Title: DIFFERENCES IN THE TIME COURSE OF SHORT-TERM DEPRESSION ACROSS RECEPTIVE FIELDS ARE CORRELATED WITH DIRECTIONAL SELECTIVITY IN ELECTROSENSORY NEURONS

Abbreviated title: Correlation between short-term depression and directional selectivity.

Maurice J. Chacron¹,², Natalia Toporikova¹, and Eric S. Fortune³,*

¹Department of Physiology, Center for Nonlinear Dynamics, McGill University
²Department of Physics, McGill University.
³Department of Psychological and Brain Sciences, Johns Hopkins University.

*Corresponding Author:
Eric S. Fortune
Department of Psychological and Brain Sciences, Johns Hopkins University
3400 North Charles Street
Baltimore, MD, 21218

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Abstract

Directional selectivity, in which neurons respond preferentially to one direction of movement ("preferred") over the opposite direction ("null"), is a critical computation that is found in the nervous systems of many animals. Here we show the first experimental evidence for a correlation between differences in short-term depression and direction selective responses to moving objects. As predicted by quantitative models, the observed differences in the time courses of short-term depression at different locations within receptive fields were correlated with measures of direction selectivity in awake, behaving weakly electric fish (*Apteronotus leptorhynchos*). Because short-term depression is ubiquitous in the central nervous systems of vertebrate animals, it may be a common mechanism used for the generation of directional selectivity and other spatiotemporal computations.

Keywords: directional selectivity, short-term synaptic depression, weakly electric fish
Introduction

Uncovering the mechanisms that are used by neural circuits to perform behaviorally relevant computations is a central goal of neuroscience. One such computation is motion processing, where brain circuits determine the direction of movement of sensory stimuli (Hubel 1959; Hubel and Wiesel 1962). Neurons that exhibit directionally selective responses are a form of motion processing unit in the brain: such neurons respond strongly to sensory stimuli moving in a given ‘preferred’ direction but weakly or not at all to the opposite ‘null’ direction. Such responses have been found in a variety of species from flies to primates (Hubel 1959; Hubel and Wiesel 1962; Priebe and Ferster 2005; Priebe and Ferster 2008; Reichardt 1969; Sillito et al. 1981). Directional selectivity has received considerable attention over the past forty years and a variety of theoretical models have been proposed and numerous physiological experiments have been conducted to understand the underlying cellular and network mechanisms (Carver et al. 2008; Chance et al. 1998; Priebe and Ferster 2008; Reichardt 1987; 1969). One such mechanism is short-term synaptic depression: mathematical models that include differences in the time courses of short-term synaptic depression across a neuron’s receptive field can lead to directional responses (Carver et al. 2008; Chance et al. 1998).

Weakly electric fish are well suited for studying directional selectivity. This system benefits from well-characterized neural circuitry (Carr and Maler 1985; Heiligenberg 1991), the ability to achieve intracellular recordings CNS neurons in awake, behaving animals (Rose and Fortune 1996), and well-characterized behaviors that require perception of moving sensory images (Cowan and Fortune 2007; Nelson and MacIver 1999). In electrosensory perception, nearby objects produce spatiotemporal distortions of the animal’s self-generated electric field. These electric “images” are sensed by an electroreceptor array on the animal’s skin (Turner et al. 1999). As in the visual system, sensory images are transmitted to the receptor array at the speed of light and there is a direct correlation between object size / position and the image that is detected on the sensor array (Fortune 2006). Information from electroreceptors is transmitted through the hindbrain electrosensory lateral line lobe (ELL) that projects to the midbrain torus semicircularis (TS), a layered structure equivalent to the mammalian inferior colliculus. Previous studies have shown that ELL-TS synapses display short-term synaptic depression (Fortune and Rose 2000). Mathematical modelling studies, however, predict that synaptic depression alone is not sufficient to generate directional
selectivity: differences in the time course of short-term synaptic depression across the receptive field are necessary (Chance et al. 1998).

We examined the contribution of short-term depression to the generation of directional selectivity by stimulating small subregions of TS neuron receptive fields with stationary stimuli that are known to elicit short-term synaptic depression in these neurons (Fortune and Rose 2000). These stimuli elicited depression with different time courses in both the postsynaptic spiking and membrane potential responses at different locations within the receptive field. These differences in the time course of depression are strongly correlated with direction selectivity in TS neurons. Further, we used a mathematical model to explore the relations between short-term depression and directional selectivity. The directional selectivity exhibited by the model neuron, which used the spatial measures of short-term depression alone to predict direction selectivity, was significantly correlated with the experimentally measured directional selectivity.

Materials and Methods

Stimulation and recording

Adult specimens (12 – 18 cm length) of the Gymnotiform weakly electric fish, Apteronotus leptorhynchus, were used in this study. The surgical and experimental procedures have been described in detail elsewhere (Bastian et al. 2002; Chacron 2006; Chacron and Bastian 2008; Ramcharitar et al. 2006; Rose and Fortune 1996; Toporikova and Chacron 2009) and were approved by the Marine Biological Laboratory’s, McGill University’s, and the Johns Hopkins University’s Institutional Animal Care and Use Committees. Animal husbandry and all experimental procedures followed guidelines established by the Society for Neuroscience and the National Research Council. Fish husbandry and experimental design followed published recommendations (Hitschfeld et al. 2009).

