Peroxidase activity was determined by following the oxidation of guaiacol for 1 min at 470 nm and was standardized by total protein content as previously described.

**Bioassay with P. syringae pv. tomato DC3000**

Fifteen replicates of each line were challenged by infiltration of three leaves with 10^9 colony-forming units per ml (D_50 = 0.902) after 3 weeks of plant growth. After 5 days, leaf samples were removed, ground and plated on KB medium to determine the concentration of bacteria.

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**letters to nature**

**Non-classical receptive field mediates switch in a sensory neuron’s frequency tuning**

**Maurice J. Chacron†, Brent Doiron†, Leonard Maler*, André Longtin† & Joseph Bastian‡**

Animals have developed stereotyped communication calls to which specific sensory neurons are well tuned. These communication calls can be discriminated from environmental signals such as those produced by prey. Sensory systems might have evolved neural circuitry to encode both categories. In weak electric fish, prey and communication signals differ in their spatial extent and frequency content. Here we show that stimuli of different spatial extents mimicking prey and communication signals cause a switch in the frequency tuning and spike-timing precision of electrosensory pyramidal neurons, resulting in the selective and optimal encoding of both stimulus categories. As in other sensory systems, pyramidal neurons respond only to stimuli located within a restricted region of space known as the classical receptive field (CRF). In some systems, stimulation outside the CRF but within a non-classical receptive field (nCRF) can modulate the neural response to CRF stimulation even though nCRF stimulation alone fails to elicit responses. We show that pyramidal neurons possess a nCRF and that it can modulate the response to CRF stimuli to induce this neurobiological switch in frequency tuning.

The complex statistical structure of many naturalistic visual and auditory stimuli makes our interpretation of neural responses to these stimuli difficult and often prevents clearcut correlations of the responses with behaviour. Weakly electric fish offer a simple system for studying the differential encoding of natural stimuli because there is a clear spatiotemporal distinction between prey and communication stimuli. Amplitude modulations (AMs) of the electric fish’s self-generated electric organ discharge (EOD) contain information relevant to both types of stimulus. Epidermal electroreceptors encode these AMs precisely and provide synaptic input to pyramidal neurons of the electrosensory lateral line lobe (ELL), whose antagonistic centre–surround CRF structure resembles that of visual neurons. Relative motion of the fish near prey during feeding produces low-frequency (less than 10 Hz) spatially localized AMs. However, communication signals from conspecifics produce high-frequency (more than 50 Hz) spatially diffuse AMs.

To provide naturalistic stimuli that mimic prey and communication signals, we used two stimulation geometries (see Methods). Local stimulus geometry provides AMs whose spatial extent is similar to that produced by prey, while global stimulus geometry produces spatially diffuse AMs similar to communication signals.

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(Fig. 1a, b). We investigated the frequency response properties of ELL pyramidal neurons by constructing EOD AM frequency tuning curves. Changing the stimulus geometry from local to global causes a shift in response preference from low to high frequencies (Fig. 1c; compare blue with red). We quantified this shift by computing the frequencies associated with the maximum vector strength (see Methods) under each stimulus geometry. These averaged 4.67 ± 2.73 Hz and 53.33 ± 16.52 Hz, respectively (mean change of 48.66 Hz, P = 0.0004, pairwise t-test, n = 7). Plots of the stimulus–spike train coherence obtained from random AM stimulation (see Methods) showed a qualitatively similar effect (Fig. 1d; compare blue with red). The average coherence values $C_{L}$ between 0 and 20 Hz and $C_{H}$ between 40 and 60 Hz were also computed. With global stimulus geometry, high-frequency coherence was significantly greater than low-frequency coherence ($P < 10^{-3}$, t-test, n = 17), but with local stimulus geometry the opposite frequency preference was seen ($P < 10^{-3}$, t-test, n = 17). Firing rates did not change as we went from local (23.12 ± 10.86 spikes s$^{-1}$) to global (23.24 ± 11.82 spikes s$^{-1}$) geometry ($P = 0.9$, pairwise t-test, n = 17). This confirms that ELL pyramidal neurons behave as low-pass filters when stimulated locally, whereas they exhibit high-pass characteristics when stimulated globally.

We used information theoretic measures (see Methods) to quantify the consequences for stimulus encoding of this shift in temporal frequency tuning. We compared results obtained with locally and globally applied low-frequency (0–20 Hz) and high-frequency (40–60 Hz) random AMs. These results are summarized in Table 1. High-frequency global stimuli (communication-like) were encoded much better (about 200%) than high-frequency local stimuli. Moreover, low-frequency local stimuli (prey-like) were encoded much better (about 100%) than low-frequency global stimuli. These results demonstrate that pyramidal neurons show improved stimulus encoding when the combination of temporal frequency content and spatial stimulus characteristics closely mimic those of natural stimuli.

