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## **Low-Frequency Transient Visual Oscillations in the Fly**

### **ABSTRACT**

Low-frequency oscillations were observed near the H1 cell in the fly. Using coherence calculations, we found that this signal is correlated with the visual stimulus. Further supporting this correlation, controls such as blocking most of the visual stimulus decreased the coherence. The high correlation was short-lived, lasting only approximately 100 ms, although the specific duration of the correlation may depend on stimulus properties.

### **INTRODUCTION**

Oscillations have been observed in many systems and have been theorized to be involved in multiple functions, ranging from attention (Fell 2003) to object recognition. Local field potentials in the fruit fly include a 20-30 Hz component that increases with the presentation of a novel stimulus (van Swinderen 2003).

While examining signals near the H1 of the fly, we observed low-frequency oscillations. We wanted to determine whether this signal was dependent on the visual stimulus. In order to isolate the low-frequency component of the signal before addressing this question, we converted the signals into the frequency domain and calculated the coherence. The coherence can be thought of as the correlation between the two vectors at each frequency. The magnitude of the coherence values varies from zero to one, where one is the maximal correlation.

We calculated the coherence while a full-field randomly varying stimulus was presented at two frequencies or when the stimulus was briefly but regularly turned on. We also calculated control coherences by shuffling the stimuli values or by blocking the majority of the stimulus from the fly. In the initial three conditions, we found high coherence, whereas it drastically decreased in each of the control conditions.

We also examined whether the signal remained coherent across time. If the oscillations occurred only during novel stimuli, they might be transient. Also, studies have shown that characteristics such as firing rate and the information rate adapt extremely quickly within the H1 neuron (Fairhall 2001), so perhaps the oscillations occur only while adapting. The high coherence was found to be transient.

### **METHODS**

## Recordings

Calliphora or Sarcophaga flies were aged approximately two days to prepare for physiological recordings. Flies were first anesthetized by placing flies in freezing temperatures for approximately six minutes and were then fixed with wax at the abdomen to a small metal plate. The antennae, limbs, proboscis, and wings were amputated and small amounts of wax were placed on these locations to prevent further movement. The posterior part of the head was pushed waxed to the body, such that the back of the head was parallel to the metal plate. Small incisions were made to expose one hemisphere of the brain. One drop of saline was applied to this hemisphere. A small incision was also made in the posterior part of the abdomen for the ground electrode. The metal plate was then secured into a clamp that positioned the fly in the center of circle composed of LEDs (described in the Visual Stimuli section). Tungsten electrodes with 3 mega-ohm impedance were used. The recording electrode was positioned in the right third neuropil, in the region of H1, whereas the ground electrode was placed in the incision mentioned above. LABVIEW programs collected data from the electrodes at a rate of 30,000 Hz.

## Visual Stimuli

Stimuli were presented on individually controlled light-emitting diodes (LEDs) that were located at multiple heights but an approximately constant radius from the fly. The LEDs extended across the majority of the circle, although a small angle behind the fly did not include any LEDs, as the hardware of the apparatus was positioned there. LABVIEW programs controlled the LEDs.

This experiment was designed to use temporally varying full-field stimuli, as the width of the LEDs had been determined to be too small to simulate continuous moving vertical bars. The full-field stimuli's luminance values were selected from a random distribution. The luminance value was modulated either at 80 or 200 Hz. The 'delta' experiment consisted of turning all of the LEDs to the maximum intensity for what would correspond to one-twentieth of a frame at either 80 or 200 Hz (.625 or .25 ms, respectively) and would then turn off for the remainder of the frames.

During one experiment, the majority of the light was blocked, by placing cardboard in front of the LEDs, although some light did escape that was located on the LEDs most posterior to the fly's head.

## Data Analysis

The voltage signal recorded from the electrodes was low-pass filtered using a Butterworth filter with a cutoff frequency of 500 Hz. The signal was then down sampled to 1000 Hz. Similarly the stimulus that was output was sampled at 1000 Hz. The coherence phase and magnitude were calculated between the first ten seconds of the voltage signal and the stimulus, unless otherwise noted. Error bars were calculated using the following equation:



where the p-value was set to equal .05.  $N$  is the number of samples that each time series interval was divided into, which for all analyses except that in figure 6 was equal to 39.  $\mu$  was set to 1 as there was no overlap between the windows of the initial time sample. Error bars are only shown in figure 2, as the error is a constant error in all other graphs, except for analyses present in figure 6. (In the figure six analysis, a time segment was used that was only one-fifth as long as in other analyses, thereby decreasing the  $N$  value and increasing the error. However, it was necessary to use short segments, as we were analyzing the dynamics of the coherence measurements.)