Extracellular recordings from ELL pyramidal cells and TS neurons were made with metal-filled micropipettes (Frank and Becker 1964) as described previously (Bastian et al. 2002; Chacron 2006; Chacron and Bastian 2008; Toporikova and Chacron 2009). Intracellular recordings from TS neurons were made with patch pipettes using previously described techniques (Rose and Fortune 1996). Recordings were made in the first 5 layers of TS which receive direct input from the ELL (Carr and Maler 1985). Data were acquired with
a Cambridge Electronic Design Power1401 hardware and Spike2 software (Cambridge, UK). The majority of recordings were made at the normal resting potential (i.e. no current injection) or less than -0.1 nA continuous current injection.

Stimuli included moving objects (Ramcharitar et al. 2005; 2006) and electrical signals from both moving and stationary dipoles (Bastian et al. 2002; Chacron 2006; Chacron et al. 2003). The moving object consisted either of a 1.8 cm wide metal plate with a plastic coating on the side opposite to the animal or of a dipole emitting a constant stimulus (see below) that was actuated with a pen plotter (HP 7010B) and moved sinusoidally along the fish’s rostro-caudal axis over a distance of 20 cm. In both cases, the sinusoid was centered at the animal’s midpoint and had a frequency of 0.25 Hz. Therefore, the object moved a total of 40 cm over 4 sec, corresponding to an average velocity of about 10 cm/sec, which is the observed mean velocity of salient sensory images during prey capture behavior (Nelson and MacIver 1999) and within the velocities of error signals that the fish experience during refuge tracking behaviors (Cowan and Fortune 2007). These stimuli were repeated at least 20 times. We did not observe significant differences in directional selectivity using these two objects (data not shown).

Receptive fields from TS neurons were mapped by using a small dipole identical to the one used previously for ELL pyramidal cells (Bastian et al. 2002). The dipole was placed near the side of the animal (within 1 mm of the skin) at various rostro-caudal positions at the level of the midline and responses were accumulated at these positions. We used 4 Hz sinusoidal AMs to map receptive fields as done previously for ELL pyramidal cells (Bastian et al. 2002). This temporal frequency is also close to the principal temporal frequency component of movement during prey capture experiments (Nelson and MacIver 1999) and the obtained maps should therefore be useful in predicting directional selectivity to moving objects mimicking this behaviorally relevant situation.

We also used 20 Hz bursts to map TS neurons’ receptive fields. These stimuli consisted of 5 cycles of a 20 Hz sinewave (250 msec duration) followed by 750 msec silence repeated 300 times for extracellular recordings and at least 6 times for intracellular recordings. These stimuli are known to elicit short-term depression at ELL-TS synapses when presented via global stimulation geometry to the entire animal (Fortune and Rose 2000).
Stationary, localized stimuli were sinusoidal or constant amplitude modulations (AMs) of the animal’s own electric organ discharge (EOD) that were generated by multiplying EOD mimic waveforms with sinusoidal signals. The EOD mimic consisted of a train of single sinusoids of a duration slightly less than that of a single EOD cycle synchronized to the zero-crossings of the animal’s own EOD. ‘Contrasts’ (depth of the AM) were similar to those used in previous studies (~10-20%) (Bastian et al. 2002; Chacron and Bastian 2008; Chacron et al. 2005). The resulting signal was presented to the animal via the local dipole as has been done in previous experiments (Bastian et al. 2002) using a custom-made linear stimulus isolation unit (Fortune Laboratory Industries, Baltimore MD).

Data analysis

Data analysis was performed using Spike2 (Cambridge Electronic Design, Cambridge, UK), Origin (OriginLabs, Northampton, MA) as well as custom-made routines in Matlab (The Mathworks, Natick, MA). Intracellularly recorded membrane potentials were either low-pass filtered (40 Hz, FIR filter in Spike2) to remove the sodium action potentials or band-passed filtered (100-800 Hz, FIR filter in Spike2) to select the action potentials. Spiking responses were accumulated as peri-stimulus time histograms (PSTHs) and low-pass filtered membrane potentials were accumulated as average membrane potential waveforms during stimulus trials. The directional selectivity index was computed as:

\[ DSI = \frac{R_{HT} - R_{TH}}{\max(R_{HT}, R_{TH})} \]

where \( R_{HT} \) and \( R_{TH} \) are the responses to the moving object in the head-to-tail and tail-to-head directions, respectively. DSI ranges from -1 (the neuron only responds when the object moves in the tail-to-head direction) to 0 (the neuron responds equally to either direction) to 1 (the neuron only responds when the object moves in the head-to-tail direction). We quantified spiking responses as the peak firing-rate in the PSTH in each direction. We quantified the subthreshold membrane potential response with action potentials removed as the difference between the maximum and baseline values in each direction (spikes removed).

