Stimulus Selectivity Is Enhanced by Voltage-Dependent Conductances in Combination-Sensitive Neurons

Bruce A. Carlson and Masashi Kawasaki

Department of Biology, University of Virginia, Charlottesville, Virginia

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Carlson, Bruce A. and Masashi Kawasaki. Stimulus selectivity is enhanced by voltage-dependent conductances in combination-sensitive neurons. J Neurophysiol 96: 3362-3377, 2006. First published September 27, 2006; doi:10.1152/jn.00839.2006. Central sensory neurons often respond selectively to particular combinations of stimulus attributes, but we know little about the underlying cellular mechanisms. The weakly electric fish Gymnarchus discriminates the sign of the frequency difference (Df) between a neighbor's electric organ discharge (EOD) and its own EOD by comparing temporal patterns of amplitude modulation (AM) and phase modulation (PM). Sign-selective neurons in the midbrain respond preferentially to either positive frequency differences (Df >0 selective) or negative frequency differences (Df <0 selective). To study the mechanisms of combination sensitivity, we made whole cell intracellular recordings from sign-selective midbrain neurons in vivo and recorded postsynaptic potential (PSP) responses to AM, PM, Df >0, and Df <0. Responses to AM and PM consisted of alternating excitatory and inhibitory PSPs. These alternating responses were in phase for the preferred sign of Df and offset for the nonpreferred sign of Df. Therefore a certain degree of sign selectivity was predicted by a linear sum of the responses to AM and PM. Responses to the nonpreferred sign of Df, but not the preferred sign of Df, were substantially weaker than linear predictions, causing a significant increase in the actual degree of sign selectivity. By using various levels of current clamp and comparing our results to simple models of synaptic integration, we demonstrate that this decreased response to the nonpreferred sign of Df is caused by a reduction in voltage-dependent excitatory conductances. This finding reveals that nonlinear decoders, in the form of voltage-dependent conductances, can enhance the selectivity of single neurons for particular combinations of stimulus attributes.

INTRODUCTION

A primary function of sensory systems is to extract behaviorally relevant information from peripheral sensory input (Heiligenberg 1991; Marr 1982). At each subsequent level of processing, individual sensory neurons become increasingly selective, often responding preferentially to specific combinations of different stimulus features. These combination-sensitive neurons play a critical role in sensory processing because they provide information that cannot be obtained by analyzing any one stimulus feature in isolation (Schnupp and King 2001). For example, the visual system must obtain information about multiple stimulus attributes such as shape, size, color, orientation, speed, and direction of motion, and then combine information about each of these different features to detect, identify, and discriminate among different visual stimuli. Certain visual neurons in the mammalian cortex display remarkable selectivity for complex stimuli, providing explicit representations of faces, individuals, landmarks, animals, objects, or spatial scenes (Ewert 1997; Fujita et al. 1992; Logothetis and Sheinberg 1996; Quiroga et al. 2005; Tanaka 1996; Tsao et al. 2006). Similarly, vertebrate auditory neurons are often highly selective for particular sounds that are defined by relative intensity, temporal sequence, and spectral characteristics (Fuzessery and Feng 1983; Margoliash 1983; Margoliash and Fortune 1992; Misawa and Suga 2001; Peña and Konishi 2001). However, the complexity of vertebrate visual and auditory systems has made it difficult to precisely characterize the mechanisms underlying the transformation from raw sensory input to single-neuron combination sensitivity.

The weakly electric fish Gymnarchus niloticus generates a nearly sinusoidal electric organ discharge (EOD) that is monitored using an array of electroreceptors distributed throughout the skin, allowing them to communicate and electrolocate (Lissman 1958). When two fish with similar EOD frequencies meet, their electrolocation abilities are impaired by electrical interference (Heiligenberg 1975). To avoid this, both fish shift their EOD frequencies away from each other, a behavior called the "jamming avoidance response" (JAR) (Bullock et al. 1975). To perform the JAR, a fish determines the sign of the frequency difference (Df) between its own EOD and its neighbor's EOD (Df = neighbor's EOD frequency - own EOD frequency) by comparing the temporal patterns of sinusoidal amplitude modulation (AM) and sinusoidal phase modulation (PM) that result from the interference (Kawasaki 1993). By themselves, both AM and PM are identical for opposite signs of Df, but the temporal relationship between them differs: when Df is positive (Df >0), PM is advanced by 90° relative to AM, but when Df is negative (Df <0), PM is delayed by 90° relative to AM (Carlson and Kawasaki 2004).

AM and PM are processed in separate electrosensory pathways that converge in the midbrain torus semicircularis (Kawasaki and Guo 1998), where a number of neurons are selective for the sign of Df (Kawasaki and Guo 2002). In response to sinusoidal AM, these sign-selective neurons typically fire during either the rising or falling portion of the stimulus and, in response to sinusoidal PM, they typically fire during either the advanced or delayed portion of the stimulus (Carlson and Kawasaki 2004). Sign selectivity is generally characterized by a nonlinear summation of the spiking responses to AM and PM that depends on their relative timing; for the preferred sign of Df, the responses to AM and PM are typically aligned, leading to a linear to supralinear summation of spike rates, but for the nonpreferred sign of Df, these responses are typically offset,

Address for reprint requests and other correspondence: B. A. Carlson, University of Virginia, Department of Biology, 277 Gilmer Hall, P.O. Box 400328, Charlottesville, VA 22904-4328 (E-mail: bc6s@virginia.edu).

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leading to a linear to sublinear summation (Carlson and Ka-wasaki 2004).

Experimental and theoretical studies on synaptic integration by single neurons suggest that several possible mechanisms could be responsible for this nonlinear processing, such as inhibitory shunting (Borg-Graham et al. 1998; Torre and Poggio 1978), presynaptic inhibition (Rudomin et al. 1998), and voltage-dependent ion channels, including both ligand-gated channels with voltage dependency (Mel 1992, 1993) and ion channels that are regulated exclusively by voltage (Magee 1999). Alternatively, a nonlinear summation of spike rates can result from a linear summation of the underlying postsynaptic potentials (PSPs) because of a nonlinear relationship between membrane potential and spike rate (Jagadeesh et al. 1993; Srinivasan and Bernard 1976). In the current study, we made whole cell recordings in vivo from sign-selective midbrain neurons in Gymnarchus to explore these possibilities and determine how the PSP responses to AM and PM interact to confer selectivity for the sign of Df.

METHODS

Animals

We used 38 *Gymnarchus niloticus* of both sexes (10–20.5 cm in total length). They were collected in West Africa at lengths of 5 to 6 cm and raised to the experimental size in our laboratory under conditions identical to those described earlier (Kawasaki 1994). After anesthesia with tricaine methanesulfonate (MS-222, 1:10,000; Sigma, St. Louis, MO), we immobilized fish with an intramuscular injection of flaxedil (gallamine triethiodide: 8 to 20 μ l of a 0.1% solution; Sigma), which greatly attenuated EOD amplitude. Activity of the EOD pacemaker command signal was recorded from the tail to monitor the fish's condition throughout each experiment.

Fish were placed inside a Plexiglas chamber, gently held with a sponge-lined clamp, and submerged in water except for a small area along the dorsal surface of the head. Oxygen-saturated water was provided to the gills with a tube inserted in the mouth. After local application of Xylocaine (2%; Barber Veterinary Supply, Richmond, VA), we removed a small portion of the skull and meninges above the midbrain. The valvula cerebelli, which lies above the torus semicircularis (Bass and Hopkins 1982), was gently displaced using the grounding wire to expose the dorsal surface of the torus. At the conclusion of experiments, fish were killed by deep anesthesia in MS-222 (1:1,000). These procedures are in accordance with the guidelines established by the National Institutes of Health and were approved by the University of Virginia Animal Care and Use Committee.

