Coding Movement Direction by Burst Firing in Electrosensory Neurons

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Abstract

Directional selectivity, in which neurons respond strongly to an object moving in a given direction (‘preferred’) but respond weakly or not at all to an object moving in the opposite direction (‘null’), is a critical computation achieved in brain circuits. Previous measures of direction selectivity compare the numbers of action potentials elicited by each direction of movement, but most sensory neurons display patterning, such as bursting, in their spike trains. To examine the contribution of patterned responses to direction selectivity, we recorded from midbrain neurons in weakly electric fish and found that most neurons responded with a combination of both bursts and isolated spikes to moving object stimuli. In these neurons, we separated bursts and isolated spikes using an interspike interval (ISI) threshold. The directional bias of bursts was significantly higher than that of either the full spike train, or the isolated spike train. To examine the encoding and decoding of bursts, we built biologically plausible models that 1) examine the upstream mechanisms that generate these spiking patterns and 2) downstream decoders of bursts. Our model of upstream mechanisms uses an interaction between afferent input and subthreshold calcium channels to give rise to burst firing that occurs preferentially for one direction of movement. We tested this model in vivo by application of calcium antagonists, which reduced burst firing and eliminated the differences in direction selectivity between bursts, isolated spikes, and the full spike train. Our model of downstream decoders used strong synaptic facilitation to achieve qualitatively similar results to those obtained using the ISI threshold criterion. This model shows that direction selective information carried by bursts can be decoded by downstream neurons using biophysically plausible mechanisms.
Introduction

Directional selectivity, in which neurons respond selectively to a given direction of movement over the opposite direction, is perhaps the most widely known computation performed by sensory neurons and is a correlate of movement perception (Hubel and Wiesel 1962). Several models have been proposed in order to explain how directional selectivity arises (Borst 2007; Derrington et al. 2004; Hock et al. 2009; Maex and Orban 1991). In particular, the Reichardt model for generating directionally selective responses to moving objects requires at least two fundamental operations (Reichardt 1987; Reichardt and Wenking 1969): asymmetric filtering of information from at least two spatial locations within the receptive field and non-linear integration of these inputs. These so-called “Reichardt detectors” have received considerable attention and have been described in neural circuits across animal species (Borst and Egelhaaf 1990; 1989; Chacron and Fortune 2010; Chacron et al. 2009; Euler et al. 2002; Haag et al. 2004; Hubel and Wiesel 1962; Jagadeesh et al. 1997; Priebe and Ferster 2008; Priebe et al. 2004; Srinivasan et al. 1999).

Directional selectivity has been traditionally characterized by considering the spike train as a whole. Previously used measures of direction selectivity compare the numbers of action potentials elicited by each direction of stimulus movement. However, most vertebrate sensory neurons display distinctive patterning in their spike trains, such as the production of bursts of spikes (i.e. the firing of packets of action potentials followed by quiescence) (Gray and Singer 1989; Krahe and Gabbiani 2004; Lisman 1997; Macleod and Laurent 1996; Sherman 2001; Sherman and Guillery 2006; Sillito et al. 1994). These patterns can arise from many biophysical sources, including a variety of voltage-gated conductances. For example, subthreshold T-type calcium channels, which can act as the necessary nonlinear integrator for generating directionally selective responses in weakly electric fish (Chacron and Fortune 2010), can also give rise to burst firing (Llinas and Jahnsen 1982; Sherman 2001; Sherman and Guillery 2006; Sherman and Guillery 2002).

Burst firing is ubiquitous in the central nervous system (CNS) circuits (Krahe and Gabbiani 2004) and may serve multiple functions including feature detection (Chacron and Bastian 2008; Chacron et al. 2001; Chacron et al. 2004; Deemyad et al. 2011; Gabbiani et al. 1996; Kepecs and Lisman 2003; Lesica and Stanley 2004; Martinez-Conde et al. 2002; Oswald et al. 2004; Sherman 2001), coding for stimulus attributes such as intensity and slope (Avila Akerberg and Chacron 2011; Avila Akerberg et al. 2010; DeBusk et al. 1997; Eyherabide et al. 2008; Gaudry and Reinagel 2008; Kepece et al. 2002; Marsat and Pollack 2010; Martinez-Conde et al. 2002; Oswald et al. 2007; Samengo and Montemurro 2010), and improving the reliability of synaptic transmission (Lisman 1997; Reinagel et al. 1999). Here we investigated the coding of movement direction by bursts and isolated spikes (i.e. the spikes that are not part of bursts) in sensory neurons.

We used a well-suited model system, weakly electric fish, which sense distortions of their self-generated electric organ discharge through an array of electroreceptor neurons on their skin (Chacron et al. 2011; Fortune and Chacron 2011; Turner et al. 1999). These electroreceptors project onto pyramidal cells within the hindbrain, which in turn project onto neurons within the midbrain Torus semicircularis (TS). The TS is homologous to the mammalian inferior colliculus.
While previous studies have shown that TS neurons display directional selectivity to moving objects (Chacron and Fortune 2010; Chacron et al. 2011; Chacron et al. 2009), these measurements were made by considering all of the action potentials in the spike train or the membrane potential and thus did not specifically examine the contributions of action potential patterns such as bursts or isolated spikes.
Methods

Animals

We used the weakly electric fish *Apteronotus leptorhynchus* in this study. Animals were obtained from tropical fish suppliers and were housed in laboratory tanks for several days in order to become acclimated to the new environment. Fish husbandry was performed according to published guidelines (Hitschfeld et al. 2009). The surgical and experimental procedures have been described in detail elsewhere (Avila Akerberg et al. 2010; Bastian et al. 2002; Chacron 2006; Chacron and Bastian 2008; Chacron et al. 2003; Chacron and Fortune 2010; Chacron et al. 2007; Chacron et al. 2005a; Chacron et al. 2009; Krahe et al. 2008; Savard et al. 2011; Toporikova and Chacron 2009). The animals were immobilized by intramuscular injection of a nicotinic receptor antagonist Tubocurarine (~4μg/g; Sigma, St. Louis, MO) and respirated via a mouth tube with aerated tank water at a flow rate of ~10 ml/min. The fish was submerged in water except for the top of its head. To expose the midbrain for recording, we first locally anesthetized the skin on the skull by applying 2% lidocaine. Then we removed ~6 mm² of skin to expose the skull to which a metal post was glued for stabilization. We drilled a hole of ~2 mm² in the skull above the midbrain. The surface of the brain was covered by saline throughout the experiment. All experimental procedures and animal husbandry followed guidelines established by the National Research Council and the Society for Neuroscience and were approved by the Marine Biological Laboratory’s, McGill University’s, and the Johns Hopkins University’s Institutional Animal Care and Use Committees.

Stimulation and recording

Recordings from TS neurons were made using previously described techniques (Chacron and Fortune 2010; Chacron et al. 2009; Rose and Fortune 1996). These consisted of both intracellular and extracellular recordings. Intracellular recordings and some extracellular recordings were made with patch pipettes as described previously (Rose and Fortune 1996). Other extracellular recordings were made with metal-filled micropipettes that were plated with gold and platinum at the tip (Frank and Becker 1964). Since no quantitative differences were seen when comparing our intracellular and extracellular datasets or when comparing our extracellular dataset obtained with patch pipettes to our extracellular dataset obtained with metal-filled micropipettes, all three datasets were pooled.