In order to determine possible errors in the DSI values obtained from our data. We ran simulations in a model that displays no directional selectivity: the rate dependent Poisson process where the rate was given by (in Hz): \( r(t)=20+40*\exp[-(t-0.5)^2/0.1] \) where \( t \in [0,1] \).
We chose a baseline rate of 20 Hz since it corresponds to the mean firing rate observed in our data sample. Here the time t is expressed in seconds. This process was simulated numerically in Matlab 300 times with binwidth $t_{bin}=1$ msec. Briefly, the content of each bin was set to zero and we generated a uniformly distributed random number between 0 and 1 for each bin: if that random number was less than the probability of obtaining a spike in this bin $p=20*0.001$, then a spike occurred in that bin. We set the value of bin i to $1/t_{bin}$ if a spike occurred in that bin and 0 otherwise. We pooled the 150 first trials and computed the PSTH from these binary sequences. Similarly, we pooled the 150 last trials and computed the PSTH from those binary sequences as well. We assumed that the former PSTH represents the response in the head-to-tail direction and the latter the response in the tail-to-head direction and computed the DSI as described above, we repeated this procedure 1000 times. Although in theory the DSI should be zero for this process, our numerical values were normally distributed around zero with mean $-0.014 \pm 0.019$ and standard deviation of $0.254 \pm 0.038$. If we instead looked at the distribution of the absolute value of DSI, we found that this distribution had a non-zero mean of $0.0936 \pm 0.0714$ whereas, in theory, we should obtain $|\text{DSI}|=0$. We therefore corrected for this bias when testing whether distributions of $|\text{DSI}|$ obtained from TS and ELL neurons were significantly different from zero. This was done by comparing each group of $|\text{DSI}|$ values to a surrogate sequence of $|\text{DSI}|$ values with the same length generated by the Poisson process using a Wilcoxon ranksum test.

We quantified the peak response, $R_1$, during the first stimulation cycle of a 250 msec long 20 Hz sinewave as either the peak firing rate for extracellular data or peak PSP amplitude for intracellular data. The peak response, $R_5$, was computed in the same fashion during the last cycle of the stimulus. The time constant $\tau$ was obtained by fitting the function $b + a \cdot \text{exp}[-(t-t_0)/\tau]$ to the data $[t_i, R_i]_{i=1,5}$ where $t_i$ is the time at which $R_i$ occurs, this was performed using the non-linear least squares fitting tool in Origin (OriginLabs, Northampton, MA). We identified the greatest and smallest $\tau$ across different positions and compute the difference (rostral-caudal) as a function of DSI.

Responses to sinusoidal stimulation delivered by a dipole located at different rostro-caudal positions were accumulated as phase histograms that were used to compute the gain $g$ and phase $\phi$ of the response at that position by fitting the function $R(t)=g \cdot \text{sin}(2\pi ft+\phi)$ to the phase histogram. We take $-\pi < \phi \leq \pi$ with $\phi=0$ corresponding to a local maximum of the sinusoid (Bastian et al. 2002).
We built X-T plots as described previously (Adelson and Bergen 1985) using either the responses to continuous 4 Hz sinusoidal stimulation or to 20 Hz bursts accumulated at different rostro-caudal positions. These positions were converted to a spatial phase with phase 0 being at the tip of the animal’s rostrum. For each neuron, we normalized the response to either of the largest value of gain \( g \) across rostro-caudal positions for sinusoidal stimuli. X-T plots for depression were generated in the following manner: the responses obtained to stationary “burst” stimuli at different positions were accumulated as PSTHs and fitted with an exponential decay. These fitted responses where normalized using the maximum response value and plotted as a function of time and position.

We computed Pearson’s correlation coefficient \( R \) and tested for statistically significantly different values from 0 using standard techniques covered in statistics textbooks (Newbold 2006). Briefly, the t-value is obtained from the correlation coefficient \( R \) and the sample size \( N \) by:

\[
    t = \frac{R}{\sqrt{1 - R^2 \frac{N-2}{N-2}}}
\]

from which one can perform a t-test to test for the hypothesis that the correlation coefficient is null.

**Modeling**

We built a mathematical model of a given TS neuron’s receptive field. This was achieved by discretizing the animal’s rostro-caudal axis into bins of length \( d = 5 \) mm. We always took \( x = 0 \) to be at the tip of the animal’s rostrum. For the model in which stationary sinusoidal stimuli were used, the response \( r_i(t) \) of bin \( i \) was given by \( G_i = g_i \Theta(t-t_i) \text{sign}(\phi_i) \) when the object is inside bin \( i \) and 0 otherwise. Where \( g_i, -\pi \leq \phi_i \leq \pi \) are the gain and phase obtained from sinusoidal stimulation experimentally, respectively. For the model with synaptic depression, the response \( r_i(t) \) of bin \( i \) was given by \( G_i = \Theta(t-t_i) g_i \exp(-t/\tau_i) \), where \( \tau_i \) is the depression time constant obtained from experimental data, when the object is inside bin \( i \) and 0 otherwise.

Here \( \Theta(\ldots) \) is the Heaviside function (\( \Theta(x) = 1 \) if \( x \geq 0 \) and \( \Theta(x) = 0 \) otherwise) and \( t_i \) is the time at which the object moving at a speed of 10 cm/sec first enters bin \( i \).
In both cases, the TS neuron was modeled as a passive leaky integrator:

\[ C \frac{dV}{dt} = -g_{\text{leak}}(V - E_{\text{leak}}) + A \sum_{i=1}^{N} R_i(t) + I \]

We used an Euler algorithm to numerically simulate the model with \( dt = 0.0025 \) msec. Other parameter values used were \( g_{\text{leak}} = 0.18 \) \( \mu \)S, \( E_{\text{leak}} = -70 \) mV, \( C = 1 \) nF, \( A = 1 \) nA, \( I = -1.1 \) nA.