Whole cell intracellular recording

We obtained whole cell recordings from torus neurons following the method of Rose and Fortune (1996). Electrodes were pulled in three stages to a tip diameter of about 1.2 μ m and filled with a tip solution containing (in mM): potassium acetate (100), KCl (2), MgCl₂ (1), EGTA (5), HEPES (10), KOH (20), and biocytin (43). The shank was filled with an identical solution except that the biocytin was replaced with mannitol. This yielded pipette resistances of 20–30 MΩ and initial seal resistances >1 GΩ. After gaining intracellular access, we estimated the series resistance and input resistance as the first and second components, respectively, of a double-exponential fit to the voltage response to square-wave current injection of -0.1 nA. The estimated median series resistance was 76 MΩ and the estimated median input resistance was 195 MΩ. We obtained recordings from a total of 67 neurons in the torus semicircularis that responded to stimulus modulation and met the following criteria for inclusion: stable resting potentials of at least -30 mV after subtracting a calculated liquid junction potential of 5.6 mV (Barry 1994) and spikes with a height of $\geq 10 \text{ mV}$. A small number of neurons (11 of 67) did not produce any spikes at rest or in response to stimulus modulation. However, these neurons had stable resting potentials, responded with robust PSPs to sensory stimulation, and generated large spikes in response to depolarizing current injection. We therefore included these neurons in our analyses of PSP responses. The mean \pm SD of the resting potentials was $-55.02 \pm 8.75 \text{ mV}$, and spike heights typically ranged from 20 to 30 mV.

Intracellular activity was amplified tenfold on an AxoClamp 2B amplifier (Molecular Devices, Palo Alto, CA) then sent to an A/D converter with a sampling rate of 20 kHz and to a Schmitt Trigger with an output to an event timer that recorded spike times at a clock rate of 1 MHz (models DA3-4 and ET1, respectively; Tucker-Davis Technologies [TDT], Gainesville, FL). Intracellular potentials and spike times were saved using custom-made software for Matlab 7.0.1 (The MathWorks, Natick, MA).

Sensory stimulation

Information about PM is extracted centrally by comparing differences in phase between different portions of the body surface (differential PM). To independently stimulate fish with AM and differential PM, we used a phase chamber to electrically isolate the head and trunk of each fish (Fig. 1A). Sinusoidal electric stimuli at a frequency within 20 Hz of the fish's EOD frequency before the experiment (ranging from 350 to 460 Hz) were delivered to both chambers using homemade isolators with field effect transistors. Both chambers received a single sinusoidal signal, in which the carrier signal and any modulations were numerically generated using custom-made software for Matlab 7.0.1, which controlled a D/A board using a sampling rate of 20 kHz (TDT model DA3-4). Two programmable attenuators (TDT model PA4) were used to set the carrier amplitude to values of 1-2mV/cm as measured near the skin surface. Four different stimuli were used (Fig. 1B): sinusoidal AM presented alone, sinusoidal PM presented alone, Df >0 (sinusoidal AM and PM, with PM advanced by 90° relative to AM), and Df <0 (sinusoidal AM and PM, with PM delayed by 90° relative to AM). In each case, one chamber (head or trunk) received the modulated signal, whereas the other chamber received an unmodulated carrier signal (Fig. 1B). The depth of AM was set at 20-25% of the carrier amplitude, and the depth of PM was set at 15-20° of the carrier cycle.

The search stimulus alternated between head Df < 0, head Df > 0, trunk Df <0, and trunk Df >0, at modulation rates of 2 Hz. After establishing an intracellular recording, we determined the modulation rate (1, 2, or 4 Hz) and the chamber that elicited the strongest response. We then obtained responses to sinusoidal AM alone, sinusoidal PM alone, Df >0, and Df <0, presented in pseudorandom order, at the best modulation rate in the preferred chamber. Time permitting, we also obtained responses to other modulation rates (1, 2, or 4 Hz), modulations in the other chamber, and responses under different levels of holding current ranging from -0.4 to +0.2 nA. When we recorded responses to the same stimuli under different holding currents, we periodically injected square pulses of -0.1 nA to ensure that there were no substantial changes in the access or input resistance. We also periodically checked responses in the absence of any holding current to ensure that the peak-to-peak PSP amplitude had not changed.

Data analysis

We ignored responses to the first modulation cycle to avoid any edge effects. Spiking responses to all four stimuli were assessed by determining mean spike rates and constructing histograms of spike Downloaded from jn.physiology.org on November 16,

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FIG. 1. Experimental setup and stimulus delivery. A: a phase chamber is used to electrically divide the fish into separate head (H) and trunk (T) compartments. Each compartment receives separate electrosensory stimulation, allowing one to independently manipulate amplitude modulation (AM) and differential phase modulation (PM) by modulating the amplitude and/or phase of the signal in either compartment. B: schematics of the 4 different stimulus modulations used in the current study, including AM, PM, frequency difference (Df) >0, and Df <0. In the examples shown, the signal in the trunk (T) is unmodulated and the signal in the head (H) is modulated, but in some cases, this was reversed (T was modulated, H was unmodulated). For each stimulus, the solid line above the H stimulus shows the amplitude envelope and the vertical dashed lines show the timing of zero-crossings in the unmodulated T stimulus. AM and PM waveforms are shown below each stimulus as solid and dashed lines, respectively, with positive values representing increased amplitude and phase delays and negative values representing decreased amplitude and phase advances. Compared with the actual stimuli used in experiments, the carrier frequency is set much lower and the PM depth is set much higher to exaggerate the shifts in zero-crossing times caused by PM.

times relative to the modulation cycle, in units of spikes per cycle (Carlson and Kawasaki 2004). To analyze the underlying membrane potentials, we removed spikes using a median filter with a width of 15 ms (Jagadeesh et al. 1993) and defined the resting potential as the mean membrane potential during the 1-s period preceding each stimulus modulation. We estimated the membrane potential derivatives by low-pass filtering the membrane potential using a cutoff frequency of 10 Hz and then differentiating the resulting waveform. To analyze PSP responses to all four stimuli, we calculated the average membrane potential and average derivative with respect to the stimulus modulation cycle and then analyzed a total of 11 different variables from these average responses, including the peak-to-peak PSP amplitude, the maximum and minimum PSP amplitudes relative to rest, the integral of the positive and negative portions of the PSP relative to rest, the maximum and minimum derivative, and the durations of the positive and negative portions of both the average PSP relative to rest and its derivative (rising and falling portions). We measured the degree of sign selectivity (SS) as

$$SS = \frac{Df > 0_{response} - Df < 0_{response}}{|Df > 0_{response}| + |Df < 0_{response}|}$$

where the response to Df >0 and Df <0 was either the mean spike rate or one of the 11 different PSP response measures (Carlson and Kawasaki 2004; Jagadeesh et al. 1993).

For both the response histograms and average PSP responses, we compared the observed responses to Df >0 and Df <0 with a linear sum of the separate responses to AM and PM presented alone. Linear summations of the AM and PM responses were obtained in the following way. First, we subtracted either the resting spike rate (for the response histograms) or the resting potential (for the average PSP responses) from the AM and PM responses. Then, we advanced the average PM response by 90° for Df >0 or delayed the average PM response by 90° for Df <0 so that the response was aligned with the combination stimulus. Finally, we added the resulting responses to AM and PM. In the case of the response histograms, negative values were then changed to 0 because spike rates cannot be negative. To determine whether observed spiking responses to Df >0 and Df <0deviated significantly from a linear sum, the observed spike rates from each modulation cycle were tested against the spike rate derived from the linear summation histogram using a single sample *t*-test. Observed spike rates that were significantly lower than a linear sum were classified as "suppressed" spike-rate responses, observed spike rates that were significantly greater than a linear sum were classified as "facilitated" spike-rate responses, and observed spike rates that were not significantly different from a linear sum were classified as "linear" spike-rate responses (Carlson and Kawasaki 2004). For the predicted linear PSP responses to Df >0 and Df <0, we measured the same 11 variables as we measured from the actual PSP responses (see above).