The moving stimulus consisted of a 1.8 cm wide metal plate coated with plastic on the side opposite to the animal that was actuated using a pen plotter (HP 7010B). This object was moved back and forth along the animal’s rostro-caudal axis over a distance of 20 cm (Chacron and Fortune 2010; Chacron et al. 2009; Ramcharitar et al. 2005; 2006) for at least 30 cycles (Fig. 1A). The sinusoid was centered at the animal’s midpoint and had a frequency of 0.25 Hz (i.e. a cycle duration of 4 sec) corresponding to an average velocity of ~10 cm/sec. These velocities are similar to those that the animal experiences during prey capture (Nelson and MacIver 1999) and are furthermore within the range of velocities seen during refuge tracking (Cowan and Fortune 2007).
To determine whether midbrain neurons were responsive to information from tuberous
electroreceptors, we also delivered amplitude modulations of the animal’s own EOD that were
applied via chloridized silver wire electrodes positioned 19 cm away from the fish on either side
of the animal (Toporikova and Chacron 2009). Such stimuli selectively activate the tuberous but
not the ampullary system (Bastian et al. 2002). The zero crossings of the amplified EOD signal
(DAM50, World Precision Instruments, Sarasota, FL; band-pass filter between 300 Hz and 3
kHz) were detected by a window discriminator, which then triggered a function generator to
output a single-cycle sinusoid of slightly higher frequency than the fish’s EOD. This created a
train of single-cycle sinusoids that were phase-locked to the EOD. The train was then multiplied
(MT3 multiplier, Tucker Davis Technologies, Gainesville, FL) with a modulation waveform
produced by a Power1401 (Cambridge Electronic Design, Cambridge, UK). The resulting signal
was attenuated (LAT45 attenuator, Leader Electronics, Cypress, CA) and fed into the tank via a
stimulus isolator (A395 linear stimulus isolator, World Precision Instruments). Depending on the
polarity of the signal relative to the fish’s EOD, the signal led to an increase or a decrease in
amplitude of the EOD. All TS neurons in this study responded to at least one AM stimulus
within a set of sinewave with frequencies 1, 2, 4, 8, 16, 32, 64, 128 Hz and thus received tuberous
input (data not shown). We did not explicitly test whether these neurons also received ampullary
input.

Data were acquired with a Cambridge Electronic Design Power1401 hardware and Spike2
software (Cambridge, UK) and analyzed using Spike2 (CED) and custom-made routines in
MATLAB (The Mathworks, Natick, MA). An amplitude threshold was used to obtain the action
potential times from the high-pass filtered (400 Hz cutoff) recorded signal (i.e. the action
potential times were taken as the times at which the signal crossed the threshold from below).
We excluded neurons that had total spike counts of less than 400 spikes over the stimulus
duration (typically 120 sec). For a stimulus whose duration is equal to 120 sec, this corresponds
to keeping neurons whose firing rates were greater than 3.33 Hz.

**Burst classification**

We used an interspike interval threshold to separate the recorded spike trains into burst and
isolated spikes (Avila Akerberg and Chacron 2011; Avila Akerberg et al. 2010; Chacron and
Bastian 2008; Deemyad et al. 2011; Kepecs and Lisman 2003; Oswald et al. 2004) (Fig. 1B).
Specifically, two consecutive action potentials that were separated by a time interval less than
the threshold were considered as part of a burst. Spikes that were not part of bursts were included
in the isolated spike train. As done previously, the burst threshold was computed as the time at
which the falling phase of initial peak of the autocorrelogram crossed the 99.9 % Poisson
confidence limit (Fig. 1C) (Avila Akerberg and Chacron 2011; Avila Akerberg et al. 2010;
Bastian and Nguyenkim 2001; Chacron and Bastian 2008). Neurons with burst or isolated spike
counts less than 100 were excluded from the data pool (note that this corresponds to neurons
whose burst or isolated spike event rates that were less than 0.83 Hz for a stimulus duration of
120 sec). This method is effective for separating bursts and isolated spikes, but is acausal in
nature because one needs to know when the next spike time $t_{i+1}$ will occur in the future in order
to assign a spike occurring at time $t_i$ as being part of a burst or not. As such, assigning spikes as
being part of a burst or not based on an ISI threshold cannot be implemented in a biologically plausible neural circuit.

Membrane potential responses to moving objects

For some neurons that were recorded from intracellularly, we plotted the average membrane potential waveform in response to the moving object and low-pass filtered (160 Hz cutoff, FIR filter in Spike2) the resulting trace in order to remove the action potentials. The membrane hyperpolarizations were quantified by computing the area between the membrane potential curve and its average value for which the membrane potential was less than average before the peak depolarization in each movement direction. The average was computed as the average membrane potential during a full cycle of movement.

Quantifying directional selectivity

The full spike train, the burst train (i.e. the train of spikes that belong to bursts) and the isolated spike train (i.e. the train of spikes that are isolated) were used to generate peri-stimulus time histograms (PSTHs) in response to the moving object. For each neuron, the preferred direction was taken as the direction of movement for which the peak firing rate was highest for the full spike train and the other direction was termed null. We then computed a measure of directional bias as (Chacron and Fortune 2010; Chacron et al. 2009):

\[
DB = \frac{R_p - R_N}{\max(R_p, R_N)}
\]

where \(R_p, R_N\) are the maximum firing rates obtained in the preferred and null directions, respectively. This measure ranges between 0 (no directional selectivity) and 1 (complete directional selectivity).

Pharmacology

As done previously, we used pressure to inject Mibefradil (1mM, Sigma-Aldrich, St. Louis), NiCl\(_2\) (1mM, Sigma-Aldrich, St. Louis) and ACSF into the brain (Chacron and Fortune 2010). NiCl\(_2\) is a broad spectrum calcium channel antagonist while Mibefradil is selective for T-type calcium channels as the concentration used here (Bloodgood and Sabatini 2007; Chacron and Fortune 2010; Mehrke et al. 1994). A patch pipette with a large tip diameter (~0.5 mm) was connected to a 50cc syringe and advanced into the TS close to the recording pipette. Gentle pressure was then applied to the syringe in order to eject the drug without disrupting the intracellular recording. Directional selectivity and burst firing were compared in neurons recorded under control conditions and after the injection of NiCl\(_2\), Mibefradil, and ACSF. Both NiCl\(_2\) and Mibefradil were effective for at least 3 hours after injection (Chacron and Fortune 2010). As both NiCl\(_2\) and Mibefradil had quantitatively similar effects on directional selectivity and burst firing, the data were pooled.

Modeling TS neurons
A TS neuron’s receptive field was modeled in one dimension by two contiguous zones of length \( d = 10 \text{ mm} \) each. A point object was then moved back and forth across the receptive field at a speed of 10 cm/s. The outputs of each zone are then given by:

\[
O_i(t) = F_i + G_i \Theta(t - \lambda_i) \exp\left(-\frac{t}{\tau_i}\right)
\]

where \( G_i \) and \( \tau_i \) are, respectively, the gain and depression time constant associated with zone \( i \) and \( \lambda_i \) is the time at which the object first enters zone \( i \) (i=‘ON’ or ‘OFF’). The “ON” zone represents the output of E-type ELL pyramidal cells that are excited by the moving object while the “OFF” zone represents the output of I-type ELL pyramidal cells that are inhibited by the moving object (Saunders and Bastian 1984). The term \( F_i \) is a bias that represents the known baseline activity from these cells which are approximately equal on average (Chacron et al. 2005b; Krahe et al. 2008). We further note that the contiguous ON and OFF zones are consistent with the receptive field structure of some TS neurons (see Fig. 4B of Chacron et al. 2009). The input \( I(t) \) to our neuron model is given by:

\[
I(t) = O_{ON}(t) + O_{OFF}(t)
\]