**Results**

**TS Neurons exhibit directional selectivity**

We recorded the responses of 49 TS neurons *in vivo* to moving objects (Fig. 1). A moving bar stimulus was moved sinusoidally back and forth along the rostro-caudal axis of the animal (Fig. 1A). Most TS neurons in our dataset had directionally biased responses to this stimulus: the peak response was greater in one direction, termed “preferred”, than the opposite direction, termed “null” (Fig. 1B). We quantified this bias using the directional selectivity index (DSI), which ranges between -1 (the neuron responds only when the object moves in the tail-to-head direction) to 1 (the neuron responds only when the object moves in the head-to-tail direction). The distribution of DSI values obtained from our data were symmetric around 0 and displayed peaks near -0.6 and 0.6 (Fig. 1C), indicating that roughly 50% of neurons preferred the tail-to-head direction and the other 50% preferred the head-to-tail direction.

**ELL neurons that project to TS do not exhibit directional selectivity**

The simplest explanation for the directionally biased responses in TS is that they are inherited from the activity of afferent neurons. In weakly electric fish, electroreceptors on the skin surface respond to electrosensory stimuli and relay this information to pyramidal cells within the electrosensory lateral line lobe (ELL) (Maler 1979; Maler et al. 1991; Maler et al. 1981) (Fig. 2A): pyramidal cells are the sole source of ascending electrosensory input to TS. We recorded pyramidal cell responses to the same moving object stimulus. Responses to the preferred and null directions were similar in ELL neurons, indicating a lack of direction selectivity (Fig. 2B). In contrast, TS neurons typically showed differences in the responses to the two directions of movement (Fig. 2C). We looked at the distributions of the absolute
directional bias in both ELL and TS neurons and tested whether their means were statistically significantly different from 0. The average absolute directional bias in ELL ($|DSI|=0.1041 \pm 0.0108$) (Fig. 2D), was not significantly different than zero ($p=0.1791$, Wilcoxon ranksum test, $N=38$), indicating that ELL neurons are not directionally selective and confirming previous results (Bastian 1981). In contrast, the absolute directional bias in TS ($|DSI|=0.4213 \pm 0.0583$) was significantly greater than zero ($p<10^{-3}$, Wilcoxon ranksum test, $N=23$). These results show that the ELL is not the source of directionally biased responses in TS.

**Directional selectivity to moving objects in TS is not correlated with center-surround receptive field properties.**

We mapped the receptive fields of TS neurons using a small non-moving dipole that was placed at different locations along the side of the animal (Bastian et al. 2002) (Fig. 3A). TS neurons often displayed large receptive fields with center-surround organization. Interestingly, we found neurons that displayed identical responses to these stationary, spatially localized stimuli that were largely independent of location along the side of the animal (Fig. 3B). We used X-T plots (Adelson and Bergen 1985; Priebe and Ferster 2008) to characterize the receptive field properties of TS neurons. The X-T plot from the same neuron as in Figure 3 shows a response that is largely independent of position (Fig. 4A). Consequently, one would predict that this neuron would show weak or no directional selectivity. Another example neuron displayed a response to stationary stimuli that were strongly dependent on the dipole’s position (Fig. 4B): inspection of this map would suggest that this neuron should display a greater response when the object moves from left to right than vice versa.

The responses of these two neurons to the same dipole used as a moving stimulus are shown in Figures 4C and 4D, respectively. The first example neuron showed a strong directional bias while the second example neuron showed no directional bias, which is opposite to what was predicted from the RF maps. Although these two examples do not support a role of the center-surround organization of excitation and inhibition in the generation of direction selectivity, there may nevertheless be a correlation between direction selectivity and the center-surround organization when we look across our dataset. To address this issue, we then built a mathematical model that incorporated the experimentally measured responses to
stationary sinusoidal stimuli at different rostro-caudal positions for a given neuron (see Methods). We used this model to predict the DSI and compared it to the experimentally measured value for that neuron. We found no significant correlation between both quantities (R=0.3131, p=0.1363, N=24) across our data. These results strongly speak against the contribution of the center-surround receptive field properties to direction selectivity in TS neurons for moving stimuli whose velocities are close to those found in behaviorally relevant prey capture (MacIver et al. 2000; Nelson and MacIver 1999) and refuge tracking situations (Cowan and Fortune 2007). We note however that they do not preclude a putative involvement of center-surround characteristics in directional responses to objects moving at higher velocities.

*Differences in the time constants of depression within the receptive field are correlated with directional selectivity*

Previous modeling studies have shown that directional selectivity can be generated by differential temporal filtering of inputs from different zones within the neuron’s receptive field (Borst 2007; Borst and Egelhaaf 1989; Carver et al. 2008; Chance et al. 1998; Reichardt 1969). Two modeling studies suggest that short-term synaptic depression can be a potential source of differential temporal filtering of inputs (Carver et al. 2008; Chance et al. 1998). The mechanism by which different time constants of depression across the receptive field can give rise to directional selectivity in summarized in Fig. 5. In the simplest form, the model consists of two inputs from two spatially distinct zones within the receptive field that are associated with different time constants of depression: the resulting post-synaptic potential (PSP) from stimulating the left zone decays with a shorter time constant than the PSP resulting from stimulating the right zone (Fig. 5A). When stimulated with a moving object, this model gives rise to an output that depends on the direction of motion: movement from right to left gives rise to a compound PSP whose maximum value is greater than when the object moves from left to right (Fig. 5B). This can explained as follows: when the object moves from left to right, the fast PSP is elicited first and the slow PSP last, leading to very little overlap and little summation. In contrast, when the object moves from right to left, the slow PSP is elicited first and the fast PSP last, leading to greater overlap in time and thus greater summation, which leads to a greater maximum value (Carver et al. 2008; Chance et al. 1998).
To experimentally test the hypothesis that differences in short-term depression across a receptive field can contribute to the generation of direction selectivity, we again stimulated small subregions within a given neuron’s receptive field (Fig. 6A) with stationary, spatially localized electrosensory signals. In this experiment, the signals were transient 20 Hz oscillations, which are known to elicit short-term synaptic depression from midbrain neurons (Fortune and Rose, 2000).