We used circular cross-correlation to examine the temporal relationship between the average PSP responses to AM and PM using custom-made software for Matlab 7.0.1 (Oppenheim et al. 1999). The circular cross-covariance (γ_{AM} . PM) between the average AM and PM responses is defined as

$$\gamma_{\text{AM} \cdot \text{PM}}(\tau) = \sum_{\theta=0^{\circ}}^{300^{\circ}} \left[\text{AM}(\theta) - \mu_{\text{AM}} \right] \cdot \left[\text{PM}(\theta - \tau) - \mu_{\text{PM}} \right]$$

where θ is defined with respect to the stimulus modulation cycle (ranging from 0° at the start of the cycle to 360° at the end of the cycle), τ is the delay of the average PM response relative to the average AM response, and μ_{AM} and μ_{PM} are the means of the average AM and PM responses, respectively. The circular cross-correlation function (ρ_{AM} . PM), which ranges from -1 (perfect negative correlation) to 1 (perfect positive correlation), is obtained by normalizing the cross-covariance by the autocovariance of the average AM and PM responses at $\tau = 0$

$$\rho_{\mathrm{AM} \cdot \mathrm{PM}}(\tau) = \gamma_{\mathrm{AM} \cdot \mathrm{PM}}(\tau) / \sqrt{\gamma_{\mathrm{AM}}(0)} \cdot \gamma_{\mathrm{PM}}(0)$$

All statistical analyses were done using Statistica 6.1 (StatSoft, Tulsa, OK) with a two-tailed $\alpha = 0.05$. Unless otherwise stated, all reported values are the mean \pm SD. To avoid the inclusion of multiple responses from individual neurons in statistical analyses, we included data obtained only from the modulation rate (1, 2, or 4 Hz) and chamber (head or trunk) that elicited the largest peak-to-peak PSP amplitude in response to either Df >0 or Df <0.

Modeling

We generated simplified, single-compartment, conductance-based models of synaptic integration for qualitative comparison with our intracellular recording data using the following equation for a leaky integrator with a sampling period of 1 ms

$$\tau_m \frac{\mathrm{d}V_m}{\mathrm{d}t} = E_L - V_m + r_m g_e P_e (E_e - V_m) + r_m g_i P_i (E_i - V_m) + R_m I_e$$

where V_m is the membrane potential (in mV), E_L is the resting potential (-65 mV), R_m is the total membrane resistance (200 M Ω), τ_m is the membrane time constant, equal to the product of R_m and the total membrane capacitance (0.15 nF), E_e and E_i are the excitatory and inhibitory reversal potentials (0 and -75 mV, respectively), I_e is extrinsic current (in nA), r_m is the specific membrane resistance (1) M $\Omega \cdot mm^2$), g_e and g_i are the excitatory and inhibitory specific conductances (in nS/mm²), and P_e and P_i describe the probability (ranging from 0 to 1) that excitatory and inhibitory channels are open (Dayan and Abbott 2001). Excitatory and inhibitory responses to sinusoidal stimulus modulations were simulated by specifying the values of g_e and g_i and varying the open probabilities (P_e and P_i) according to half-wave-rectified sine waves (the rectification prevents negative values) with an amplitude of 1 and a period of 1 s (corresponds to a modulation rate of 1 Hz). P_e had a start angle of 0°, whereas P_i had a start angle of 180°, so that increases in excitatory and inhibitory conductances were offset. To avoid edge effects, we simulated three cycles of stimulation and used the data from the second cycle.

To simulate the effect of current injection on responses to a single feature (either AM or PM), we modeled responses in the presence of various values of I_e , which ranged from -0.4 to +0.4 nA. To simulate the integration of responses to two different features (AM and PM), we modeled separate responses to each feature individually and then summed the weighted conductances from the two inputs and modeled the resulting response to both features. To simulate responses to both signs of Df, the conductances from the two inputs were summed in phase (neither response is shifted, simulating the preferred sign of Df) and out of phase (one response is shifted by 180°, simulating the nonpreferred sign of Df). The resulting responses were compared with a linear sum of the two voltage responses, in the same way that the responses of actual neurons to Df >0 and Df <0 were compared with a linear sum of the responses to AM and PM (see above).

To study the effects of a voltage-dependent excitatory conductance, we used the same model, except that the value of g_e at any given time was scaled as a sigmoidal function of the voltage at the previous time step (t - 1)

$$g_{e,scaled} = g_e \cdot \{0.5 + \frac{1}{1 + e^{-[V_m(t-1)+45]/4}}\}$$

Thus the sigmoidal function is symmetric around -45 mV, at which voltage $g_{e,scaled} = g_e$, whereas more negative voltages asymptote toward $g_{e,scaled} = 0.5g_e$, and less negative voltages asymptote toward $g_{e,scaled} = 1.5g_e$. The denominator of the exponential, which describes the slope of the sigmoidal function (smaller values yield a steeper slope), was arbitrarily set at 4. This type of voltage dependency simulates the effects of a voltage-dependent, ligand-gated conductance, analogous to a conductance resulting from *N*-methyl-D-aspartate (NMDA) –type glutamate receptors, although models based on adding an additional conductance that is purely voltage dependent and not synaptically driven yield qualitatively similar results. All modeling was done using custom-made software for Matlab 7.0.1.

RESULTS

We recorded intracellular responses to sinusoidal AM, sinusoidal PM, Df >0, and Df <0 from a total of 67 neurons located throughout the torus semicircularis (Bass and Hopkins 1982). Of these 67 neurons, 56 (83.58%) generated spikes during stimulus modulation and 11 (16.42%) did not. To investigate the basis of sign selectivity (SS), as measured from spike rate, we analyzed the PSP responses to Df >0 and Df <0

using 11 different measures (Table 1, METHODS). Among those neurons that generated spikes, there was a significant correlation between spike-rate SS and only two of these measures of PSP SS, i.e., the peak-to-peak PSP amplitude and maximum derivative (Table 1). Using peak-to-peak PSP amplitude as a measure of response, neurons with a SS >0.05 were categorized as Df >0 selective and neurons with a SS < -0.05 were categorized as Df <0 selective, resulting in a total of 28 Df >0-selective neurons, 23 Df <0-selective neurons, and 16 nonselective neurons. Unless otherwise stated, all reported SS values are based on peak-to-peak PSP amplitude.

The responses of sign-selective neurons typically consisted of smooth fluctuations in membrane potential that followed the sinusoidal modulations in amplitude and phase (Fig. 2). Small, fast PSPs and action potentials rode on top of these larger fluctuations (Fig. 2). The responses to AM and PM presented alone typically consisted of alternating hyperpolarizing and depolarizing responses relative to rest (Fig. 2). For example, the neuron shown in Fig. 2A was depolarized during amplitude decreases and phase advances and was hyperpolarized during amplitude increases and phase delays. In some cases, the responses to AM alone and PM alone were comparable in magnitude (Fig. 2A). In many cases, however, the response to PM was substantially weaker than the response to AM. For example, the neuron shown in Fig. 2B gave robust responses to AM, but only weak, subthreshold responses to PM. Nevertheless, when AM and PM were combined, the response to PM was sufficient to modify the AM response and give rise to a strong preference for Df < 0 over Df > 0.