As such, both zones project unto our model TS neuron in an excitatory fashion consistent with known anatomical data (Carr and Maler 1985). \( I(t) \) was then convolved with an alpha function with time constant 20 msec to mimic synaptic EPSPs in order to obtain the input \( I_c(t) \). The TS neuron was modeled using the Hodgkin-Huxley formalism. We included leak, spiking sodium, delayed rectifier potassium, and low threshold calcium (T-type) conductances based on available experimental data (Chacron and Fortune 2010). The model is described by the following equations:
\[
C \frac{dV}{dt} = -g_{\text{leak}} (V - E_{\text{leak}}) - g_T s^3 h (V - E_{\text{Ca}}) - g_Na m^3 (V)(0.85 - n)(V - E_{\text{Na}}) - g_K n^4 (V - E_K) \\
+ A I_c(t) + I_{\text{bias}} + \sigma \xi(t)
\]
\[
dh = \frac{\Phi h_{\infty}(V) - h}{\tau_h}
\]
\[
dn = \frac{n_{\infty}(V) - n}{\tau_n}
\]
\[
m_{\infty}(V) = \frac{\alpha_m(V)}{[\alpha_m(V) + \beta_m(V)]}
\]
\[
n_{\infty}(V) = \frac{\alpha_n(V)}{[\alpha_n(V) + \beta_n(V)]}
\]
\[
\tau_h(V) = 0.05/[\alpha_h(V) + \beta_h(V)]
\]
\[
\alpha_m(V) = 0.1(V + 40.7)/(1 - \exp[-0.1(V + 40.7)])
\]
\[
\beta_m(V) = 4 \exp[-0.05(V + 49.7)]
\]
\[
\alpha_n(V) = 0.01(V + 40.7)/(1 - \exp[-0.1(V + 40.7)])
\]
\[
\beta_n(V) = 0.125 \exp[-0.0125(V + 50.7)]
\]
\[
s_{\infty}(V) = \frac{1}{1 + \exp[-(V + 69)/7.8]}
\]
\[
h_{\infty}(V) = \frac{1}{0.5 + \sqrt{0.25 + \exp[(V + 82)/6.3]}}
\]

where C is the membrane capacitance, V is the transmembrane potential difference, \( g_{\text{leak}} \) is the leak conductance with reversal potential \( E_{\text{leak}} \). Here \( g_T, g_{\text{Na}}, \) and \( g_K \) are the voltage-gated calcium, sodium, and potassium conductances with reversal potentials \( E_{\text{Ca}}, E_{\text{Na}}, \) and \( E_K \), respectively. A is the synaptic weight and \( I_{\text{bias}} \) is a constant bias current, \( \sigma \xi(t) \) is zero mean low-pass filtered Gaussian white noise with standard deviation \( \sigma \) that mimics sources of synaptic input (Manwani and Koch 1999). These equations were previously used to model burst firing in Thalamic relay neurons and a full description of the burst mechanism in the deterministic regime (i.e. \( \sigma = 0 \)) can be found in (Rush and Rinzel 1994).

We simulated this model numerically using an Euler-Maruyama Algorithm (Kloeden and Platen 1999) with integration time step \( dt = 0.0025 \) msec. Other parameter values used, unless otherwise stated, were \( g_{\text{leak}} = 0.18 \) \( \mu \)S, \( \tau_h = 30 \) ms, \( g_T = 0.32 \) \( \mu \)S, \( g_{\text{Na}} = 30 \) \( \mu \)S, \( g_K = 10 \) \( \mu \)S, \( E_{\text{leak}} = -65 \) mV, \( E_{\text{Ca}} = 120 \) mV, \( E_{\text{Na}} = 60 \) mV, \( E_K = -85 \) mV, \( C = 1 \) \( \mu \)F, \( A = 0.75 \) nA, \( \Phi = 2 \), \( G_{\text{ON}} = 1 \), \( G_{\text{OFF}} = -1 \), \( F_{\text{ON}} = F_{\text{OFF}} = 2 \), \( I_{\text{bias}} = -4.3 \) nA, \( \tau_{\text{ON}} = 100 \) ms, \( \tau_{\text{OFF}} = 10 \) ms. We note that the negative value of \( G_{\text{OFF}} \) should not be taken to imply that TS neurons receive direct inhibition from ELL pyramidal neurons. Rather, it reflects the fact that I-type ELL pyramidal cells within that zone that excite TS neurons are inhibited by the moving object stimulus. These parameter values are comparable to those used in previous modeling studies (Chacron and Fortune 2010; Chacron et al. 2009; Rush and Rinzel 1994) and to the experimentally observed time constant of depression (Chacron et al. 2009). The simulated spiking responses were then used to build PSTHs over 2000 presentations of the moving stimulus and were segregated into bursts and isolated spikes as described above using an ISI threshold of 10 ms (Oswald et al. 2004). The directional bias \( DB \) was then computed in the same way as we did for the data.
Modeling biophysically plausible mechanisms to extract bursts

While the interspike interval threshold procedure described above is a simple computational method for segregating bursts from isolated spikes, the method is acausal and therefore cannot be implemented in brain circuits on a spike by spike fashion as it is necessary to know when the next spike will occur in order to classify a spike happening at the present time as being part of a burst or not. It was previously proposed that a synapse that exhibited strong facilitation would be largely unresponsive to isolated spikes but would be sensitive to bursts (Kepecs and Lisman 2003; Kepecs et al. 2002; Lisman 1997). We thus considered such a circuit using well-established techniques to model and quantify short-term synaptic plasticity (Dayan and Abbott 2001; Gabbiani and Koch 1998; Rieke et al. 1996). Specifically, we consider the presynaptic spike train as a sum of delta functions:

\[ X(t) = \sum_{i=1}^{N} \delta(t - t_i) \]

where \( t_i \) is the \( i \)th spike time. The amplitude of the synaptic EPSP caused by the \( i \)th spike time is given by the product of a facilitation term \( F(t_i) \) and a depression term \( D(t_i) \) that obey the following dynamics (Harvey-Girard et al. 2010; Lewis and Maler 2002; 2004; Lindner et al. 2009):

\[
\frac{dD}{dt} = \frac{1 - D}{\tau_D}; \quad t = t_i \Rightarrow D(t_i) \rightarrow D(t_i)[1 - F(t_i)]
\]

\[
\frac{dF}{dt} = -\frac{F}{\tau_F}; \quad t = t_i \Rightarrow F(t_i) \rightarrow F(t_i) + \Delta F(t_i - t_{i-1})
\]

\[
\Delta F(I) = \frac{I_i}{I}
\]

At the time of an input spike \( t_i \), \( D \) is first decreased by an amount \( F(t_i)D(t_i) \); then \( F \) is updated by an increment \( \Delta F \). The increment \( \Delta F \) is inversely proportional to the time interval between the current action potential and the last one. As such, short time intervals such as those that occur during burst firing will cause more potentiation than longer ones. We have also introduced an upper bound for \( F \) (i.e. \( F(t) \leq 1 \)) to prevent negative values for the update factor of the depression variable. We used an Euler algorithm to simulate this model with integration time step 0.025 msec. The output of the model was convolved with an alpha function with time constant \( \tau_\alpha = 4 \) ms in order to mimic synaptic PSPs. Thus, the output is given by:

\[
Y(t) = \sum_{i=1}^{N} \Theta(t - t_i)D(t_i)F(t_i) \frac{t - t_i}{\tau_\alpha^2} \exp \left( -\frac{t - t_i}{\tau_\alpha} \right)
\]

where \( \Theta(t) \) is the Heaviside function (\( \Theta(t) = 1 \) if \( t \geq 0 \) and \( \Theta(t) = 0 \) otherwise). Other parameter values used were \( \tau_\alpha = 70 \) msec, \( \tau_D = 500 \) msec. The transfer function \( TF \) of the postsynaptic neuron is given by the following:
Thus, the output of the postsynaptic neuron is given by:

\[ Z(t) = TF[Y(t)] \]

We then took experimentally recorded spike sequences, and segregated them into bursts and isolated spikes using both our decoding model and ISI threshold methods. Then, we compared the sequences of burst and isolated spikes obtained from each model in the following way. We used signal detection theory (Green and Swets 1966) in order to quantify the decoding model’s performance at detecting bursts as defined by the ISI threshold. We computed the probability of correct detection (P_D) as the fraction of spike times deemed to be part of a burst according to the decoding model that were also deemed part of a burst using the ISI threshold criterion (i.e. that were “correctly” classified). The probability of false alarm (P_FA) was computed as the fraction of spike times deemed to be part of a burst according to the decoding model that were deemed to be isolated using the ISI threshold criterion (i.e. that were “incorrectly” classified). The overall performance can then be quantified by computing the probability of correct classification (P_{cc}) as:

\[ P_{cc} = \frac{P_D}{2} + \frac{(1-P_{FA})}{2} \]

A value of P_{cc}=0.5 implies that our model performs at chance level compared to the ISI threshold criterion (i.e. that any given spike is randomly assigned as being part of a burst or isolated). In contrast, P_{cc}=1 indicates that the model performs identically to the ISI threshold criterion. We note that this does not imply that the ISI threshold criterion is optimal in any way as segregating bursts and isolated spikes, merely that our biophysically plausible decoding model performs as well. As such, signal detection theory is used here to determine how well the decoding model performs relative to the ISI threshold criterion. In order to investigate the robustness of the model’s performance, we computed the probability of correct classification of bursts over a wide range of physiologically plausible facilitation (\tau_F) and depression (\tau_D) time constants.