Strongly directionally selective neurons typically displayed slowly decaying peak responses at one end and rapidly decaying responses at the other end of their receptive field (Fig. 6B). We quantified this depression in the responses by fitting an exponential to the peak response during each stimulus cycle (see methods). The resulting depression time constants were then superimposed with the responses (Fig. 6B). In contrast, non-directionally selective neurons did not show differences in the depression time constants at different locations in the receptive field and tended to show slower depression time constants (Fig. 6C). In fact, we found a strongly significant negative correlation between $|\text{DSI}|$ and the fastest depression time constant measured across the receptive field for our data ($R=-0.78$, $p<<10^{-3}$, $N=18$). We next explored the relationship between directional selectivity and differences in depression time constants across the receptive field: we found a strong positive correlation between DSI and the greatest difference in depression time constants across the receptive fields (Fig. 6D; $R=0.77$, $N=18$, $p<10^{-3}$).

To further examine the relations between the spatial arrangement of short-term depression within the receptive field and direction selectivity, we computed X-T plots using the experimentally measured depression time constants at several different locations within the receptive field of a TS neuron. These were obtained by plotting the exponential fit as a function of time and position. An example X-T plot from a neuron that displayed slow/fast depression in the rostral/caudal parts of its receptive field show a strong asymmetry that would suggest that this neuron was directionally selective for movement from spatial phase 0 to spatial phase 360 (i.e. head-to-tail direction) (Fig. 7A). This neuron did indeed show a strong directional bias in the head-to-tail direction with DSI=0.667. In contrast, an X-T plot from a neuron that displayed fast depression at its receptive field edges separated by slower depression near the middle shows relative symmetry in the X-T plot that suggests weaker direction selectivity (Fig. 7B). This neuron showed much weaker directional selectivity with DSI=0.13.
To quantitatively assess whether the differences in depression time constants measured across the receptive field could predict the directional responses of TS neurons, we built a mathematical model that consisted of passive integration of convergent inputs from across the receptive field (Fig. 7C). We performed numerical simulations using this passive model with the depression time constants obtained experimentally. The role of the differences in short-term depression in generating direction selectivity was supported by a strongly significant correlation between the predicted directional bias from the model and experimentally obtained measures ($R=0.88$, $N=18$, $p<10^{-3}$).

Sources of depression and direction selectivity: comparisons of extracellular spiking activity and intracellular PSPs

What is the source of depression in the spiking response? To answer this question, we performed intracellular recordings from TS neurons and compared their spiking and subthreshold membrane potential responses to stationary stimuli. These recordings revealed that the depression in the spiking response to stationary local stimuli was largely due to depression of the underlying PSP amplitude: more or less pronounced depression in the spiking response was accompanied by more or less pronounced depression in the subthreshold membrane potential response (Figs. 8A and 8B). As has been described previously (Rose and Fortune 1999), the amplitudes of PSPs elicited by these depressing stimuli typically varied from cycle to cycle, and in most neurons showed a weak oscillation.

We quantified the decay in the PSP amplitude by fitting an exponential function to the peaks of the response (see experimental procedures). We observed a strong correlation between the depression time constants obtained from the membrane potential and spiking responses (Fig. 8C, $R=0.81$, $p<10^{-3}$, $N=13$). Furthermore, the data were not significantly different from the identity line ($p=0.80$, Wilcoxon ranksum test, $N=13$) indicating that depression seen in the spiking activity is due to depression of PSP amplitudes. The time constants appear to form two clusters – a fast cluster around 10 ms and a longer cluster with values in the 100s of ms. In vitro recordings will be necessary to investigate the mechanisms underlying these differences, which may have important functional consequences (see Discussion).
In the same light, we also quantified the directional bias in the membrane potential and compared it to the directional bias in the spiking response to moving objects (Fig. 8D) and found a strong positive correlation between both quantities ($R=0.99$, $N=18$, $p<<10^{-3}$) that was not significantly different from the identity line (Fig. 8E, $p=0.38$, Wilcoxon ranksum test, $N=18$). These data show that the membrane potential response already contains all the directional information and further supports that differences in the short-term depression time constants across the receptive field of TS neurons mediate their directionally biased responses and justifies the use of a linear integrator for the mathematical model.

Source of short-term depression in TS

What are the mechanisms underlying the observed differences in short-term depression time constants in TS? One possibility is that these differences originate in the presynaptic patterns of action potentials of ELL neurons: input from different subregions within the receptive field could come from ELL neurons with different adaptation in their spiking responses. The ELL is composed of three parallel maps of the body surface that all project to TS (Carr and Maler 1985; Krahe et al. 2008; Shumway 1989a; b). A recent study of the differential temporal filtering properties of ELL neurons in all three maps has revealed adaptation time constants within the ranges of depression time constants observed in TS neurons (Krahe et al. 2008): neurons within the centro-medial segment (CMS) had the longest (>200 msec) adaptation time constants whereas neurons within the lateral segment (LS) had the shortest (<20 msec) adaptation time constants.