The higher mutual information rates, indicating an increased signal-to-noise ratio, obtained with high-frequency global stimuli as compared with high-frequency local stimuli indicate a possible dependence of spike train variability on the spatial extent of the stimulus. Neurons can display low trial-to-trial variability to repeated stimuli both in vitro and in vivo. Using ‘frozen noise’ (see Methods), we explored the reliability of spike timing displayed by ELL pyramidal neurons. Results show high trial-to-trial variability under local stimulus geometry (Fig. 2a) and low trial-to-trial variability under global stimulus geometry (Fig. 2b). The average reliability of spike timing, measured as the fraction of spikes occurring reproducibly during high-frequency ‘events’, increased from 0.16 ± 0.17 under local stimulation to 0.72 ± 0.12 with global stimulation ($P = 0.004$, pairwise t-test, n = 7). The mean spike time precision (average standard deviation of spike times within ‘events’) decreased from 1.44 ± 0.16 ms under local stimulation to 1.08 ± 0.23 ms with global stimulation ($P = 0.009$, pairwise t-test, n = 7). Pyramidal neurons display high reliability and high spike timing precision to stimuli mimicking the frequency content of communication signals, but only when the stimulus is presented globally. Such timing precision does not occur for low-frequency stimuli (data not shown), presumably because accurate encoding of low-frequency stimuli does not require such high temporal precision.

**Table 1** Summary of results with different information-theoretic measures

<table>
<thead>
<tr>
<th></th>
<th>Local</th>
<th>Global</th>
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<tbody>
<tr>
<td>High frequency</td>
<td>$I = 0.08 ± 0.08$</td>
<td>$I = 0.25 ± 0.17$</td>
</tr>
<tr>
<td>Low frequency</td>
<td>$I = 0.29 ± 0.28$</td>
<td>$I = 1.14 ± 0.52$</td>
</tr>
<tr>
<td>Statistical significance</td>
<td>$P = 0.0031$ (n = 12)</td>
<td>$P = 0.0035$ (n = 12)</td>
</tr>
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The coding fraction, $I$, is the fraction of the stimulus waveform correctly estimated by the neuron; the mutual information rate, $I$, in bits per spike gives the amount of information transmitted by the neuron by each spike. $P_{L}$ and $P_{H}$ are the respective $P$ values obtained from a pairwise t-test comparing the $I$ values obtained with high-frequency (40–60 Hz) stimuli with those obtained with low-frequency (0–20 Hz) stimuli.
Anatomical studies\textsuperscript{17,19} predict that local and global stimulation will activate different constellations of synaptic inputs to ELL pyramidal neurons. If these differing synaptic inputs cause the shift in frequency tuning, this should be reflected in the cell’s membrane potential. We recorded intracellularly from pyramidal neurons to test this hypothesis. Figure 2 shows the membrane potential response under local (Fig. 2c) and global (Fig. 2d) stimulation geometry with random AM stimulation. The membrane potential response (black) does not track the higher-frequency components of the stimulus (blue) during local stimulation but does so during global stimulation. We emphasize this point by computing the coherence between the membrane potential response with spikes removed (green) (see Methods) and the stimulus (Fig. 2e, f). For local stimulus geometry, \( C_{\text{low}} \) was significantly greater than \( C_{\text{high}} \) (\( P = 0.024, t\text{-test, } n = 6 \)) whereas the opposite was true for global stimulus geometry (\( P = 0.012, t\text{-test, } n = 6 \)). These results show that the change in frequency tuning is seen in the membrane potential response itself and thus probably originates from differing synaptic inputs under local and global stimulus geometries.

We have previously mapped the antagonistic centre–surround CRF organization of ELL pyramidal neurons\textsuperscript{8}. Local stimuli affect only a fraction of the CRF centre (Fig. 1a) whereas global stimuli influence the entire CRF as well as the nCRF (Fig. 1b). In the visual system, the nCRF is known to modulate CRF centre responses\textsuperscript{7,8}. To test for the presence of nCRF effects and their possible role in pyramidal neuron frequency tuning shifts, we performed partition experiments (see Methods) illustrated in Fig. 3a. The thin rubber partition electrically isolated the fish’s head region from its trunk region, allowing each to be independently stimulated. We recorded from pyramidal neurons whose CRF centre was sufficiently distant from the partition to ensure that the CRF was entirely within the trunk region. Thus, stimuli applied to the head region influenced the responsiveness of the recorded cell only through the nCRF. Local stimulation of the CRF centre alone with 0–60 Hz random AMs produced results identical to those obtained under local stimulus geometry without a partition (compare Figs 1d and 3b, blue). We then paired the local CRF centre stimulation with in-phase global stimulation of the head chamber (Fig. 3b, red). Simultaneous stimulation of the nCRF decreased the cell’s response to low frequencies only. The measure of low-frequency coherence, \( C_{\text{low}} \), was significantly decreased (\( P < 10^{-3}, \) pairwise \( t\text{-test, } n = 15 \)). This decrease is similar to that seen in the transition from local to global geometries. However, nCRF stimulation phase-shifted by 180° relative to the CRF had no effect on coherence (Fig. 3b, green); there was
no significant change in $C_{\text{low}}$ and $C_{\text{high}}$ ($P = 0.36$ and $0.16$ respectively, pairwise t-tests, $n = 19$). Stimulation of the head region alone was ineffective in driving the recorded cell (Fig. 5b, black).