Interaction of postsynaptic potential responses to AM and PM

We tested the hypothesis that the observed sign selectivity resulted from a simple linear addition of the responses to AM and PM. After advancing the average PM response by 90° for Df >0 and delaying the average PM response by 90° for Df <0, we generated these predicted linear responses by simply summing the average responses to AM and PM, as illustrated in Fig. 3 (see METHODS). Responses to the preferred sign of Df were typically equal to or slightly weaker than the responses

TABLE 1. Correlation between sign selectivity (SS) of spike rates	
and SS of various postsynaptic potential measurements	
(n = 56 neurons)	

Postsynaptic Potential Measurement	Spearman Rank <i>R</i>	Р
Peak-to-peak amplitude, mV	0.3733	0.0019
Maximum amplitude, mV	0.1724	0.1630
Minimum amplitude, mV	-0.0708	0.5693
Integral of positive values, mV · ms	0.0949	0.4447
Integral of negative values, mV · ms	-0.1080	0.3844
Maximum derivative, mV/ms	0.3463	0.0041
Minimum derivative, mV/ms	-0.1413	0.2539
Positive duration, fraction of cycle	-0.0483	0.6981
Negative duration, fraction of cycle	0.1125	0.3648
Rising duration, fraction of cycle	-0.2283	0.0631
Falling duration, fraction of cycle	0.2342	0.0565

Among neurons that generated spikes, there was a significant correlation between spike-rate SS and only two measures of PSP SS, indicated in boldface type.



FIG. 2. Responses of 2 sign-selective neurons to AM, PM, Df >0, and Df <0. A: 4-s segments of raw data from a Df >0-selective neuron (SS = 0.1352). Below each trace, the solid line represents AM and the dashed line represents PM (modulation rate = 1 Hz), as in Fig. 1B. Arrowheads to the *left* of each trace show the resting potential in the absence of any stimulus modulation. B: 2-s segments of raw data from a Df <0-selective neuron (SS = -0.0841), in the same format as A (modulation rate = 4 Hz).

predicted by a linear sum (Fig. 3). By contrast, responses to the nonpreferred sign of Df were generally much weaker than the predicted responses (Fig. 3). In some cases, the response to the nonpreferred sign of Df was characterized by a reduction in peak-to-peak PSP amplitude compared with a linear sum (Fig. 3, A-C). In other cases, the peak-to-peak PSP amplitude was not substantially different from a linear sum, but the entire response was shifted in a hyperpolarizing direction (Fig. 3*D*).

To quantify the interaction between the responses to AM and PM, we compared the observed responses to Df >0 and Df <0 with the responses predicted by a linear sum of the responses to AM and PM using 11 different PSP measures (see METHODS and Table 1 for a list of these measures). The direction of sign selectivity was generally predicted by a linear sum (predicted SS of Df <0-selective neurons = -0.0384 ± 0.1768 ; predicted SS of Df >0-selective neurons = 0.0798 ± 0.1400). However, the observed peak-to-peak PSP amplitudes were smaller than a linear sum for both the preferred and nonpreferred signs of Df (Fig. 4A) and this deviation was significantly greater for the nonpreferred sign of Df [Wilcoxon matched-pairs test, z(51) = 3.252, P = 0.001]. As a result, the observed SS of Df <0-selective neurons (-0.1815 ± 0.1111) was

significantly greater than the SS predicted by a linear sum of the responses to AM and PM [z(23) = 3.011, P = 0.003]. Similarly, the observed SS of Df >0-selective neurons (0.1240 ± 0.0823) was significantly greater than the SS predicted by a linear sum [z(28) = 2.368, P = 0.018].

For the preferred sign of Df, the observed maximum PSP amplitudes did not generally deviate from a linear sum of the responses to AM and PM (Fig. 4A). For the nonpreferred sign of Df, however, the observed maximum PSP amplitudes were substantially weaker than a linear sum (Fig. 4A), resulting in a significant difference between the preferred and nonpreferred sign of Df [z(51) = 3.056, P = 0.002]. Similarly, there was a significant difference between the preferred and nonpreferred sign of Df in the integral of positive membrane potential values [z(51) = 3.665, P =0.0002]). By contrast, both the preferred and nonpreferred sign of Df showed similar deviations of the minimum PSP amplitude from a linear sum (Fig. 4A) and there was no significant difference for this measure [z(51) = 0.150, P =0.88] or for the integral of negative membrane potential values [z(51) = 0.555, P = 0.56]. Therefore the difference in peak-to-peak PSP amplitude was primarily related to a





3367

FIG. 3. Comparing observed responses to Df >0 and Df <0 with responses predicted by a linear sum of AM and PM responses. Shown are the average postsynaptic potential (PSP) responses of 4 sign-selective neurons to AM, PM, Df >0, and Df <0, along with the responses to Df >0 and Df <0 that would have resulted from a linear sum of the responses to AM and PM. For each trace, the average PSPs are repeated for 2 cycles to illustrate the periodic nature of the responses. Responses to AM and PM are presented twice, once with the average PM response advanced by 90° for alignment with the Df >0 stimulus (*left column*) and once with the average PM response delayed by 90° for alignment with the Df <0 stimulus (*right column*). Below each trace, the solid line represents AM and the dashed line represents PM, as in Fig. 1*B*. Arrowheads to the *right* of each trace show the resting potential in relation to the average PSP responses. *A*: a Df <0-selective neuron (SS = 0.1111). *C*: a Df <0-selective neuron (SS = 0.4940).

reduction in the depolarizing portion of the response to the nonpreferred sign of Df.

The maximum and minimum derivatives also deviated from the values predicted by a linear sum of the responses to AM and PM (Fig. 4*B*). The maximum derivative was slightly larger than predicted for the preferred sign of Df, but substantially smaller than predicted for the nonpreferred sign of Df (Fig. 4*B*), resulting in a significant difference between the preferred and nonpreferred sign of Df [z(51) = 2.859, P = 0.004]. The minimum derivative was slightly less negative than predicted for the preferred sign of Df and was substantially less negative than predicted for the nonpreferred sign of Df (Fig. 4*B*), also resulting in a significant difference [z(51) = 2.943, P = 0.003]. Therefore responses to the preferred sign of Df were characterized by much more rapid increases and decreases in membrane potential. No significant differences were found for the durations of the positive and negative portions of the responses [z(51) = 1.096, P = 0.27] or for the durations of the rising and falling portions of the responses [z(51) = 0.942, P = 0.35].

Relationship between spike-rate nonlinearities and PSP nonlinearities

For Df >0-selective neurons, the sign selectivity of spike rates (0.1689 \pm 0.3516) was slightly larger than the sign



3368

FIG. 4. Deviation of responses from a linear sum of the responses to AM and PM for the preferred and nonpreferred signs of Df. Values show the observed response minus the response predicted by a linear sum of the responses to AM and PM (mean \pm SE, n = 51 neurons). Bars above each pair of measurements denote significant differences determined using a Wilcoxon matched-pairs test. A: deviations of peak-to-peak PSP amplitude ($V_{\text{peak-to-peak}}$), maximum PSP amplitude relative to rest (V_{max}), and minimum PSP amplitude relative to rest (V_{min}). B: deviations of the maximum and minimum membrane potential derivatives (dV/dt_{max} and dV/dt_{min} , respectively).

selectivity of peak-to-peak PSP amplitudes (0.1127 ± 0.0399) , although this difference was not significant [z(25) = 0.0942, P = 0.92]. The sign selectivity of spike rates for Df <0selective neurons (-0.1889 ± 0.4130) was almost identical to that of peak-to-peak PSP amplitudes (-0.1911 ± 0.1155), also a nonsignificant difference [z(25) = 0.44802, P = 0.65]. This finding indicates that the spike-generating mechanism does not confer additional selectivity to these neurons.