**Results**

**Bursts preferentially code for movement direction in TS neurons**

We performed in vivo extracellular recordings from TS neurons (N=43) while moving an object back and forth along the rostro-caudal axis of the animal (Fig. 1A). We found that most neurons (N=36 or ~70%) fired bursts of action potentials in response to the moving object (Fig. 1B). This firing pattern was reflected in a large peak in the autocorrelogram (Fig. 1C, brown line) that exceeded the 99.9% confidence interval (Fig. 1C, green line) around the autocorrelogram from a Poisson process with the same firing rate (Fig. 1C, cyan line). Such a peak has been taken to be a sign of burst firing (Abeles 1982; Bastian and Nguyenkim 2001). We used an ISI threshold criterion to separate the train of action potentials into bursts and isolated spikes (Fig. 1B). Specifically, when the difference between the times of two consecutive spikes was less than the
threshold, the two spikes were labeled burst spikes (Kepecs and Lisman, 2003; Lesica and Stanley, 2004; Oswald et al., 2004). The remaining spikes were labeled isolated spikes (Fig. 1B).

This criterion was used to separate the spike train into the burst spike train (i.e. the train of action potentials that belong to bursts) and the isolated spike train (i.e. the train of action potentials that do not belong to bursts). The burst event train was defined as the train of action potentials that consisted of only the first spike within each burst (Oswald et al. 2004). As in previous studies, we chose the burst threshold to be equal to the time at which the autocorrelation function first crosses the 99.9% confidence interval from above (Fig. 1C) (Bastian and Nguyenkim, 2001; Chacron and Bastian, 2008; Avila-Akerberg et al., 2010). This value also corresponds to the trough in the bimodal ISI distribution from this neuron (Fig. 1D), which is frequently taken as the threshold to segregate bursts and isolated spikes (Deemyad et al. 2011; Doiron et al. 2003; Turner et al. 1994).

The remainder of neurons in our dataset (N=16 or ~30%) did not preferentially produce bursts, but approached a Poisson distribution (Fig. 1E). The autocorrelogram (Fig. 1F, brown line) did not display a large peak as seen in bursting neurons, and instead slowly rose towards the value computed from a Poisson process with the same firing rate (Fig. 1F, cyan line). Further, the ISI distributions of these neurons were unimodal in nature (Fig. 1G), which indicates their tendency to not fire bursts of action potentials (Doiron et al. 2003).

Approximately 50% of directionally selective TS neurons have the tail-to-head direction as their preferred direction while the other 50% have the head-to-tail direction as their preferred direction when the full spike train is considered (Chacron et al. 2009). The data obtained from a representative bursting neuron are shown in Fig. 2A. This neuron responded best when the object was moving from tail to head (preferred direction) and displayed a weaker response when the object was moving from head to tail (null direction) (Fig. 2A, Fig. 2B). We quantified this difference using a directional bias (DB) index and found that this neuron displayed strong directional selectivity (DB=0.62) (DB=0 indicates no directional selectivity and that DB=1 indicates complete directional selectivity).

For bursting neurons, we observed a qualitatively different sensitivity to movement direction when considering either the burst spike train (i.e. only the spikes belonging to bursts) or the isolated spike train. We found that burst spikes almost exclusively occurred when the object was moving in the preferred direction (Fig. 2A arrows; Fig. 2B), thereby giving the burst spike train a larger directional bias (DB=0.91) than the full spike train. Similar results were seen when considering the burst event train (DB=0.91) (Fig. 2A arrows, Fig. 2B). In contrast, isolated spikes were more evenly distributed (Fig. 2A arrows; Fig. 2B) and, as a result, the isolated spike train displayed a weaker directional bias (DB=0.33). This result is not dependent on the specific threshold for separating burst and isolated spikes: we found that the directional biases of the burst, burst event, and isolated spike trains were largely unaffected by varying the burst threshold over a wide range of values (Fig. 2C).

Bursting neurons displayed a wide range of directional biases when the full spike train was considered (0-0.91). As such, the example neuron showed in Figure 2 was in the upper range with a directional bias of 0.62. Despite these large heterogeneities, the directional bias of bursts...
was significantly higher than that of all spikes (p=0.006, sign rank test, N=32) across these neurons. Further, the directional bias for all spikes was significantly higher than for isolated spikes (p<<10^-3, sign rank test, N=32) (Fig. 3A). We conclude that bursts are overall more reliable indicators of the direction of movement than either the entire spike train or the isolated spikes. Moreover, the directional bias of burst events was not significantly different from that of bursts (p=0.1577, sign rank test, N=32). Since burst and burst events did not display significantly different levels of directional bias across our dataset, we only present results obtained with the burst train from now on.

As a methodological control, we set an arbitrary threshold of 30 msec in the subset of neurons that did not produce bursts. In these neurons, the differences between the directional biases of “bursts”, all spikes, and “isolated spikes” were not significantly different (bursts-allspikes: p=0.1; allspikes-isolated spikes: p=0.2; bursts-isolated spikes: p=0.07, sign rank tests, N=32) (Fig. 3B). The directional biases of the burst and isolated spike trains were qualitatively similar for a wide range of burst threshold values (data not shown). Moreover, the directional bias from all spikes of these neurons was not significantly different than that computed from bursting neurons (p=0.6557, Wilcoxon ranksum test, df=42). We shall return to this point in the discussion.

Mechanisms for the generation of burst and isolated spike responses to moving objects

Theoretically, direction selectivity can rely on two fundamental operations (Reichardt and Wenking, 1969; Reichardt, 1987). The first operation involves generating a directional bias by asymmetric filtering of information from at least two separate spatial locations within the receptive field. Many mechanisms can give rise to a directional bias, including dendritic integration (Euler et al. 2002), temporal delays (Haag et al. 2004; Jagadeesh et al. 1997), and synaptic depression (Carver et al. 2008; Chacron et al. 2009; Chance et al. 1998). The second operation involves the nonlinear interaction of these inputs and various types of nonlinearities have been considered such as multiplication (Reichardt, 1987; Haag et al., 2004), squaring (Adelson and Bergen, 1985), nonlinear synaptic integration (Chacron and Fortune 2010), shunting (Euler et al., 2002), and thresholding (Priebe et al., 2004; Priebe and Ferster, 2008).

In TS neurons, asymmetric filtering by different time constants of synaptic depression across the receptive field creates a directional bias (Chacron et al., 2009) and these inputs are nonlinearly integrated by a subthreshold T-type conductance (Chacron and Fortune, 2010). Since T-type calcium channels have been shown to give rise to burst firing in other systems (Llinas and Jahnsen 1982; Sherman 2001; Sherman and Guillery 2006), we hypothesized that these might be also responsible for burst firing in TS neurons. T-type calcium channels are inactivated at resting membrane potential values (~ -60 mV) and require ~100 ms hyperpolarization to (~ -70 mV) in order to remove their inactivation after which a subsequent depolarization will lead to a subthreshold calcium spike, leading to nonlinear integration of synaptic input. Bursts of sodium action potentials can occur on top of these calcium spikes (Rush and Rinzel, 1994; Sherman and Guillery, 2006). However, a simple depolarization from the resting potential will not lead to burst firing as the calcium channel will still be inactive and will instead lead to tonic firing (Sherman and Guillery 2006).
We therefore examined intracellular recordings from TS neurons and looked at whether a membrane hyperpolarization indeed preceded the peak firing response in each direction of movement. The data show that the peak response in both the preferred and the null directions were preceded by a period of spiking silence (Fig. 4A). Further, plots of average membrane potential waveforms also revealed, critically, that membrane hyperpolarizations were responsible for the lack of spiking activity and preceded the peak depolarizations (Fig. 4B). These hyperpolarizations were significantly larger in magnitude in the preferred direction (Fig. 4C, p=0.031, sign rank test, N=6). These results show that membrane hyperpolarization removes the inactivation of the T-type conductance, thereby giving rise to burst firing. The data also show that the hyperpolarizations are larger when the object moves in the preferred direction. This larger hyperpolarization would presumably more effectively remove the inactivation of the T-type conductance, thereby increasing the probability of burst firing when the object moves in the preferred direction.