The cells’ firing rates decreased by 3.9 and 2.1 spikes $s^{-1}$ on average under in-phase and out-of-phase paired stimulation of CRF plus nCRF, respectively ($P = 0.003/0.001$, pairwise t-tests, $n = 15$). Because, in each case, the neurons received the same amount of primary afferent excitation by means of the CRF centre, the cells must be inhibited by nCRF stimulation. Furthermore, because the nCRF stimulus must be in phase with the CRF stimulus to decrease a neuron’s response to low frequencies, the inhibition must act on a moderately fast timescale. Paired stimulation of the CRF centre plus the nCRF in phase did not on average alter high-frequency response because $C_{\text{high}}$ did not change significantly with in-phase nCRF stimulation ($P = 0.58$, pairwise t-test, $n = 15$).

Either global stimulus geometry or paired stimulation of the nCRF plus the CRF centre in the partition experiment reduced low-frequency coherence. However, only global stimulation (no partition) resulted in an improvement in high-frequency coherence. Global geometry not only results in nCRF stimulation but also ensures that the entire CRF centre is stimulated (spatial saturation). Hence, the complete shift in frequency tuning seen with the transition from local to global geometry (Fig. 1c, d) might require both stimulation of the nCRF as well as spatial saturation of the CRF centre. To test the latter hypothesis we repeated the partition experiment but increased the CRF centre area influenced by the local stimulus by adding a second dipole (Fig. 3c; see Methods). As shown in Fig. 3d (compare blue and red), addition of the second dipole increased $C_{\text{high}}$ by about 70% ($P = 0.0027$, pairwise t-test, $n = 7$). Thus, combining saturation of the CRF centre with in-phase nCRF stimulation is sufficient to induce both the increase in the cell’s high-frequency response and the decrease in its low-frequency response (Fig. 3d, green) just as in the normal transition from local to global geometry. Stimulation of the CRF surround did not alter the frequency tuning to stimulation of the CRF centre (data not shown).

We have demonstrated that ELL pyramidal neurons can switch their tuning properties on the basis of the spatial extent of a stimulus. Specifically, pyramidal neurons responded maximally to temporal frequencies below 10 Hz under spatially local stimulation and to frequencies over 50 Hz under spatially global stimulation. These responses are well matched to the observed temporal frequency content of prey and communication stimuli, respectively. Maximum information transfer was obtained when the stimulus matched the spatiotemporal content of prey and communication stimuli. In particular, high information transfer was obtained for high-frequency global stimuli. This differs from previous results that showed poor information transfer under a global-like geometry. However, both previous studies used mainly low-frequency stimuli, to which pyramidal neurons respond poorly under global stimulation.

Intracellular recordings revealed that a change in synaptic input was probably responsible for the change in frequency tuning. The reduced response to low frequencies under global stimulation is due primarily to nCRF stimulation, probably acting through inhibition because a decrease in firing rate was observed. This decreased response is thus unlikely to occur with global yet spatially heterogeneous environmental stimuli such as those caused by a root mass, because it requires spatially homogeneous communication-like signals. ELL pyramidal neurons receive inhibition from several sources. Inhibition is known to modulate neural frequency tuning and can lead to oscillations and synchrony in a neural population. We have recently shown that ELL pyramidal neurons displayed inhibition-mediated oscillatory dynamics under global stimulation but not under local stimulation. The emergence of this oscillation can sometimes accompany the shift in frequency response found here, but is not required to induce it (data not shown).

Our study shows that the structure of the receptive field of ELL pyramidal neurons is well adapted to categorical coding of their natural stimulus environment. This is achieved through the differential spatial extent of prey and communication stimuli that will differentially activate the structure of the receptive field of pyramidal neurons. Prey-like stimuli activate only a fraction of the CRF centre, whereas communication stimuli spatially saturate the CRF centre and also activate the nCRF, thus producing different synaptic input from that for prey stimuli. This neurobiological switch in synaptic input allows pyramidal neurons to encode both prey and communication stimuli optimally.