Previous extracellular single-unit recordings from sign-selective toral neurons revealed that spike-rate responses to AM and PM frequently sum nonlinearly (Carlson and Kawasaki 2004). For example, the Df <0-selective neuron shown in Fig. 5A responded to Df >0 with a spike rate of 32.55 spikes/s, significantly lower than the spike rate of 42.17 spikes/s that was predicted by a linear sum of the responses to AM and PM [t(21) = 4.822, P = 0.00009]. By contrast, the spike rate of this neuron in response to Df <0 was 42.67 spikes/s, not significantly different from the predicted spike rate of 42.61 spikes/s [t(20) = 0.057, P = 0.95]. We recorded from 56 neurons that generated spikes in response to stimulus modulation, resulting in a total of 112 responses to Df >0 and Df <0. Of these 112 responses, 12 (10.71%) were characterized as facilitated spike-rate responses (observed spike rate significantly greater than a linear sum), 51 (45.54%) were characterized as suppressed spike-rate responses (observed spike rate significantly lower than a linear sum), and 49 (43.75%) were characterized as linear spike-rate responses (no significant difference between observed spike rate and linear sum).

Facilitated spike-rate responses were characterized by peakto-peak PSP amplitudes that were approximately equal to the peak-to-peak PSP amplitude predicted by a linear sum of the AM and PM responses (Fig. 5B). By contrast, the peak-to-peak PSP amplitudes of linear spike-rate responses were somewhat lower than predicted and the peak-to-peak PSP amplitudes of suppressed spike-rate responses were substantially lower than predicted (Fig. 5B). However, these differences were not significant [F(2,109) = 2.105, P = 0.13]. Among the three categories of spike-rate responses, there was, however, a significant difference in the deviations of the maximum PSP derivatives [F(2,109) = 4.057, P = 0.02] (Fig. 5C). Thus linear spike-rate responses were characterized by maximum PSP derivatives approximately equal to the values predicted by a linear sum, whereas facilitated and suppressed spike-rate responses had maximum PSP derivatives that were greater and less than the values predicted by a linear sum, respectively (Fig. 5*C*). No significant difference among the three categories of spike-rate responses was found for deviations of the minimum PSP derivatives [F(2,109) = 0.326, P = 0.73].

Postsynaptic potential interactions depend on the relative timing of the depolarizing and hyperpolarizing responses to AM and PM

The responses to AM and PM were typically aligned for the preferred sign of Df, whereas they were offset for the nonpreferred sign of Df (Fig. 3). We used circular cross-correlation to quantify the relative timing of the depolarizing and hyperpolarizing responses to AM and PM for the preferred and nonpreferred signs of Df (see METHODS). To do so, we first defined the average PSP responses to AM and PM as periodic functions of the angle θ , which ranges from 0 to 360° (Fig. 6, A and B), and then calculated the correlation between these two responses as a function of their relative delay, τ (Fig. 6C), which was arbitrarily set as the delay of the average PM response relative to the average AM response. The cross-correlation coefficients at $\tau = 90$ and 270° give estimates of the degree of AM and PM response alignment for Df >0 and Df <0, respectively, because PM is advanced by 90° relative to AM for Df >0, whereas PM is delayed by 90° relative to AM for Df < 0 (see Fig. 3).

Figure 6C shows the circular cross-correlation coefficients for a Df >0-selective neuron and a Df <0-selective neuron. In both cases, the cross-correlation coefficients are high at the delay corresponding to the preferred sign of Df (white arrowheads in Fig. 6C), whereas they are low at the delay corresponding to the nonpreferred sign of Df (gray arrowheads in Fig. 6C). This general trend held across the population of sign-selective neurons (Fig. 6D), with positive correlations between the AM and PM responses for the preferred sign of Df and negative correlations between the AM and PM responses for the nonpreferred sign of Df [Wilcoxon matched-pairs test, z(51) = 2.315, P = 0.02]. Therefore the depolarizing and hyperpolarizing responses to AM and PM were in phase for the preferred sign of Df, but for the nonpreferred sign of Df, the depolarizing response to one feature coincided with the hyperpolarizing response to the other feature.

COMBINATION SENSITIVITY AND ACTIVE CONDUCTANCES



FIG. 5. Comparing deviations of PSP responses and spike-rate responses from a linear sum of the responses to AM and PM. A: histograms of action potential times relative to the modulation cycle for a Df <0-selective neuron (SS = -0.0917), along with the responses to Df >0 and Df <0 that would have resulted from a linear sum of the responses to AM and PM. Histograms are presented using the same format as the average PSP responses in Fig. 3. Response to Df >0 is weaker than a linear sum (suppressed spike-rate response), whereas the response to Df < 0 is not significantly different from a linear sum (linear spike-rate response). B: deviation of peak-to-peak PSP responses from a linear sum of the responses to AM and PM for facilitated spike-rate responses, linear spike-rate responses, and suppressed spike-rate responses. Bar below the plot shows the result of an ANOVA. C: deviation of maximum PSP derivatives from a linear sum of the responses to AM and PM for facilitated spike-rate responses, linear spike-rate responses, and suppressed spike-rate responses. Bar above the plot denotes a significant difference determined using an ANOVA.

Conductance-based models of postsynaptic potential responses to AM and PM

We constructed simplified models of neuronal responses to changes in excitatory and inhibitory conductances and compared their behavior to the responses of sign-selective neurons to AM, PM, Df >0, and Df <0. These models are not meant to reproduce the actual responses of particular neurons, but simply to serve as conceptual models to help interpret the data from our intracellular recordings and better understand the interaction between synaptic responses to two stimulus attributes.

Figure 7A shows an example of two different inputs that give rise to exclusively excitatory responses. When both inputs occur in phase (corresponding to the preferred sign of Df), the response is weaker than a linear sum of the separate responses to each input, because of a mutual reduction in driving force (Johnston and Wu 1996). When both inputs occur out of phase (corresponding to the nonpreferred sign of Df), however, the response does not deviate from a linear sum because the depolarizations induced by both inputs are offset in time and the response to one input does not affect the driving force of the other. As seen in the graph to the *right*, this finding holds true regardless of the actual magnitude of the excitatory conductances. The same general result occurs when the two inputs give rise to exclusively inhibitory responses (Fig. 7B), also because of driving-force effects. These results are in stark contrast to the responses of actual sign-selective neurons, for which the deviation from a linear sum was greater when the AM and PM responses were out of phase. This indicates that the suppression of responses to the nonpreferred sign of Df cannot be accounted for by solely excitatory or solely inhibitory inputs.

Including both excitation and inhibition, however, can give rise to responses that are qualitatively similar to the responses of actual sign-selective neurons. Figure 7*C* shows an example of one input driving purely excitatory responses and the other input driving purely inhibitory responses. When the depolarizing and hyperpolarizing portions of these two responses are in phase (preferred sign of Df, excitation and inhibition are offset), the response does not deviate from linearity. When they occur out of phase (nonpreferred sign of Df, excitation and inhibition are in phase), however, the response can be much weaker than a linear sum because the inhibitory conductance serves to shunt the excitatory conductance (Johnston and Wu 1996; Torre and Poggio 1978).