## **RESULTS & DISCUSSION**

### **Recordings from the optic lobe included low-frequency components**

Flies were placed in the center of a circle composed of light-emitting diodes (LEDs). The differential voltage was recorded between an electrode placed in the right optic lobe and a ground electrode located in the fly's abdomen. All LEDs were then changed to randomly selected luminance values that varied as a function of time. The luminance values were modulated either at 200 Hz or 80 Hz. A segment of the voltage trace obtained during the presentation of the 200-Hz stimulus is shown in Figure 1a. This trace seems to include spikes (probably from H1) that were not well isolated from the noise and low frequency oscillations (Fig. 1a). These characteristics were observed in both of the two flies tested and in both the 200- and 80-Hz stimulus conditions.

In order to better analyze the low-frequency oscillations, the signal was filtered by a Butterworth, low-pass filter, with a cutoff frequency of 250 Hz (Fig. 1b). Finally, in order to save on computational time, the signal was down sampled from 30,000 to 1,000 Hz (Fig. 1c).

### **The low-frequency signal's high coherence with the stimulus suggests that the signal is visual**

The low-frequency signal could be attributed to many sources, including noise in the apparatus. Coherence is a measure of how correlated each frequency component of the signal is with another signal. The coherence measurement varies between zero and one, with a coherence of one indicating maximal correlation. In this instance, we were interested in the coherence of the low-frequency band between the signal and the visual stimulus. We calculated the coherence during the first ten seconds of each of the two signals. Figure 2a shows that there is indeed high coherence within the low-frequency band (less than 100 Hz), regardless of whether the 200- or the 80-Hz stimulus was presented.

As a control, the coherence was also calculated between the signal and a shuffled stimulus. The stimulus was shuffled in two manners. The first shuffling maintained the times at which the stimulus changed, but the luminance values to which the stimulus changed were shuffled. For example, if the stimulus initially changed to the arbitrary intensity values of .5, 1, and .75 at the arbitrary times of 1, 6, and 11, then the shuffled stimulus would still change at times 1, 6, and 11, but the intensity values might be shuffled to .75, .5, and 1. This was termed the frame-shuffling control.

The second shuffling shuffled the stimulus values at every millisecond of the data (which is the sampling rate used in order to calculate the coherence). Extending the above example, this new shuffling would cause the stimulus to change every time value, from 1 to 15, with 1/3 of the frames being assigned to each of the three contrasts. This was termed the ms-shuffling control.

Figures 2b and 2c show the results from the coherence calculated between the signals and the frame- and ms-shuffling controls, respectively, for both the 200- and the 80-Hz stimuli. (The non-shuffled coherence is plotted as a dotted line for comparison.) Both forms of shuffling seem to eliminate any significant coherence in the low-frequency band.

In order to further explore whether the low-frequency oscillations were due to the fly's response to the visual stimulus (as opposed to external noise picked up on the electrode), the light was partially blocked using cardboard. Some of the LEDs, located in the extreme periphery escaped, and was probably seen by the fly, as their visual angle is so wide. This test was only performed using the 200-Hz stimuli. The coherence in the low-frequency band decreased significantly (Fig. 3), although it still appears to be more coherent than baseline. This may be due to the light that was not blocked.

The high coherence at the low frequencies suggests that the signal depends on the visual stimulus. The low coherence in the same frequency band with the shuffled stimulus further supports this fact. The ms-shuffling stimulus controls for the possibility that the signal would have caused this coherence with any given stimulus. The frame-shuffling stimulus controls for the possibility that the high coherence is due to the stimulus changing at particular times within the stimulus, rather than the actual luminance values within those frames. Therefore, the low-frequency component of the signal does seem to be controlled by the specific stimulus characteristics.

### **The phase of the coherence is similar at frequencies with high coherence, suggesting that the high coherence was due to one single signal**

If there is a visually driven signal in the noted low-frequency range, then phase of the coherence with the visual stimulus across these frequencies should be similar. This is because the high coherence at that range of frequencies is likely due to a single signal with a single phase, rather than a collection of signals with similar frequencies but independent phases (and therefore likely to be independent signals). The coherence phases were found to be rather constant (especially compared to the large fluctuations in phase at high frequencies) in the low-frequency band during both the 80- and 200-Hz stimuli (Fig. 4).