Previously used models of directional selectivity in TS neurons did not incorporate spike firing (Chacron and Fortune 2010) and furthermore assumed only excitatory input from afferent neurons. These models thus cannot reproduce the experimentally observed membrane hyperpolarization that precedes the peak depolarization. As such, we built a mathematical model that incorporates the known mechanisms that lead to directional selectivity in the electrosensory system and that gives rise to a larger membrane hyperpolarization when the object moves in the preferred direction.

There are two types of ELL pyramidal cells (Bastian et al. 2002; Maler 2009; Saunders and Bastian 1984), E and I, that correspond to the ON and OFF type cells seen in other systems. While E-type pyramidal cells are excited by our metal moving object stimulus, I-type pyramidal cells are instead inhibited by it. The receptive field is modeled in one dimension as two contiguous zones, one that represents the output of I-type ELL pyramidal cells and the other represents the output of E-type ELL pyramidal cells that have time constants of depression $\tau_{OFF}$ and $\tau_{ON}$, respectively (Fig. 5A). Both the ON and OFF zones then make excitatory contact unto our model TS neuron, which is consistent with anatomical data (Carr and Maler 1985). The summed input is then convolved with an alpha function to mimic the synaptic EPSP shape and fed into a Hodgkin-Huxley model with leak, spiking sodium, delayed rectifier potassium, and T-type calcium conductances (Fig. 5A). It is important to note that our model includes the effects of the significant spontaneous activity of afferents that occurs in vivo (Destexhe et al. 2003) by including a noise term that induces membrane potential fluctuations. In particular, the noise term can give rise to a mixture of burst and isolated action potential firing as observed under in vivo conditions in other systems (Wolfart et al. 2005) as well as our experimental data (Fig. 1B).

The model stimulus was an object that moved across the receptive field in both directions. Figure 5B shows the responses of both the OFF and ON zones to this stimulus. When the object moves from the OFF to the ON zone, a decrease in excitation from when the object is in the OFF zone precedes an increase in excitation when the object is in the ON zone. However, when the object moves in the opposite direction, the increase in excitation is truncated by the decrease in excitation (Fig. 5C). The membrane potential responses of the model neuron to these moving stimuli are shown in Fig. 5D. Interestingly, when the object moves from the OFF to the ON zone, the decrease in excitation removes the inactivation of the calcium conductance and the
subsequent increase in excitation then activates it: as a result there is a strong tendency to have a calcium spike which gives rise to a burst of action potentials (Fig. 5D, purple). In contrast, when the object moves from the ON zone to the OFF zone, the increase in excitation from the ON zone only gives rise to isolated spikes as the calcium conductance is then inactivated. Moreover, the weaker decrease in excitation is less effective at removing the inactivation of calcium channels. This and the fact that this decrease is not followed by an increase in excitation both contribute to our model having a lesser tendency to display burst firing in that direction (Fig. 5D, orange). The preferred direction is therefore when the object moves from the OFF zone to the ON zone and the null direction is the opposite direction.

Role of T-type calcium channels in generating bursts in response to moving objects

We systematically varied the T-type calcium channel conductance $g_T$ in order to gauge the effects of the active burst mechanism on responses to a simulated moving object. Our results show that varying $g_T$ can lead to qualitatively different regimes (Fig. 6A). As in the experimental data, we segregated bursts and isolated spikes using an ISI threshold. For lower values of $g_T$, bursts and isolated spikes showed similar levels of directional selectivity to all spikes (Figs. 6B,C,D). In contrast, for higher values of $g_T$, bursts displayed greater directional selectivity than either all spikes or isolated spikes (Figs. 6E,F,G). In contrast, a model that only includes ON zones but with different time constants of depression cannot reproduce this critical feature of our data (data not shown). Thus, the removal of inactivation of the T-type calcium conductance by membrane hyperpolarization resulting from the decrease in excitation coming from the OFF zone is necessary in order for our model to reproduce the current dataset.

These results show that our relatively simple mathematical model can qualitatively reproduce the experimentally observed directional biases of bursts and isolated spikes. How does this work? The inputs from the two zones for $\tau_{ON}=100$ ms, $\tau_{OFF}=10$ ms are shown in Fig. 7A. As shown above (Fig. 5C), a decrease in excitation is followed by an increase in excitation when the object moves in the preferred direction (Fig. 7A, purple). In contrast, the increase in excitation is truncated by weaker decrease in excitation when the object moves in the null direction (Fig. 7A, orange). As a result, for weaker values of the calcium conductance $g_T$, the weaker hyperpolarization followed by no depolarization when the object moves in the null direction results in a mixture of bursts and isolated spikes (Fig. 7B). However, the larger hyperpolarization when the object moves in the preferred direction more effectively removes the inactivation of calcium channels and the following depolarization elicits more action potential firing (Fig. 7C). However, this action potential firing still consists of bursts and isolated spikes with roughly the same proportion as when the object moves in the null direction. As such, the directional biases of bursts and isolated spikes are roughly equal.

For larger values of the calcium conductance $g_T$, qualitatively different results were observed. When the object moves in the null direction, the resulting firing pattern is similar to that observed with lower values of $g_T$ (Fig. 7D). In contrast, when the object moves in the preferred direction, the stronger decrease in excitation more effectively removes the inactivation of the now stronger calcium conductance, and the following depolarization now gives rise to a stronger calcium spike and thus a greater probability of obtaining burst firing and lesser probability of
obtaining isolated spike firing (Fig. 7E). As a result, the directional bias of bursts increases while the directional bias of isolated spikes decreases with respect to their values obtained for lower \( g_T \).

**Role of T-type calcium channels in vivo**

To test the model’s prediction that the T-type calcium channel mediated burst mechanism can produce bursts that display significantly more directional selectivity than either all spikes or isolated spikes, we injected the antagonists NiCl\(_2\) and Mibefradil (Mib), as was done in previous studies (Chacron and Fortune 2010), to block the activity of T-type calcium conductances in TS neurons. As both NiCl\(_2\) and Mib had quantitatively similar results on directional selectivity and burst firing, the data were pooled. We found that fewer neurons displayed burst firing after injection of either NiCl\(_2\) or Mib into the TS. In contrast, the percentage of neurons that display burst firing after saline injection was similar to that observed for control conditions (Fig. 8A).

We quantified the directional biases of bursts, isolated spikes, and all spikes before (Fig. 8B) and after injection of saline (Fig. 8C), or Mib and NiCl\(_2\) (Fig. 8D). As expected, the directional bias values obtained for bursts were significantly greater than those obtained for all spikes or isolated spikes before injection (Fig. 8B) and injection of saline had no significant effect (compare Fig. 8C to Fig. 8B). However, after injection of NiCl\(_2\) or Mib, the measures of direction selectivity for bursts, isolated spikes, and all spikes were dramatically altered – they were not significantly different from each other (Fig. 8D). Further, NiCl\(_2\) or Mib significantly reduced the directional bias of bursts (p=0.02, Wilcoxon Ranksum test, n=12) and all spikes (p<10\(^{-7}\), Wilcoxon Ranksum test, n=12) but not of isolated spikes (p=0.24, Wilcoxon Ranksum test, n=12) whereas saline injection did not significantly alter the directional bias of either bursts (p=0.51, Wilcoxon Ranksum test, n=12) or isolated spikes (p=0.32, Wilcoxon Ranksum test, n=12). As such, these data support our modeling prediction that an active burst mechanism based on T-type calcium conductances promotes selective coding of directional information by bursts.