Voltage-dependent conductances provide another possible mechanism for the suppression of responses to the nonpreferred sign of Df (Magee 1999; Mel 1993). Figure 7D shows an example where the two inputs both consist of a combination of excitation and inhibition. The two panels show results from the same combination of excitatory and inhibitory conductances, except that the excitatory conductance of the example to the *right* is adjusted as a sigmoidal function of voltage (see METHODS). In the absence of this voltage dependency, there is a suppression of the combination response when the two inputs are either in phase or out of phase, resulting from driving-force reduction and inhibitory shunting, respectively. The addition of voltage dependency to the excitatory conductance, however, serves to linearize the combination response when the two inputs are in phase because the mutual reduction in driving



FIG. 6. Responses to AM and PM are in phase for the preferred sign of Df and out of phase for the nonpreferred sign of Df. A and B: average AM (A) and PM (B) responses of a Df >0-selective neuron (*left column*) and Df <0-selective neuron (*right column*), expressed as periodic functions of θ , which varies from 0° at the start of the modulation cycle to 360° at the end of the cycle. Below each trace, the solid line represents AM and the dashed line represents PM, as in Fig. 1B. C: circular cross-correlation functions obtained from the average AM and PM responses shown in A and B. The cross-correlation coefficient expresses the correlation between the average AM and PM responses at a given value of τ , which represents the delay of the average PM response relative to the average AM response. Below the abscissae, the white arrowheads show the delay corresponding to the preferred sign of Df and the gray arrowheads show the delay corresponding to the preferred sign of Df and nonpreferred sign of Df for all sign-selective neurons (mean \pm SE, n = 51). Bar above the plot denotes a significant difference determined using a Wilcoxon matched-pairs test.

force is somewhat mitigated by a voltage-dependent increase in excitatory conductance. On the other hand, when the two inputs are out of phase, the subtractive voltage effect is enhanced by a voltage-dependent reduction in excitatory conductance.

3370

These modeling results suggest that both shunting inhibition and voltage-dependent conductances are potential mechanisms that could give rise to the observed responses of sign-selective midbrain neurons. To distinguish between these two possibilities, we modeled the effect of injecting various levels of holding current on the membrane potential responses to a single input. Figure 8 illustrates PSP responses in the presence of several holding currents for models consisting solely of excitation (Fig. 8A), solely of inhibition (Fig. 8B), a combination of inhibition and excitation (Fig. 8C), and a combination of inhibition and voltage-dependent excitation (Fig. 8D). To quantify the changes in model PSP responses caused by applying various holding currents, we determined the timing of the maximum and minimum values of the average PSP responses during no current injection (t_{max} and t_{min} , respectively, as illustrated in the *left panels* of Fig. 8, A-D), and then determined the difference in membrane potential measured between these two time points at each holding current [V(t- $_{\rm max}) - V(t_{\rm min})$]. For all possible combinations of excitatory and inhibitory conductances without any voltage dependency, the relationship between $V(t_{max}) - V(t_{min})$ and holding current is linear, with the direction and slope of the relationship depending on the relative balance of excitation and inhibition (Fig. 8, A-C). The addition of voltage dependency to the excitatory conductance, however, makes the relationship between $V(t_{\rm max}) - V(t_{\rm min})$ and holding current nonlinear, with $V(t_{\rm max}) - V(t_{\rm max})$ $V(t_{\min})$ peaking at a value that depends on the quantitative

characteristics of the voltage dependency and the magnitudes of the excitatory and inhibitory conductance changes (Fig. 8D).

Voltage-dependent conductances enhance sign selectivity

We stimulated several neurons (n = 28) with sinusoidal AM and PM while subjecting the neurons to various holding currents ranging from -0.4 to +0.2 nA and obtained the values of $V(t_{max}) - V(t_{min})$ for each level of holding current. Figure 9A shows examples of the average PSP responses of three neurons to sinusoidal AM at several holding currents and Fig. 9B shows plots of $V(t_{max}) - V(t_{min})$ as a function of holding current for 10 additional neurons, along with the spike rates of the same ten neurons during sinusoidal AM stimulation. In each case, the relationship between $V(t_{max}) - V(t_{min})$ and holding current is nonlinear, with maximum values of $V(t_{max}) - V(t_{min})$ occurring between -0.1 and 0 nA (Fig. 9B). We recorded similar responses to sinusoidal PM under different holding currents (Fig. 9, *C* and *D*), indicating that the PSP responses to both AM and PM are shaped by voltage-dependent conductances.

As described above, responses in the absence of current injection were typically characterized by alternating depolarizing and hyperpolarizing responses (Fig. 9, A and C). With sufficient levels of hyperpolarizing current, however, the responses were often depolarized relative to rest throughout the entire duration of the response (Fig. 9, A and C). This observation indicates that the hyperpolarizing portions of the responses are caused by inhibitory PSPs that reversed in direction when sufficient current was injected to hyperpolarize the neuron beyond the inhibitory reversal potential. Only in a few cases were we able to induce a purely hyperpolarizing response through depolarization



FIG. 7. Conductance-based models of responses to 2 synaptic inputs occurring in phase and out of phase. A: integration based on 2 excitatory inputs $(g_{e1}$ and g_{e2}). To the *left* is an example of 2 responses occurring in phase and out of phase. The linear sum of responses is determined by summing the responses of input 1 and input 2 (as in Fig. 3), and the observed response is determined by running the model using the summed conductances from input 1 and input 2. Right graph: deviation of peak-to-peak PSP amplitudes from a linear sum of the responses to both inputs as a function of variation in g_{e1} and g_{e2} . B: integration based on 2 inhibitory inputs (g_{i1} and g_{i2}), presented as in A. C: integration based on one excitatory input (g_{e1}) and one inhibitory input (g_{i2}) , presented as in A. D: integration based on 2 inputs that each give rise to both inhibitory and excitatory conductances. The two examples (left and right) are identical, except that the excitatory conductance of the example to the *right* is adjusted as a sigmoidal function of voltage.

3371



FIG. 8. Conductance-based model responses to a single synaptic input under various holding currents. Four different conditions are shown: A: excitation only ($g_e = 0.5 \text{ nS/mm}^2$). B: inhibition only ($g_i = 0.5 \text{ nS/mm}^2$). C: inhibition and excitation ($g_e = 0.6 \text{ nS/mm}^2$; $g_i = 0.3 \text{ nS/mm}^2$). D: inhibition with a voltage-dependent excitation ($g_e = 0.6 \text{ nS/mm}^2$; $g_i = 0.3 \text{ nS/mm}^2$), in which the excitatory conductance is adjusted as a sigmoidal function of voltage. Left panels: PSP responses under various holding currents. Vertical dashed lines show the timing of the maximum membrane potential (t_{max}) and minimum membrane potential (t_{min}) in the absence of current injection. Right panels: plots of $V(t_{max}) - V(t_{min})$ as a function of holding current. In C, there are 2 traces in addition to the one obtained from the data shown in the left panel ($g_e > g_i$), one in which g_e and g_i are both equal to 0.3 nS/mm² ($g_e = g_i$) and one in which g_e is equal to 0.3 nS/mm² and g_i is equal to 0.6 nS/mm² ($g_e < g_i$).

(Fig. 9, A and C), most likely because we were generally unable to inject equally large magnitudes of depolarizing current and still maintain a stable recording.

The evidence for voltage-dependent conductances shaping the responses to AM and PM, combined with the model results illustrating the effects that voltage-dependent conductances can have on the synaptic integration of two inputs, suggest the following hypothesis for the nonlinear enhancement of sign selectivity: when the depolarizing response to AM coincides with the hyperpolarizing response to PM, and vice versa, the subtractive effect of the hyperpolarization is enhanced because of a reduction in voltage-dependent excitatory conductance. To test this hypothesis, we compared the responses of signselective neurons to Df >0 and Df <0 during no current injection with the responses obtained while the neurons were subjected to hyperpolarizing and depolarizing holding currents.