### **Fast “delta” signals still exhibit high low-frequency coherence, refuting the possibility that the signal was stimulus-dependent noise**

Perhaps the low-frequency coherence was due to noise that depended on the luminance of the LEDs. In order to test this possibility, the LEDs were briefly turned to the maximum luminance value for one-twentieth of the 40-Hz frame (1.25 ms). The LEDs turned off

for the remainder of the frame. Therefore, a stimulus change was still occurring at the same time as during previous stimuli, but if the signal directly depended on the stimulus luminance, then the frequency region of high coherence would be at a higher frequency than before. Figure 5 shows that when the delta stimulus was presented, the region of high coherence remains within the low-frequency region seen using previous stimuli, countering the idea that the signal is stimulus-dependent noise.

### **The high coherence of the low-frequency signal is transient**

The coherence between the voltage signals and the stimuli were calculated using a smaller, 2-second sliding window that slid at 10-ms intervals. The low-frequency coherence decreased as a function of time. In order to quantify, a frequency range was chosen for each stimulus that initially had high coherence. (The ranges were 10-50 Hz and 30-70 Hz for the full-field stimuli modulated at 80- and 200-Hz, respectively.) The mean coherence was calculated within this frequency range for each window. The mean coherence could then be plotted as a function of the beginning time of the window. Similar averages were calculated from coherences calculated with the frame-shuffled, control stimuli.

Figure 6 shows that at times later after the stimulus start, the coherence of the signal decreased, eventually becoming indistinguishable to that of the controls. From this data, we can roughly estimate that the coherence of the low-frequency signal becomes indistinguishable from baseline at approximately 100-ms after the stimulus onset for these stimuli.

## **SUMMARY**

Signals recorded in the optic lobe of flies revealed a low-frequency signal. This signal was analyzed by examining the coherence of the signal with various visual stimuli. The signal's low-frequency band exhibited high coherence with three full-field stimuli, including random temporally varying 200- and 80-Hz stimuli and "delta" stimuli, consisting of brief, bright LED flashes. The controlled coherences, obtained either by introducing shuffled stimuli or blocking the stimuli decreased the coherence. Therefore, the signal seems to be a visual, neural signal – not noise.

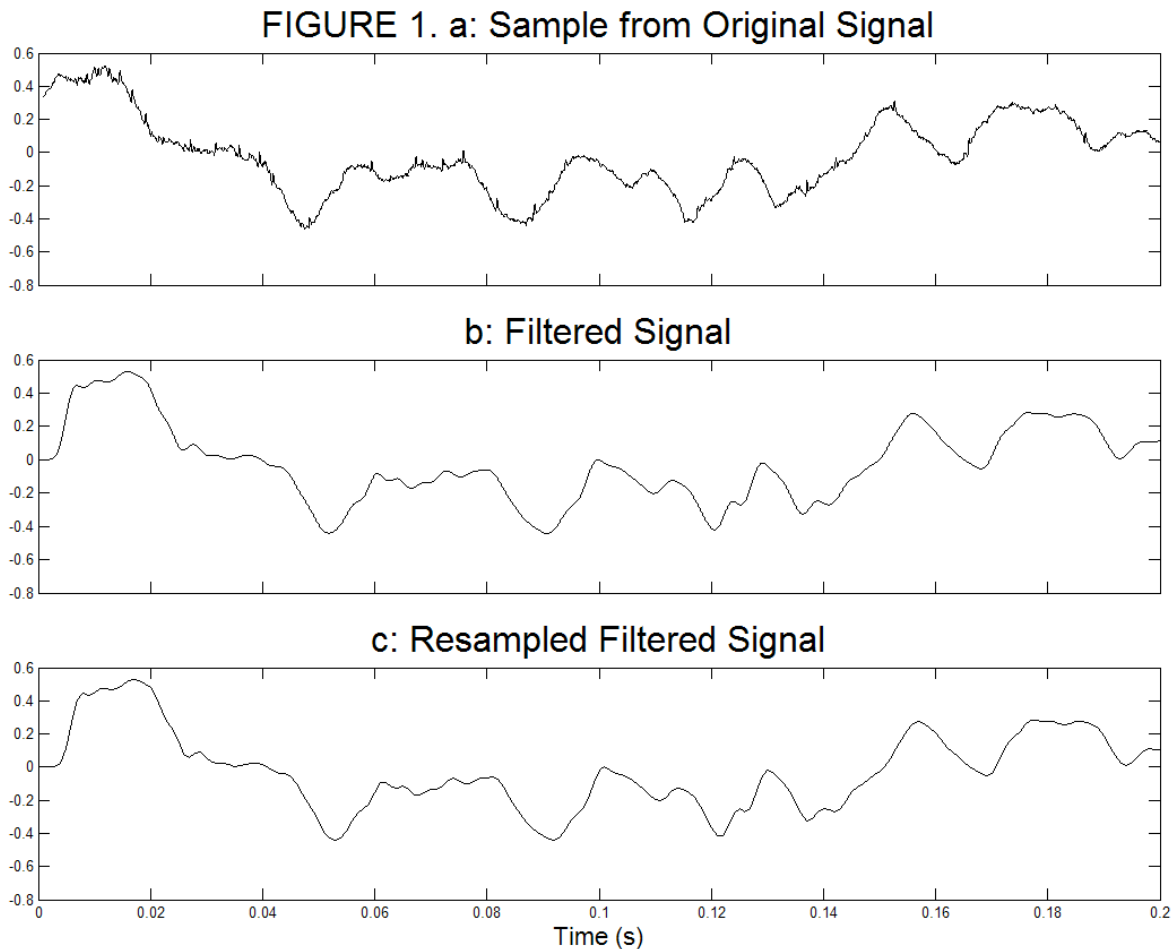
The fact that the coherence of the low-frequency components of the signal exist for only 100 ms after the onset of the stimulus suggests that these oscillations are likely influenced by other factors. Perhaps the oscillations occur only while attending or perhaps the oscillations are quickly adapted away. Previous studies mentioned in the introduction conclude that oscillations within the fly depend on specific stimulus features. However, these oscillations do seem to contain information valuable in decoding a visual scene.