**Methods**

**Stimulation and recording**

The experimental protocol has been described previously. The stimuli consisted of three types of band-limited random AMs of an animal’s own EOD presented with local or global geometry. When two local stimulus dipoles were used, the same stimulus waveform was fed to independent stimulus isolation units, each of which drove one dipole. Recordings were limited to E-type pyramidal neurons of the centrolateral and lateral pyramidal areas. Prey-like stimuli activate only a fraction of the cells required for the jamming avoidance response evoked by low-frequency global stimuli, is not considered here. Extracellular single-unit recordings were made with metal-filled microelectrodes, and intracellular recordings were made with 40–100 MΩ KCl-filled micropipettes. Standard methods of preamplification were used and data were acquired with Cambridge Electronic Design 1401 plus hardware and Spike2 software. All surgical procedures were performed in accordance with the University of Oklahoma animal care and use guidelines.

**Data analysis**

 Spike trains during sinusoidal AM stimulation were accumulated as cycle histograms and the responses was quantified by the vector strength, which measures the degree of phase locking and ranges between 0 (no phase locking) and 1 (perfect phase locking). Responses to random AMs were analysed by computing the coherence, $C(f)$, between the spike train and stimulus, where $C(f) = |P_{xx}(f)|^2 / (P_{xx}(f)P_{ss}(f))$. $P_{xx}(f)$ and $P_{ss}(f)$ are the power spectra of the stimulus and spike train respectively, and $P_{ss}(f)$ is the cross-spectrum between the stimulus and the spike train. $C(f)$ ranges from 0 to 1 and indicates the strength of the response to the stimulus at a frequency $f$. Animals often displayed electrocommunication responses to random AMs, but only when these were applied globally (data not shown), indicating that these stimuli are good communication signal mimics.

We used the ‘frozen noise’ technique to examine trial-to-trial variability: the same random AM (40–60 Hz, duration 2 s) was delivered at least 30 times. We computed the reliability and precision measures from post-stimulus time histograms (binwidth 3 ms) as described previously.

**Information theoretic measures**

A lower bound on the mutual information rate (in bits s$^{-1}$) is given in refs 14 and 30:

$$I = \int_{f_{\text{low}}}^{f_{\text{high}}} \frac{df}{f \log(1 + C(f))}$$

where $f_{\text{low}}$ and $f_{\text{high}}$ define the bandwidth of the stimulus. We obtained the mutual information rate in bits per spike by dividing $I$ by the cell’s mean firing rate during stimulation.

We performed linear stimulus reconstruction as previously. Note that this provides an absolute lower bound on information transmission. The quality of the reconstruction was quantified as vector strengths. The Rayleigh statistic was also calculated to determine whether the cycle histogram showed statistically significant phase-locking to the AM.

**Partition experiment**

The animal’s head was electrically isolated from the trunk by a thin rubber partition so that stimuli could be presented to the head and trunk regions independently. The partition decreased the normal EOD amplitude in the head region because it partly blocked EOD current flow. To compensate for this a continuous unmodulated EOD mimetic signal was delivered to the head region between a single electrode in the dorsal musculature and electrodes lateral to either side of the fish. This restored the EOD amplitude in the head region to values measured with the partition short-circuited. $C_{\text{low}}$ and $C_{\text{high}}$ values with the partition in place for local stimulation were not statistically different from those obtained without the partition ($P = 0.4359$ and $P = 0.95$ respectively, t-tests, $n = 15$).

Single electroreceptor afferents were recorded and stimulated with 4 Hz sinusoidal AMs to gauge the effectiveness of electrical isolation. Each animal’s responses to stimuli presented in the head and trunk regions were summarized as cycle histograms and quantified as vector strengths. The Rayleigh statistic was also calculated to determine whether the cycle histogram showed statistically significant phase-locking to the AM. Receptor afferent receptive field positions ranged from 8 to 45 mm rostral or caudal to the receptor.

**Design**

Single electroreceptor afferents were recorded and stimulated with 4-Hz sinusoidal AMs to gauge the effectiveness of electrical isolation. Each animal’s responses to stimuli presented in the head and trunk regions were summarized as cycle histograms and quantified as vector strengths. The Rayleigh statistic was also calculated to determine whether the cycle histogram showed statistically significant phase-locking to the AM. Receptor afferent receptive field positions ranged from 8 to 45 mm rostral or caudal to the receptor.
The average distance between the partition and the CRF centre was 5.6 cm and was always greater than 4 cm. Using results reported previously, we estimated the maximal distance from the centre of the receptive field to the CRF boundary was then estimated as the radius of a circle having this area (2.4 cm). This conservative estimate is lower than 4 cm. Along with the lack of pyramidal-cell responses to stimulation of the head chamber alone, this indicates that it is very unlikely that the CRF surround extends past the partition.

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