Figure 10, A and B, shows the responses of two Df <0selective neurons to Df >0 and Df <0 in the absence of current injection and in the presence of various holding currents. In both cases, the responses at rest consist of the typical alternation between hyperpolarization and depolarization and the response to Df < 0, but not Df > 0, is characterized by a sharp depolarization that appears to ride on top of the underlying smooth depolarization. In response to hyperpolarizing current injection, this enhanced response to Df < 0 is reduced in magnitude, thereby decreasing sign selectivity. By contrast, injecting moderate levels of depolarizing current appears to boost the response to Df > 0 by recruiting the enhanced depolarization, again reducing sign selectivity. Injecting a greater amount of depolarizing current (in Fig. 10A) eliminates this enhanced depolarization from the responses to both Df > 0and Df < 0.

Of the 28 neurons subjected to various holding currents, there were 19 sign-selective neurons, 16 of which were subjected to hyperpolarizing currents and 13 of which were subjected to depolarizing currents. To quantify the effect of current injection on the sign selectivity of these 19 neurons, we determined the mean sign selectivity of each neuron during hyperpolarizing and depolarizing current injection and divided this value by the sign selectivity of the neuron during no current injection (Fig. 10C). Hyperpolarizing current injection reduced the mean sign selectivity by a factor of 0.2175 [Wilcoxon matched-pairs test, z(16) =2.844, P = 0.004 and depolarizing current injection reduced the mean sign selectivity by a factor of 0.3563 [z(13) = 2.062, P = 0.039]. For the same 19 sign-selective neurons, we also determined the deviation of peak-to-peak PSP amplitudes from the values predicted by a linear sum of the responses to AM and PM in the absence of current injection and during both depolarizing and hyperpolarizing current injection. At rest, the nonpreferred sign of Df was characterized by significantly greater sublinear summation compared with the preferred sign of Df [Wilcoxon matchedpairs test, z(19) = 2.173, P = 0.03], as shown above (Fig. 4A). However, this difference was eliminated by both depolarizing current injection [z(13) = 1.013, P = 0.31] and hyperpolarizing current injection [z(16) = 1.138, P = 0.26];in both cases, the preferred and nonpreferred signs of Df were characterized by similar levels of sublinear summation (Fig. 10D).



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FIG. 9. Responses to AM and PM are shaped by voltage-dependent conductances. A: examples of the average responses to AM for 3 different neurons under various holding currents. Below each trace, the solid line represents AM and the dashed line represents PM, as in Fig. 1B. Three arrowheads to the *right* of each set of traces show the resting potential in relation to the average PSP responses for the response with no holding current and the responses during the strongest depolarizing and hyperpolarizing currents. B: plots of $V(t_{max}) - V(t_{min})$ and average spike rates as a function of holding current from 10 additional neurons during AM stimulation (each trace represents AM and the dashed line represents PM for 3 different neurons under various holding currents. Below each trace, the solid line represents AM and the dashed line represents PM, as in Fig. 1B. Three arrowheads to the *right* of each set of traces show the responses of the average responses to PM for 3 different neurons under various holding currents. Below each trace, the solid line represents AM and the dashed line represents PM, as in Fig. 1B. Three arrowheads to the *right* of each set of traces show the resting potential in relation to the average PSP responses for the response with no holding current and the responses during the strongest depolarizing and hyperpolarizing currents. Below each trace, the solid line represents AM and the dashed line represents PM, as in Fig. 1B. Three arrowheads to the *right* of each set of traces show the resting potential in relation to the average PSP responses for the response with no holding current and the responses during the strongest depolarizing and hyperpolarizing currents (for the 2 examples without any depolarizing holding currents, only 2 arrowheads are shown). D: plots of $V(t_{max}) - V(t_{min})$ and average spike rates as a function of holding current from 10 additional neurons during PM stimulation (each trace represents on enuron).

DISCUSSION

Similar to combination-sensitive neurons in other modalities, sign-selective electrosensory neurons in the midbrain of *Gymnarchus* are highly selective for specific combinations of different stimulus features (Kawasaki and Guo 2002). Our earlier extracellular recordings from sign-selective neurons revealed that the integration of responses to AM and PM is often nonlinear and, furthermore, that this nonlinearity depends on the relative timing of responses to the two features (Carlson and Kawasaki 2004). By combining experimental results from intracellular recordings with simplified models of synaptic integration, we show that this timing-dependent, nonlinear integration results from a combination of voltage-dependent excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs). The responses to both AM and PM presented alone are characterized by alternating EPSPs and IPSPs. For the preferred sign of Df, these alternating responses to AM and PM are in phase and the additive effect of simultaneous EPSPs causes a voltage-dependent increase in excitatory conductance, which somewhat mitigates the effects of reduced driving force. For the nonpreferred sign of Df, these alternating responses are offset and the subtractive effect of



FIG. 10. Voltage-dependent conductances enhance sign selectivity. *A* and *B*: average responses to Df >0 and Df <0 in the presence of various holding currents for 2 different Df <0-selective neurons (SS = -0.1779 in *A*; SS = -0.1631 in *B*). *C*: mean sign selectivity of all SS neurons in the presence of depolarizing and hyperpolarizing holding currents relative to sign selectivity with no holding current (mean \pm SE). Bars above the plot denote significant differences determined using a Wilcoxon matched-pairs test. *D*: deviation of peak-to-peak PSP amplitudes from a linear sum of the responses to AM and PM for the preferred and nonpreferred signs of Df (mean \pm SE), measured at rest and during both depolarization and hyperpolarization. Bars below each pair of measurements denote significant differences determined using a Wilcoxon matched-pairs test.

IPSPs coinciding with EPSPs is enhanced because of a voltagedependent decrease in excitatory conductance. The net result is that the voltage dependency causes an increase in sign selectivity, revealing that a voltage-dependent excitatory conductance can act as a nonlinear decoder for enhancing combination sensitivity.

The evidence that voltage-dependent conductances are responsible for the nonlinear integration of responses to AM and PM comes from two primary pieces of evidence. First we found a nonlinear relationship between holding current and our measure of $V(t_{max}) - V(t_{min})$ for the responses to both AM and PM presented alone (Fig. 9); our modeling results reveal that this relationship is linear in the absence of voltage dependency (Fig. 8). Second, the degree of sign selectivity and the linearity of AM and PM response summation were significantly affected by current injection (Fig. 10), which would not be the case if nonlinear integration resulted solely from driving-force effects, inhibitory shunting, or extrinsic processes such as presynaptic inhibition.

The spike-rate data indicate that neurons were still operating within a physiologically relevant voltage range at holding currents for which $V(t_{max}) - V(t_{min})$ was maximal (Fig. 9, *B* and *D*), which is supported by the fact that 0.05 nA injected into a neuron with an input resistance of 200 M Ω corresponds to a voltage shift of only 10 mV. Therefore although it is not possible to exclude a role for additional mechanisms, our results clearly implicate voltage-dependent conductances in the enhancement of sign selectivity. Future studies will seek to determine whether these conductances arise from synaptically driven, ligand-gated channels, such as NMDA-type glutamate receptors, or from channels sensitive only to voltage, such as Na⁺ channels (Fortune and Rose 2003). The relatively long time course of PSPs that we observed (on the order of hundreds of milliseconds) would seem to implicate NMDA receptors, because voltage-gated ion channels typically have much more rapid activation and inactivation kinetics (on the order of milliseconds; Johnston and Wu 1996). NMDA receptors play an important role in sensory processing for both visual and auditory systems (Daw et al. 1993; Feldman and Knudsen 1994; Zhang and Kelly 2001) and their unique pharmacological characteristics make them ideally suited for nonlinear processing and for fine-tuning the temporal dynamics of synaptic responses (Gasic and Hollman 1992; Mel 1992, 1993).