## **ACKNOWLEDGEMENTS**

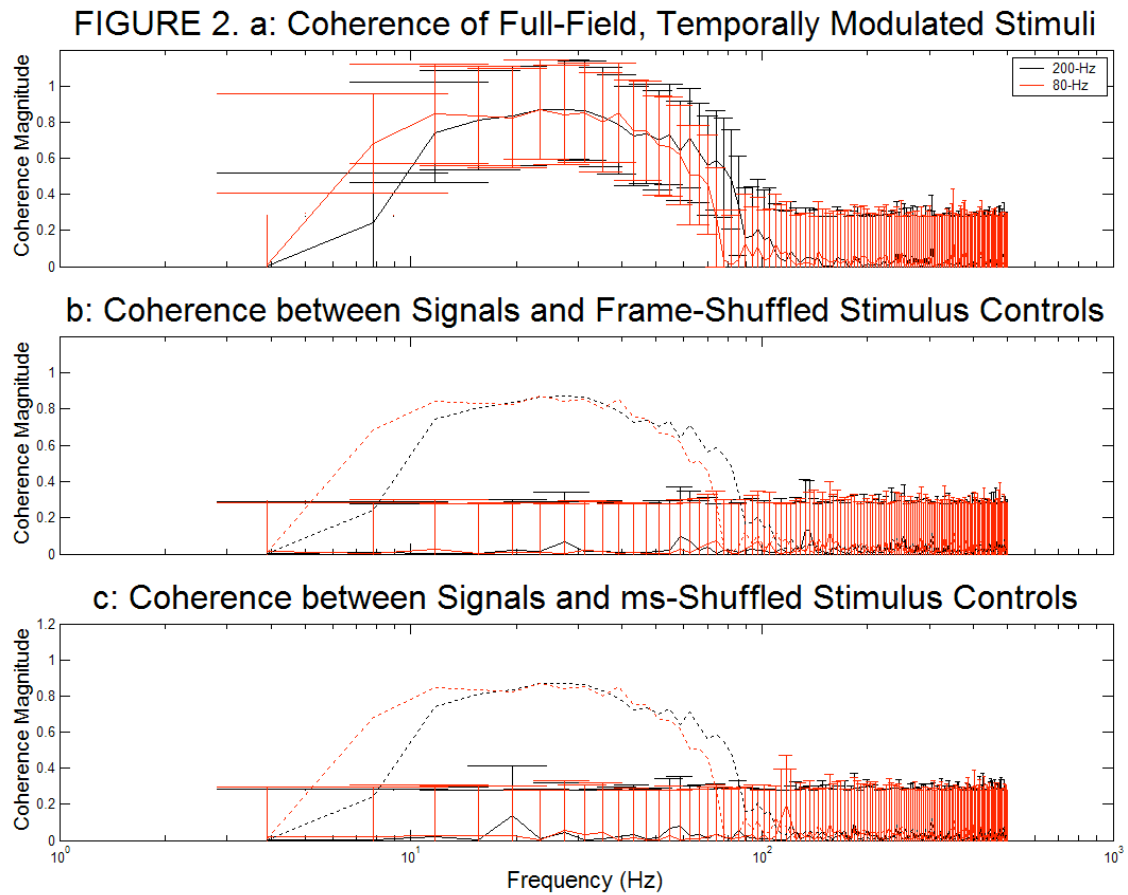
We thank Evren Turner for his help in performing the experiments presented in this paper and writing all LABVIEW programs used in gathering data and presenting the stimulus.