Since strongly directionally selective TS neurons had both short and long depression time constants, this would imply that they should receive input from both the CMS and LS maps of the ELL. However, as weakly directionally selective TS neurons had longer depression time constants on average, they should then preferentially receive inputs from the CMS map. Activity in these ELL maps also differ in another overt way: LS neurons displayed strong phase locking to the EOD whereas CLS and CMS neurons do not (Krahe et al. 2008).

We therefore quantified the degree of phase locking to the EOD to examine the contributions of the maps to the responses of individual TS neurons. While some neurons displayed strong phase locking to the EOD, suggesting that they receive input from LS, these neurons tended to have the least directional selectivity. Indeed, there was a significant negative correlation
between phase locking to the EOD and directional selectivity ($R=-0.57$, $p=0.0045$, $N=23$), indicating that neurons that showed the most directional selectivity tended to show the least phase locking to the EOD. These results speak against a possible contribution of the computational features found across ELL maps for the generation of directional selectivity in TS. Nevertheless, because we do not know the relative distribution of depressing and non-depressing efferent synapses from particular maps and/or classes of ELL neurons, we currently cannot assess particular hypothetical functional organizations of inputs to TS neurons. Future experiments, including direct stimulation of individual ELL maps and in vitro studies, will be necessary to assess the relative contributions and functional organization of ELL efferents to the TS.

**Discussion**

**Summary of results**

We investigated the directional responses of TS neurons in a species of weakly electric fish, *Apterонотус leptorrhynchus*, to moving electrosensory objects. Most neurons in the TS were directionally selective, with roughly an equal distribution of neurons that preferred movement in the head-to-tail direction and tail-to-head directions. In contrast, neurons in the ELL, which are the sole source of ascending electrosensory information to TS neurons, did not exhibit directional selectivity. Therefore, directional selectivity emerges at the level of the TS.

We first investigated whether differential steady-state filtering across the receptive field of TS neurons (e.g. center-surround) could account for their directionally biased responses. Although many TS neurons displayed center-surround organization that could theoretically underlie to direction selectivity, just as many TS neurons with strong directional selectivity did not exhibit center-surround receptive field organization. We built a simple mathematical model that incorporated the responses at different rostro-caudal positions and used it to predict the directional bias for each neuron in the study. We did not find any significant correlation between predicted and experimentally measured directional selectivity in relation to center-surround organization of receptive fields.

This led us to investigate whether differential filtering of transient stimuli across the receptive field could account for directional selectivity in TS neurons. We found that some TS neurons...
could display large variations in depression time constants to transient stimuli across their receptive fields. We then built a mathematical model incorporating the depression time constants measured at different rostro-caudal positions and used this model to predict the directional bias for a given neuron. We found a strong and significant correlation between the predicted and experimentally measured directional selectivity in relation to differences in short-term depression across the receptive field.

Intracellular recordings then revealed that depression in the spiking response was caused by depression of PSPs in the subthreshold membrane potential response. The directional bias of the subthreshold membrane potential was in fact equal to that of the spiking response. Finally, our data speaks strongly against ELL pyramidal cell heterogeneities being the cause for directional selectivity in TS neurons.

Short-term depression as a mechanism for generating a directional bias

Previous modelling studies have shown that differential temporal filtering of input coming from different areas within the receptive field is sufficient to give rise to a directionally biased response properties (Reichardt 1969). This differential temporal filtering could come from a multitude of mechanisms including, but not restricted to, delay lines (Reichardt 1969), differential excitation and inhibition, and short-term depression (Carver et al. 2008; Chance et al. 1998). Our results show experimentally, for the first time, that the differential temporal filtering created by short-term depression correlates with directional biases. The most likely source of differences in short-term depression is short-term synaptic depression: homosynaptic depression has been demonstrated to occur in ELL to TS synapses (Fortune and Rose 2000).

This conclusion is supported by the fact that differences in the ELL segments do not seem to contribute to directional selectivity in TS. Further, the depression time constants that we measured appear to form two clusters (Figure 7C), suggesting that there may be two classes of ELL-TS neuron synapses: one group displays fast short-term depression on the ~10 msec timescale while the other displays slower short-term depression on the ~100-300 msec timescale. Further studies preferably conducted in vitro are however necessary to test this hypothesis. In Eigenmannia virescens, short-term depression in TS neurons were typically best fit by a double exponential with time constants on the order of 10s of milliseconds and seconds (Fortune and Rose 2000). This is consistent with results obtained in cortical neurons.
(Varela et al. 1997). Further, a modelling study suggests that these two clusters of time
constants could serve two functions – the shorter time constant mediating direction selectivity
and the longer time constant mediating an enhancement of direction selectivity (Carver et al.
2008).