We have not directly demonstrated the presence of inhibitory synapses on sign-selective neurons. By injecting sufficient levels of hyperpolarizing current, however, it was possible to change responses that consisted of alternating depolarizations and hyperpolarizations into purely depolarized responses (Fig. 9, A and C), strongly indicating that inhibition is responsible for these hyperpolarizations. Preliminary results using anti- γ aminobutyric acid (GABA) and anti-glutamic acid decarboxylase (GAD) immunocytochemistry reveal substantial numbers of GABAergic neurons, fibers, and terminals within the electrosensory torus (Y. Zhang, B. A. Carlson, and M. Kawasaki, unpublished observation), providing further support for this hypothesis. In the auditory forebrain of songbirds, the strong selectivity of combination-sensitive neurons for the bird's own song is also dependent on inhibitory processing (Lewicki 1996; Mooney and Prather 2005; Rosen and Mooney 2006).

Although early models of dendritic integration based purely on the passive cable properties of dendrites have proven quite powerful in understanding many features of synaptic integration (Rall 1964), it is becoming increasingly clear that these passive properties are insufficient to account for the impressive computational power of single neurons (Koch and Poggio 1992; Magee 1999, 2000; Rall 1999). Voltage-gated ion channels are now known to occur in the dendrites of many different types of neurons in the CNS of vertebrates (Llinás 1988; Magee 1999; Reyes 2001). The effects of these voltagedependent conductances on the electrical properties of neurons and the resulting implications for their input-output functions have primarily been studied in vitro (Häusser et al. 2000; Magee 2000; Reyes 2001). The role of voltage-dependent conductances in the processing of natural, behaviorally relevant sensory stimuli in vivo has not received a great deal of attention, largely as a result of the difficulty of manipulating ion channel conductance in intact preparations. However, studies on electrosensory neurons of the weakly electric fish Eigenmannia (Fortune and Rose 1997) and visual interneurons of the blowfly Calliphora (Haag and Borst 1996) showed that voltage-dependent membrane conductances can play an important role in shaping the frequency filtering of sensory neurons by either enhancing or counteracting the underlying passive filtering properties. In *Calliphora*, a fast, inward, voltage-dependent sodium current gives rise to a frequency-dependent amplification of visual synaptic inputs that allows neurons with these currents to respond to much higher frequencies of stimulation than neurons that lack these currents (Haag and Borst 1996). By contrast, voltage-dependent conductances in *Eigen*mannia serve to boost the underlying passive filtering properties of electrosensory neurons and thereby sharpen frequency tuning (Fortune and Rose 1997). There appear to be two distinct voltage-dependent conductances in these electrosensory neurons, one arising from voltage-gated Na⁺ channels that give rise to a constant duration potential and the other possibly resulting from NMDA-type glutamate receptors that give rise to variable-duration potentials (Fortune and Rose 1997, 2003).

Our findings in the electrosensory system of Gymnarchus add another function for voltage-dependent conductances in the processing of natural, behaviorally relevant stimuli, that is, enhancing selectivity for particular temporal combinations of stimulus attributes. Our modeling results reveal that voltagedependent conductances can serve to linearize the summation of two inputs occurring in phase with each other by partially mitigating the effects of reduced driving force. A linearizing effect of voltage-dependent conductances on simultaneous synaptic inputs was previously shown using more formal, biophysically detailed compartmental models (Bernander et al. 1994) and was also explicitly demonstrated in vitro (Cash and Yuste 1999). On the other hand, our modeling results also reveal that voltage-dependent conductances can act to suppress the summation of two inputs occurring out of phase with each other by enhancing the subtractive effect of hyperpolarizations on depolarizations. Along these same lines, intracellular recordings in vitro and from cultured neurons showed that voltage-dependent conductances can enhance sensitivity to differences in the relative timing of multiple synaptic inputs (Margulis and Tang 1998; Nettleton and Spain 2000). The current study provides evidence that these characteristics of voltage-dependent conductances play an important role in the processing of behaviorally relevant sensory information in vivo.

Previously, we demonstrated that the spiking responses of sign-selective toral neurons in Gymnarchus are often nonlinear (Carlson and Kawasaki 2004). Although nonlinear spiking responses can arise from a linear summation of the underlying PSPs (Ferster 1994; Jagadeesh et al. 1993), the current study reveals that the spiking nonlinearities of sign-selective neurons in Gymnarchus are associated with nonlinear interactions between the PSP responses to AM and PM. Indeed, we did not find any significant difference between spike-rate sign selectivity and peak-to-peak PSP amplitude sign selectivity. However, facilitated spike rates were not associated with peak-topeak PSP amplitudes that were greater than a linear sum of the responses to AM and PM; instead, the peak-to-peak PSP amplitudes were approximately equal to a linear sum (Fig. 5B). Linear spike-rate responses were characterized by peak-topeak PSP amplitudes that were smaller than a linear sum and suppressed spike-rate responses were characterized by even greater sublinear deviations (Fig. 5B). This finding is not surprising, given the nonlinear relationship between membrane potential and spike rate. By contrast, spiking nonlinearities were directly related to deviations of the maximum PSP derivatives (Fig. 5C). Thus facilitated spike rates may be caused by faster increases in membrane potential, which may in turn arise from activation of voltage-dependent conductances in response to the preferred sign of Df. By contrast, suppressed spike rates may be caused by slower increases in membrane potential associated with the reduced activation of voltagedependent conductances.

The weakly electric fish Eigenmannia is only distantly related to Gymnarchus and the available evidence indicates that they share no common electrogenic or electroreceptive ancestors (Bullock et al. 1983). However, both species perform a JAR that is based on the same computational algorithm of comparing the temporal relationship between AM and PM to determine the sign of Df (Bullock et al. 1975; Fortune et al. 2006; Heiligenberg 1991; Kawasaki 1993). Like Gymnarchus, *Eigenmannia* has separate electrosensory pathways devoted to encoding these two stimulus features that converge onto signselective neurons in the midbrain torus semicircularis (Fortune et al. 2006; Heiligenberg and Rose 1986; Rose and Heiligenberg 1986). Sign-selective neurons in *Eigenmannia* also show a nonlinear summation of the spike-rate responses to AM and PM and the preferred sign of Df is likewise the one in which these two responses are aligned. Although the underlying postsynaptic potentials have not been explored, the demonstrated existence of voltage-dependent conductances in electrosensory midbrain neurons in Eigenmannia (Fortune and Rose 1997, 2003) suggests that they may also play a role in enhancing the sign selectivity of combination-sensitive neurons in Eigenmannia.

Combination-sensitive neurons are widespread in the auditory and visual systems as well and they exhibit similar nonlinear summations of spike-rate responses to multiple stimulus features (Fuzessery and Feng 1983; Margoliash 1983; Misawa and Suga 2001; Peña and Konishi 2001; Quiroga et al. 2005; Tsao et al. 2006). The widespread occurrence of voltagedependent conductances in the dendrites of vertebrate neurons and the straightforward mechanism by which these conductances can enhance stimulus selectivity indicate that our findings are likely to prove broadly relevant as a mechanism for extracting information about behaviorally relevant combinations of different stimulus features. Nonlinear decoders, in the form of voltage-dependent conductances, are important candidates for the dynamic enhancement of responses to particular temporal patterns of multiple synaptic inputs.

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3376

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