance set (same cell as Fig. 7). PSPs computed from the experimental conductance set with the (b) integral differences preserved (both ge and gi), and the (c) timing differences preserved (ge shape, gi shape and the delay between ge and gi). (d) Preserving the integral differences resulted in stronger positive correlations between the control and experimental PSP heights than preserving the timing differences.

Figure 9. Inhibition adjusts PSP gain, thereby narrowing response selectivity. In the 10 cells with derived synaptic conductances, we modeled the effects of blocking inhibition by comparing PSPs computed with inhibition (gray, dashed) and without inhibition (black) for downward and upward FMs with velocity or intensity varied as shown. (a, b) Responses of two example cells to the preferred sweep direction, velocity varied. The control PSPs computed with excitation (ge) and inhibition (gi) were either sub- or near-threshold (spike threshold ± 1 SD, dashed line ± gray box). The PSPs computed without inhibition reached or exceeded threshold. Cell 1 (a) is from Fig. 4. Cell 2 (b) is from Fig. 7. (c-f) Compared to controls, eliminating inhibition increased the absolute PSP heights without changing the height sequence (largest to smallest) except for 4/50 PSPs (c-f, circled). (c, d) 5/10 cells fired spikes to sweeps (firing probability as shown). 10/14 subthreshold control PSPs reached or exceeded threshold when inhibition was eliminated, suggesting that blocking inhibition would reduce selectivity. (c) Velocity varied. (d) Intensity varied. (e, f) 5 cells that did not fire to sweeps. (e) 3 cells had voltage-gated channel blockers in the recording pipette. (f) Two cells fired to current steps, but the sweeps tested evoked only subthreshold PSPs. (For each cell, all FMs swept one octave with either velocity or intensity varied as shown on plot, and intensity or velocity fixed as listed: cell 1, 45 dB SPL; cell 2, 15 dB SPL; cell 3, 15 dB SPL; cell 4, 150 oct/ s; cell 5, 175 oct/ s; cell 6, 35 dB SPL; cell 7, 75 dB SPL; cell 8, 75 dB SPL; cell 9, 45 dB SPL; cell 10, 50 oct/ s)
a) Graph showing the number of cells with best velocities for downward and upward velocities (oct/s). The x-axis represents velocity (oct/s) ranging from -320 to 320, and the y-axis represents the number of cells.

b) Scatter plot showing the number of velocities tested that evoked spikes versus the number of velocities tested that evoked PSPs. The graph includes data points for $n = 1$, $n = 2$, and $n = 3$.

c) Scatter plot showing the number of intensities tested that evoked spikes versus the number of intensities tested that evoked PSPs. The graph includes data points for $n = 1$ and $n = 5$. 
measured PSPs
predicted PSPs

-42 mV
-58 mV
-67 mV
-86 mV

1.4 0.3 0.6

50 ms
15 oct/ s 60 oct/ s 240 oct/ s

-80 -60 -40
measured (mV)

-80 -60 -40
240 oct/ s
a. Control conductance set and computed PSPs

b. All parameter differences eliminated

Experimental conductance sets and computed PSPs

c. Ge integral preserved

d. Delays preserved:

Ge shape preserved