**Mechanisms for decoding movement information from burst and isolated spike trains**

Any information is only functionally relevant if it is decoded by downstream neurons. As such, we examined biologically plausible mechanisms by which information from the burst and isolated spike trains might be discriminated in downstream decoders. This is a potentially difficult problem, because the identity of a spike as a burst or isolated spike is only determined by the time interval separating it from the next action potential firing. As such, it is necessary to know when the next spike occurs in order to classify any given spike as part of a burst or not if using the simple ISI threshold procedure that was described above. It is, at best, unclear how a sensory system would implement this.

**Decoding bursts using synaptic facilitation**

Previous studies have proposed that synapses with strong facilitation and/or high probability of failure would be insensitive to isolated spikes, but would respond to bursts (Lisman, 1997; Kepecs et al., 2002; Kepecs and Lisman, 2003). These theoretical results have been confirmed in experimental studies that have shown that bursts of action potentials are indeed more reliable at
eliciting plasticity in synapses (Harvey-Girard et al., 2010). However, it is not clear whether such
a system would preferentially extract the burst spikes in responses to moving objects.

We tested whether a synapse with strong facilitation would be sufficient to decode directional
information transmitted by a presynaptic spike train (Fig. 9A). The model displays different
levels of facilitation in response to tetanic stimulation consisting of five presynaptic action
potentials separated by an interspike interval I as I is systematically varied (Fig. 9B). The model
displayed strong facilitation for I=10 ms, weaker facilitation for I=100 ms, and no significant
facilitation for I=1000 ms (Fig. 9C, compare top, middle, and bottom panels). This was
confirmed by plotting the paired-pulse facilitation ratio (i.e. the amplitude of the second EPSP
minus the amplitude of the first EPSP normalized to the amplitude of the first EPSP) as a
function of I: this ratio decreases monotonically to zero as a function of increasing I (Fig. 9B)
and illustrates this model’s ability to respond preferentially to bursts by effectively implementing
a high-pass filter on the inverse interspike interval sequence.

We next tested whether this simple model could segregate bursts from isolated spikes and
compared its performance to that of an ISI threshold. A representative example is shown in Fig.
9D: this model can effectively segregate bursts from isolated spikes. We quantified the model’s
performance by using signal detection theory and found that this model could give about ~75%
probability of correct classification for a wide range of parameters (Fig. 9E). We next presented
experimentally measured spike trains from TS neurons to the model and computed the PSTH.
Our results show that this model was indeed sensitive primarily to bursts. When the full spike
train of the neuron shown in Figure 2A was used as input, the output PSTH displayed a reduction
of the peak response in the null direction (Fig. 9F, arrow) and was similar to that obtained by
using only the bursts (compare Fig. 9F, black with Fig. 2A blue).

Consequently, the output directional bias of the model was larger than the input directional bias
present in the input spike train (Fig. 9G). Finally, we computed the directional bias of bursts
obtained with our model against that computed from bursts obtained with the ISI threshold
criterion across our dataset (Fig. 9H) and observed a significant positive correlation between
both quantities (R=0.606, p=0.0002, N=32). In conclusion, our results show that a biophysical
realistic model of plasticity can reliably extract bursts from incoming spike trains. As such, the
directional information carried by the bursts of TS neurons can in theory be decoded by
downstream neurons.
**Discussion**

We have shown that most direction selective neurons in the midbrain of weakly electric fish preferentially encode the direction of movement using bursts. We found that ~70% of the neurons in our dataset fired bursts of action potentials in response to moving object stimuli and that, for these neurons, bursts carried significantly greater directional information than either of all spikes or isolated spikes. It is unlikely that this result is an artifact of the ISI burst threshold technique that we used to segregate bursts from isolated spikes, as qualitatively different results were obtained in non-bursting neurons. In non-bursting neurons arbitrary divisions of spikes into bursts and isolated spikes did not result in increased direction selectivity by bursts.

In order to better understand these results, we built a mathematical model of directional selectivity in TS neurons that incorporated a T-type calcium conductance. This model predicted that bursts of action potentials could result from the transient de-inactivation of the T-type conductance by membrane hyperpolarization, and that this mechanism could preferentially elicit bursts in the preferred direction. Moreover, the model predicted that, after blocking the T-type conductance, bursts would not carry greater directional information than either all spikes or isolated spikes.

We tested this prediction experimentally by injecting calcium channel antagonists in TS. We found that TS neurons, after this injection, displayed significantly less tendency to burst. Further, the directional biases of bursts, isolated spikes and all spikes were quantitatively similar under these conditions, which was reminiscent of results obtained for non-bursting TS neurons under control conditions. These results support our model’s prediction that directionally biased interactions between afferent input and T-type calcium channels mediate the enhancement of direction selectivity by burst spikes.

Finally, we have shown that a model downstream decoder, using simple biologically realistic neural circuits, can selectively extract bursts from spike trains consisting of both bursts and isolated spikes. This model decoder was shown to perform similarly to an ISI threshold. As such, our results show that downstream neurons could actually decode motion information that is carried by bursts of action potentials.

**Burst and isolated spikes in direction selective circuits**

We have shown that the standard method for measuring direction selectivity, in which the firing rates elicited by each direction of movement are compared, can underestimate direction selectivity and fail to capture salient information transmitted by the neurons. Indeed, bursts were almost always more directionally selective than the full spike trains in bursting neurons, which was largely due to the fact that isolated spikes carried little or no directional information. Our results thus show that burst firing caused by T-type calcium channels can amplify this bias. However, burst firing alone will not necessarily make a neuron more directionally selective. Indeed, previous studies have shown that TS neurons receive varying degrees of input directional bias (Chacron et al. 2009) and these differences may explain why non-bursting and bursting neurons displayed similar levels of directional selectivity in their full spike trains on average. Further studies are needed to understand this.
Our results may have implications for the analysis of direction selectivity in mammalian visual cortex. Indeed, the electro-sensory system has many parallels with thalamocortical pathways (Krahe and Gabbiani 2004). In particular, thalamic relay neurons within the lateral geniculate nucleus (LGN) display an intrinsic burst mechanism that is mediated by subthreshold T-type calcium channels that have been both biophysically characterized and modeled (Jahnsen and Llinas 1984; Lu et al. 1992; McCormick and Huguenard 1992; Mukherjee and Kaplan 1995; Rush and Rinzel 1994; Sherman 2001; Sherman and Guillery 2006; Sherman and Guillery 2002; Smith et al. 2000). These neurons have two modes of action potential firing in vitro (Sherman 2001), and studies performed in vivo have shown that their spike trains in vivo consist of both bursts and isolate spikes in awake behaving animals (Lesica and Stanley 2004; Reinagel et al. 1999; Wolfart et al. 2005).

While previous studies have shown that these neurons are not directionally selective (Hubel and Wiesel 1962), these did not consider action potential patterns such as bursts. We hypothesize that bursts of action potentials from thalamic relay neurons in LGN carry specific directional information that is then used by postsynaptic neurons within the primary visual cortex to generate directionally biased responses. This hypothesis is supported by the fact that thalamocortical synapses display strong depression, and sustained isolated action potential firing from thalamic relay neurons activates this depression (Sherman 1996; 2001; Sherman and Guillery 2006). Nevertheless, after ~100 ms of membrane hyperpolarization, T-type calcium currents in these neurons can cause burst firing, which could amplify the post-synaptic responses to bursts (Sherman 1996; 2001; Sherman and Guillery 2006). Studies performed within the LGN are necessary to validate this hypothesis.