The evidence for the involvement of short-term synaptic depression in generating directional
selectivity in TS neurons presented here is correlative – the phenomenon is only understood
across the population of neurons in the study. In the best of cases, we would be able to
manipulate short-term synaptic depression in a single recording and directly show an effect
on direction selectivity. At present, however, this is not possible in the intact organism.
Short-term synaptic depression at ELL-TS synapses is believed to be presynaptic (Fortune
and Rose 2000), and therefore it is currently impossible to block it with pharmacological
agents. Further, because short-term synaptic depression is an activity-dependent process, any
manipulation of the afferent activity that would affect short-term synaptic depression will
also necessarily involve a change in the spatiotemporal pattern of action potentials arriving at
the direction selective neuron. This is problematic because the direction selective
computation is performed on the spatiotemporal pattern of activity arriving at the TS neuron.
Nevertheless, our correlative evidence involves several methods of analysis (namely the
greatest difference in time constants across the receptive field, X-T plots, and a mathematical
model incorporating the experimentally measured depression time constants) and matches
previous predictions from modelling studies (Carver et al. 2008; Chance et al. 1998).

Comparing the mechanisms of directional selectivity across species

Differences in temporal filtering of input coming from different zones within the receptive
field lead to directionally biased responses (Reichardt 1969). It is therefore not surprising that
the mechanisms that mediate directional selectivity to movement are quite diverse and vary
across sensory modalities and species. In fact, these can take the form of different low-pass
filters as observed in insect visual system (Borst 2007; Borst and Egelhaaf 1989), different
dendritic integration in rabbit retinal ganglion cells (Taylor et al. 2000; Yang and Masland
1992), and differential delays in mammalian visual cortex (Jagadeesh et al. 1993; Jagadeesh
et al. 1997). These mechanisms could be used in other spatiotemporal computations. For
example, selectivity to ascending or descending frequency modulated acoustic sweeps has
been observed in mammalian inferior colliculus (IC) and auditory cortex (Fuzessery and Hall
1996; Fuzessery et al. 2006; Razak and Fuzessery 2008; 2006; Suga 1965). In particular, it

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has been shown that three mechanisms, facilitation, sideband inhibition, and duration tuning, mediate directional selectivity in the auditory system. That TS and IC are homologous structures make comparisons between directional selectivity to movement in TS and directional selectivity to frequency sweeps in IC particularly interesting. Previous studies have shown the presence of facilitation in ELL-TS synapses (Fortune and Rose 2000) but further studies are needed to ascertain its potential role in directional selectivity in TS.

Conclusion

We have provided the first experimental demonstration that differential time constants of short-term depression across the receptive field are correlated with responses to a moving stimulus. This mechanism is ubiquitous in vertebrate neural circuits (Boudreau and Ferster 2005) and may thus contribute to directional selectivity and other spatiotemporal computations across sensory modalities and species.
Figure Legends

Figure 1: TS neurons display directional selectivity. A) Schematic of the moving object stimulus. A metal bar with plastic backing was moved back and forth along the side of the animal’s body. Black indicates tail-to-head movement and gray head-to-tail. B) PSTH of a TS neuron to the moving object. This neuron display a greater peak firing rate when the object moved in the head-to-tail direction (preferred) as opposed to the tail-to-head direction (null). C) Distribution of DSI values for our data sample. The distribution did not have a mean that is significantly different than zero (p=0.1149, sign-test, N=49) but did display peaks at around -0.6 and 0.6.

Figure 2: Directional selectivity emerges at the level of the TS. A) Primary electrosensory receptor afferents converge unto ELL neurons, which in turn project onto TS neurons. B) Population-averaged responses in the preferred and null directions were roughly equal for ELL neurons. C) Population-averaged responses displayed a greater maximum in the preferred direction for TS neurons. D) Population-averaged |DSI| values were significantly different from zero for TS neurons but not for ELL neurons (see text for details).

Figure 3: Mapping the receptive field properties of TS neurons. A) Schematic of the experimental setup: a small dipole (black lines) is positioned at different rostro-caudal positions (black dots) throughout the receptive field of the TS neuron (gray area). B) Responses to 4 Hz sinusoidal stimulation were accumulated at each position (in mm) and are shown as cycle histograms: these were used to compute the gain $G_i$ and phase $\phi_i$ at that position. Responses of this particular neuron to sinusoidal stimulation were largely independent of the dipole’s position.

Figure 4: Center-surround organization does not predict directional selectivity in TS neurons. A) Receptive field map from the same neuron as in Figure 3 showing a response that is largely independent of position within the receptive field. One would predict weak directional selectivity from this map as can be seen by comparing the responses to moving stimuli in both directions as represented by the white arrows. B) Receptive field map from another example neuron that showed a strong center-surround receptive field organization. In contrast, one would predict strong directional preference going from spatial phase 0 to spatial
phase 360 deg (i.e. head-to-tail motion) for this neuron by comparing the responses to moving stimuli in both directions as represented by the white arrows. C) Response of the same neuron as in A) to the dipole moving: this neuron showed significant directional selectivity that does not fit the prediction from the receptive field map. D) Response of the same neuron as in B) to the dipole moving: in contrast, this neuron showed no significant directional selectivity, which again does not fit the prediction from the receptive field map.

Figure 5: Summary of the mechanism by which different time constants of depression can give rise to directional selectivity. (A) Model diagram in the case where inputs from two spatially distinct zones within the receptive field are differentially processed with two different time constants of depression and give rise to post-synaptic potentials (PSPs) with different temporal profiles. Stimulating the left zone gives rise to a PSP that decays with a fast time constant (10 msec) while stimulating the right zones gives rise to a PSP that decays with a slow time constant (100 msec). The model’s output is taken to be the sum of these two inputs. (B) Model output when an object moves from right to left (black) and from left to right (gray) across the receptive field. The height of the compound PSP is given by $R_p$ and $R_n$ when the object moves from right to left and from left to right, respectively.

Figure 6: Directionally selective TS neurons display variations in their short-term depression to stationary stimuli delivered at different spatial locations within the receptive field. A) A spatially localized stimulus is delivered via a dipole (black lines) that stimulates small regions (black and light gray dots) within the receptive field (dark gray area) of a TS neuron. The two example stimuli were delivered at two different locations and are shown in red and blue. B) Response of a directionally selective TS neuron to a stationary 20 Hz sinusoidal stimulus delivered rostrally at the gray position (left) and black positions (right) with best exponential fits. Note that the response at the black position shows more pronounced decay and thus gives rise to a lower decay time constant. C) Response of a non-directionally selective TS neuron to the same sinusoidal stimulus at the red position (left) and the blue position (right). Note that both responses have similar time courses and thus similar time constants of decay. D) The directional bias was strongly correlated with the difference in decay time constants measured from stationary stimuli across different rostro-caudal positions. ($R=0.77$, $N=15$, $p<10^{-3}$).
**Figure 7: Differences in time constants of depression across the receptive field correlate with the observed directional bias.** A) Example X-T map of depression across the receptive field from a strongly-directionally selective neuron. This map was obtained by plotting the exponential fits obtained at different rostro-caudal positions as a function of time (y-axis) and position (x-axis). The directional selectivity can be assessed by comparing the responses to moving stimuli in both directions as represented by the white arrows. B) Example X-T map from a weakly-directionally selective neuron. The directional selectivity can again be assessed by comparing the responses to moving stimuli in both directions as represented by the white arrows. C) Schematic of a model for the generation of direction selectivity using depression time constants measured across rostro-caudal positions along the animal. Yellow is a receptive field in which 5 measurements of the time constant of depression were made (green dots, $\tau_1 - \tau_5$). In the model, we sequentially stimulated the subregions of the receptive field in both directions (red and blue arrows). This information was transmitted to a post-synaptic neuron through model synapses. The short-term depression at these synapses matched experimentally obtained measures. The direction selectivity of a summing post-synaptic neuron (orange) was measured.

**Figure 8: TS neurons display similar characteristics in their spiking and membrane potential responses.** A) Comparison between the spiking (top) and subthreshold membrane potential (bottom) responses from a directionally selective TS neuron. It is seen that the depression in the spiking response is matched by a depression (i.e. a decrease in the PSP amplitude) in the subthreshold membrane potential. B) Comparison between the spiking (top) and subthreshold membrane potential (bottom) responses from the same neuron when stimulated at a different location within the receptive field. The faster depression in the spiking response is matched by a faster depression of the PSP amplitude in the subthreshold membrane potential response. C) Time constants of depression obtained from spiking responses and membrane potential responses to stationary stimuli at different spatial locations within the receptive field are strongly correlated ($R=0.81$, $p<10^{-3}$, $N=13$) and equal in magnitude as most points lied on the identity line (solid line). This indicates that the depression of the spiking response is mediated by depression of the subthreshold membrane potential response. D) Comparison of the spiking (top) and subthreshold (i.e. with spikes removed) membrane potential (bottom) responses of the same neuron to a moving object.
stimulus. It is seen that increases in firing rate are accompanied by strong membrane
depolarizations and that both responses display similar directional biases. E) DSI values
obtained from the spiking response were strongly correlated to the DSI values obtained from
the subthreshold membrane potential response across our data sample (R=0.99, N=18, p<<10−3). Moreover, all points lied across the identity line (solid line) indicating that the directional
selectivity of the spiking response is equal in magnitude to the directional selectivity present
in the subthreshold membrane potential response.

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Figure 1, Chacron et al.

Panel A: Image of a fish with gray and red arrows indicating spatial phase.

Panel B: Bar graph showing firing rate (Hz) against spatial phase (deg). X-axis: spatial phase (deg) from 0 to 300, Y-axis: firing rate (Hz) from 0 to 20.

Panel C: Bar graph showing # of counts against DSI. X-axis: DSI from -1 to 1, Y-axis: # of counts from 0 to 8.
**Figure 2, Chacron et al.**

A. Diagram showing the flow of receptors to ELL to TS.

B. Graph showing firing rate (Hz) over time (msec) with preferred and null responses.

C. Graph showing firing rate (Hz) over time (msec) for TS with preferred and null responses.

D. Bar graph showing $|DSI|$ for TS and ELL with significance indicated by **.
Figure 3, Chacron et al.
Figure 4, Chacron et al.

A

Normalized response

Temporal phase (deg)

Spatial phase (deg)

B

Normalized response

Spatial phase (deg)

C

Firing rate (Hz)

Time (sec)

Preferred

Null

|DSI|=0.49

D

Firing rate (Hz)

Time (sec)

Preferred

Null

|DSI|=0.07
A  Receptive field

\[ \tau = 10 \text{ msec} \quad \tau = 100 \text{ msec} \]

B

\[ R_p, R_n \]

150 msec

preferred, null

Figure 5, Chacron et al.
Figure 6, Chacron et al.

\[ \tau = 138 \text{ msec} \]

\[ \tau = 54 \text{ msec} \]

\[ \tau = 323 \text{ msec} \]

\[ \tau = 345 \text{ msec} \]
Figure 7, Chacron et al.
Figure 8, Chacron et al.