Further, these results might also be relevant in understanding auditory processing within the inferior colliculus (IC), as it is homologous to the Torus semicircularis in gymnotiform weakly electric fishes. IC neurons display a form of direction selectivity, responding either to ascending or descending frequency modulated acoustic sweeps (Fuzessery and Hall 1996; Fuzessery et al. 2006; Razak and Fuzessery 2008; 2006; Suga 1965). We hypothesize that T-type calcium channel mediated burst firing within the IC could be used to improve the degree of response selectivity to ascending vs. descending frequency modulated acoustic sweeps. Further studies are needed to test this hypothesis.

Removing T-type calcium channel inactivation by hyperpolarization

We have shown that a period of hyperpolarization is seen immediately before burst firing in TS neurons. Such hyperpolarization is needed in order to remove the inactivation of the T-type conductance, thereby leading to a burst of action potentials riding on top of the calcium spike (Llinas and Jahnsen 1982). Our previous experimental results indicate that the T-type conductance is predominantly activated in the preferred direction (Chacron and Fortune 2010). Our current results show that there is a greater membrane hyperpolarization when the object moves in the preferred direction which in turn leads to the greater activation of the T-type calcium conductance. Where does this hyperpolarization come from? A priori, such hyperpolarization could come from inhibitory connections within the TS. However, this would be inconsistent with our previous results showing that GABAergic and glycinergic antagonists...
do not alter directional selectivity in TS neurons (Chacron and Fortune 2010). Alternatively, this
hyperpolarization could come from a decrease in excitation. Indeed, TS neurons receive
exclusively excitatory afferent input from ELL pyramidal cells (Carr and Maler 1985). We
therefore hypothesized that the membrane hyperpolarization seen experimentally is caused by a
decrease in the excitation coming from I-type pyramidal cells that are inhibited during the
passage of the moving object in their receptive fields. We note that models in which the outputs
of ON and OFF cells are spatially offset have been proposed to explain directional selectivity in
the visual system (Hubel and Wiesel 1962). However, further studies are needed in order to
validate this hypothesis in the electrosensory system and are beyond the scope of this report.

Role of active burst dynamics in generating directional selectivity

Previous studies have suggested that bursts are more likely to transmit behaviorally relevant
information than isolated spikes because they can overcome synaptic unreliability in downstream
neurons (Lisman 1997). Indeed, bursts of action potentials have been shown to be more reliable
at inducing changes in the postsynaptic neuron such as synaptic plasticity than isolated spikes
(Gall et al. 2005; Harvey-Girard et al. 2010; Lisman 1997) and can be more reliable indicators of
stimulus features such as orientation in the visual system of mammals (Lisman 1997; Martinez-
Conde et al. 2002). However, we are not aware of previous results showing that bursts could give
rise to enhanced directional selectivity. Our results have shown that, in most TS neurons, bursts
were more directionally selective than the full spike train or the isolated spike train. This
supports the point of view that bursts of action potentials carry salient information. Our study has
shown that an active burst mechanism based on a low threshold calcium conductance gives rise
to preferential encoding of directional information by bursts. However, there are multiple
mechanisms that can give rise to burst firing in neurons (Izhikevich 2000; Krahe and Gabbiani
2004). Further studies are needed to explore whether burst dynamics that are different than the
one considered here would also give rise to the same phenomenon.

Selective burst extraction by downstream circuits

We have proposed a biologically plausible neural circuit that can selectively extract bursts.
While it is frequently assumed that bursts can be segregated from isolated spikes in the brain
(Avila Akerberg and Chacron 2011; Avila Akerberg et al. 2010; Bastian and Nguyenkim 2001;
Chacron and Bastian 2008; Doiron et al. 2007; Gabbiani et al. 1996; Kepecs and Lisman 2003;
Oswald et al. 2007; Oswald et al. 2004), biophysically plausible implementations by which such
segregation can be achieved have not been widely considered. Previous reports have suggested
potential circuits that could extract bursts (Lisman 1997), which were the theoretical basis for the
model for burst extraction used here. Our results have shown that such a model can perform
similarly to the ISI criterion that has been frequently used by previous investigators. Our results
therefore suggest that downstream neurons can decode directional information carried in the
burst firing from TS neurons. In Apterodonotus leptorhynchus, many neurons in the TS project to
the optic tectum (OT) where some neurons have been shown to respond selectively to moving
objects in a directionally biased fashion (Bastian 1982). However, the dynamics of TS-OT
synapses are not known and further studies should concentrate on whether OT neurons respond
selectively to bursts from TS neurons and on determining whether TS-OT synapses display
strong paired-pulse facilitation that would enable them to preferentially signal the occurrence of these bursts.

Functional relevance of independent burst and isolated spike codes

For most of the neurons in our dataset, bursts were best at encoding the direction of movement while isolated spikes carried little, if any, directional information. It is possible that, for these neurons, isolated spikes could code for a stimulus not related to movement while bursts would code for the movement direction. For example, weakly electric fish produce communication calls called chirps during aggressive and courtship situations in which both animals move with respect to one another (Hupe and Lewis 2008; Zakon et al. 2002): recent studies have shown that TS neurons can respond to such stimuli with well-timed isolated spikes (Vonderschen and Chacron 2009). Such parallel coding is entirely consistent with an emerging general picture in which bursts and isolated spikes code for different stimulus attributes (Avila Akerberg and Chacron 2011; Avila Akerberg et al. 2010; Deemyad et al. 2011; Kepecs and Lisman 2003; Lesica and Stanley 2004; Marsat and Maler 2010; Marsat and Pollack 2004; Marsat et al. 2009; Oswald et al. 2004).

Conclusion

We have shown that bursts could convey information about movement direction more reliably than either the full spike or isolated spike trains in electrosensory midbrain neurons. As such, burst firing can be used to amplify a given directional bias. It is likely that burst firing is used to convey information about movement direction in other systems as well.
Figure Legends

Figure 1: TS neurons respond to a moving object with a combination of bursts and isolated spikes. **A)** Schematic showing the stimulation protocol. The gray bar represents the moving object that was moved sinusoidally about 1 cm lateral to the fish. The purple arrow indicates the tail-to-head direction while the orange arrow indicates the head to tail direction. **B)** (top) Example recording from a bursting TS neuron *in vivo*. Action potentials with interspike intervals that were less than the burst threshold were identified as belonging to bursts (blue tics) while those that did not were identified as isolated spikes (red tics). The set of first spike of each burst was identified as the burst event train (black tics). **C)** Spike train autocorrelogram from this same neuron. The initial peak in the autocorrelogram (brown line) is indicative of burst firing. The time lag for which the autocorrelogram falls down the 99.9% confidence interval (green line) of the expected bin contents assuming a Poisson spike train with the same mean firing rate (blue line) was chosen as the burst threshold. **D)** Bimodal ISI histogram from this same neuron. The burst threshold derived from the autocorrelation function is located approximately at the trough between the two peaks, purple arrow. **E)** Example recording from another TS neuron *in vivo*. In contrast, this neuron tended to fire isolated action potentials. **F)** The spike train autocorrelogram from this same neuron did not display a prominent peak and instead tended towards that of a Poisson process with the same firing rate. **G)** Unimodal ISI histogram from this same neuron.

Figure 2: Bursts carry directional information. **A)** Top: Raster plot from an example directionally selective bursting TS neuron. The spikes that belong to bursts are shown in blue while isolated spikes are shown in red. **Bottom:** Peristimulus time histogram (PSTH) for this same neuron computed from all spikes (both bursts and isolated spikes, green), bursts (blue), burst events (dotted black), and isolated spikes (red). Arrows highlight similarity between measures for bursts and burst events. **B)** PSTHs from burst events (left), bursts, all spikes, and isolated spikes were aligned with respect to the maximum event rates in the preferred (pink) and null (orange) directions. Directional bias (DB) values were 0.91, 0.91, 0.62, and 0.33 for burst events, bursts, all spikes, and isolated spikes, respectively. **C)** DB values were largely insensitive to the value of the ISI burst threshold chosen.

Figure 3: Summary of directional biases obtained for bursts, all spikes, and isolated spikes for bursting and non-bursting neurons. **A)** Population-averaged directional bias values for bursts (black), all spikes (light grey), and isolated spikes (dark grey) for bursting neurons. **B)** Same as **A**, but for non-bursting neurons. Horizontal bars indicate statistical significance at the p=0.05 level using a signed-rank test.

Figure 4: A membrane hyperpolarization precedes the peak response in a directionally biased way. **A)** Peristimulus time histogram (PSTH) of an example direction selective neuron. Periods of silence preceded the peak firing rates in both the preferred and null directions (arrows). **B)** Average membrane potential waveform of the same neuron. Hyperpolarizations in the membrane potential precede the peak depolarizations in both movement directions. These hyperpolarizations are indicated with light grey in the null direction and dark grey in the preferred. Horizontal dashed line indicates the average membrane potential. The membrane hyperpolarization is stronger in the preferred direction than in the null direction (inset). **C)** Population average of the hyperpolarization magnitude that is computed as the area for which the
membrane potential is lower than average in the preferred (dark grey) and null (light grey)
directions preceding the peak depolarizations (N=6). The horizontal bar indicates statistical
significance at the p=0.05 level using a signed-rank test.

**Figure 5**: Modeling directional selectivity in TS neurons. **A)** Schematic of the receptive field of
our model neuron that is composed of two zones: the OFF Zone represents output from I-type
ELL pyramidal cells that are inhibited by the moving object and has a synaptic depression time
constant $\tau_{OFF}$ while the ON Zone represents output from E-type ELL pyramidal cells that are
excited by the moving object and has a synaptic depression time constant $\tau_{ON}$. The responses
from each zone are then summed and convolved with an alpha function with time constant 20
msec and fed into a Hodgkin-Huxley model with spiking sodium ($g_{Na}$), delayed rectifier
potassium ($g_{K}$), leak ($g_{leak}$), and T-type calcium ($g_{T}$) conductances. Noise and a constant bias
current are also added to this model in order to mimic synaptic input. The output membrane
potential $V(t)$ from the model is then thresholded to obtain the spike times that were analyzed in
the same fashion as the experimental data. **B)** Inputs from the OFF zone (gray), ON zone
(black), and the sum of the two (dashed purple) for $\tau_{OFF}=\tau_{ON}=500$ msec when the object moves
from left to right (i.e. enters the OFF zone first and then enters the ON Zone). Note that the traces were aligned with respect to their baseline values in order to better compare their time
courses. **C)** Summed input when the object moves from left to right (purple, identical to trace in B) and when the object moves from right to left (orange). **D)** Example membrane potential traces from the model responding to the inputs shown in C. Burst firing is dramatically increased when
the object moves in the preferred direction (purple) as compared to when the object moves in the
null direction (orange).

**Figure 6**: Effects of burst dynamics on directional selectivity. **A)** Directional biases obtained
from the model from all spikes (green), burst spikes (blue), and isolated spikes (red) as a function
of $g_T$. While bursts and isolated spikes showed similar levels of directional selectivity to all
spikes for low values of $g_T$ (left), a regime where bursts displayed greater directional bias than
either of all spikes or isolated spikes was observed for larger values of $g_T$ (right). **B-D)** Model
data for $g_T=0.2 \mu S$. **B)** Raster plot (top) in response to the moving object stimulus. We used an
interspike interval threshold of 10 msec in order to segregate bursts (blue) from isolated spikes
(red). PSTH (bottom) obtained from all spikes (green), bursts (blue), and isolated spikes (red).
The curves have been normalized to their maximum value for easier comparisons. **C)** PSTH
values near the maximum values in the preferred (purple) and null (orange). **D)** Directional
biases computed from bursts (blue), all spikes (green), and isolated spikes (red) were similar. **E-G)** same as B-D but for $g_T=0.28 \mu S$. **E)** Raster plot (top), PSTH (bottom) for burst spikes (blue),
all spikes (green), and isolated spikes (red). **F)** PSTH values near the maximum values in the
preferred (purple) and null (orange) directions for burst spikes (left), all spikes (middle), and
isolated spikes (right). **G)** Directional biases computed from burst spikes (blue), all spikes
(green), and isolated spikes (red). Bursts displayed a greater bias than all spikes which displayed
a greater bias than isolated spikes.

**Figure 7**: Explanation of the different regimes observed in the model. **A)** Summed input currents
from both zones when the object moves from right to left (orange) and from left to right (purple).
**B,C)** Example output from the model with $g_T=0.2 \mu S$. **D,E)** with $g_T=0.28 \mu S$. **B)** An example
membrane potential trace when the object moves from right to left, null direction, that elicited a...
mix of bursts (black) and isolated spikes (gray). C) An example trace when the object moves from left to right (preferred direction) that elicited a greater number of spikes than in the null direction. D) Changing $g_T$ from 0.20 $\mu$S to 0.28 $\mu$S has little effect on the response in the null direction. E) The increase in the $g_T$ current dramatically increases burst firing when the object moves in the preferred direction.

**Figure 8:** Testing the model’s prediction. A) Percentage of neurons that exhibited bursting under control conditions, after injection of either NiCl$_2$ or Mibefradil (Mib), and saline (ACSF). B) Directional biases computed from bursts (left), all spikes (center), and isolated spikes (right) under control conditions. C) Directional biases computed from bursts (left), all spikes (center), and isolated spikes (right) after saline injection. D) Directional biases computed from bursts (left), all spikes (center), and isolated spikes (right) after NiCl$_2$/Mib injection. Horizontal bars indicate statistical significance at the $p=0.05$ level using a signed-rank test.

**Figure 9:** Decoding information carried by bursts. A) Schematic of our decoding model. It consists of a facilitating excitatory synapse followed by half-wave rectification. B) Response of the model to a tetanus consisting of five presynaptic spikes with time interval $I$ (top) quantified by the paired-pulse facilitation ratio as a function of $I$. C) Model responses to tetani with $I=10$ msec (top), $I=100$ msec (middle), and $I=1000$ msec (bottom). D) Comparing the performances of the decoding model to an ISI threshold criterion. Shown are the original spike train (green) and the spikes that belong to bursts according to the ISI threshold (blue) and the spikes that belong to bursts according to the decoding model (purple). E) Probability of correct classification $P_{cc}$ as a function of the facilitation and depression time constants $\tau_f$ and $\tau_d$. F) Input PSTH (gray) and output PSTH (black) from the model when the input consists of the full spike train from the neuron shown in Fig. 2A. G) Output directional bias (black) and input directional bias (gray) computed from the PSTHs in F. H) Directional bias of bursts computed from the decoding model as a function of the directional bias of bursts computed from the ISI threshold criterion. There was a significant positive correlation between both quantities ($R=0.606$, $p=0.0002$, $N=32$).
References:


A

- Preferred
- Null
- Bursts
- Isolated spikes

Firing rate (Normalized)

0.5
0
1

B

- Burst events
- Bursts
- All spikes
- Isolated spikes

Firing rate (Normalized)

C

Directional bias

0.9
0.6
0.3

Burst threshold (ms)
A  bursting

<table>
<thead>
<tr>
<th></th>
<th>bursts</th>
<th>all spikes</th>
<th>isolated spikes</th>
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<tbody>
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<td>directional bias</td>
<td>0.4</td>
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B  non-bursting

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<tbody>
<tr>
<td>directional bias</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>
A. Receptive field

OFF Zone  ON Zone

\[ \text{Input} I(t) \]

\[ \text{Output} V(t) \]

\[ g_T, g_{\text{leak}}, g_{\text{Na}}, g_K \]

B. 100 ms

\[ 0.5 \text{nA} \]

\[ \text{OFF Zone} \quad \text{ON Zone} \quad \text{sum} \]

C. 100 ms

\[ 0.5 \text{nA} \]

D. 100 ms

\[ 10 \text{mV} \]
A

% bursting neurons

Control  Nickel  Mibefradil  Saline

B

directional bias

bursts  all spikes  isolated spikes

C

Saline

D

Nickel/Mibefradil

directional bias

bursts  all spikes  isolated spikes