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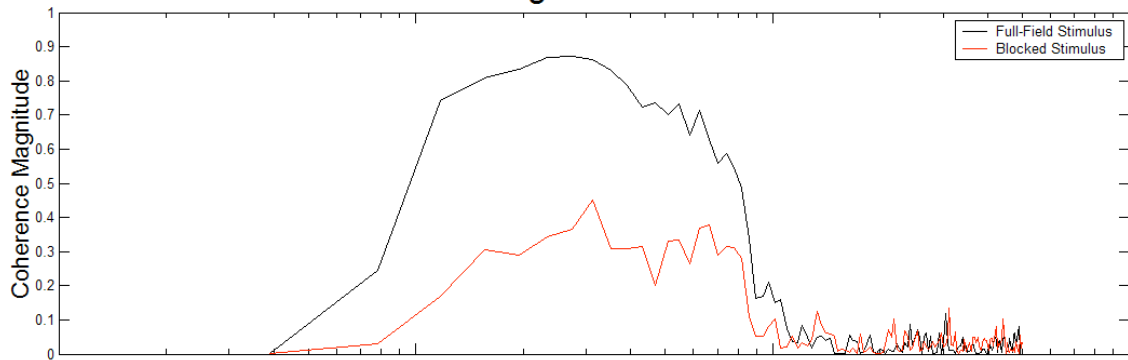


**FIGURE 1.** (a) Segment of the electrode's signal when the 200-Hz full-field stimulus was presented. The signal was low-pass filtered (b) and resampled (c), as described in the text.



**FIGURE 2.** (a) Coherence between a signal and the stimulus when a full-field temporally modulated stimulus, modulated either at 200 Hz (black line) or 80 Hz (red line), was presented. (b) Coherence calculated between the signal and a stimulus with luminance values that were shuffled over all frames (where a frame depends is 5 ms for the 200 Hz stimulus and 12.5 ms for the 80 Hz stimulus). The coherence values shown in (a) are plotted as the dotted lines. (c) Coherence calculated between the signal and a stimulus with luminance values shuffled over all millisecond bins. Error bars are calculated as described in the methods.

FIGURE 3. a: Coherence during Full-Field and Blocked 200-Hz Stimuli



b: Coherence during Full-Field and Blocked 80-Hz Stimuli

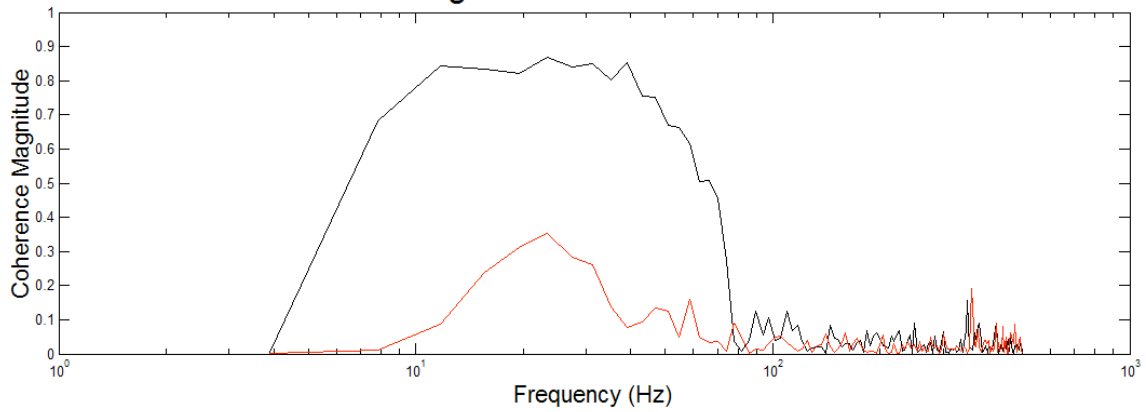


FIGURE 3. (a) Coherence between the signal and the stimulus when a full-field stimulus was modulated at 200 Hz when either all of the LEDs were visible to the fly (black line) or the majority of the LEDs were blocked (red line). (b) Similar coherence measurements using an 80-Hz stimulus.



FIGURE 4. Coherence Phase during Full-Field Stimuli

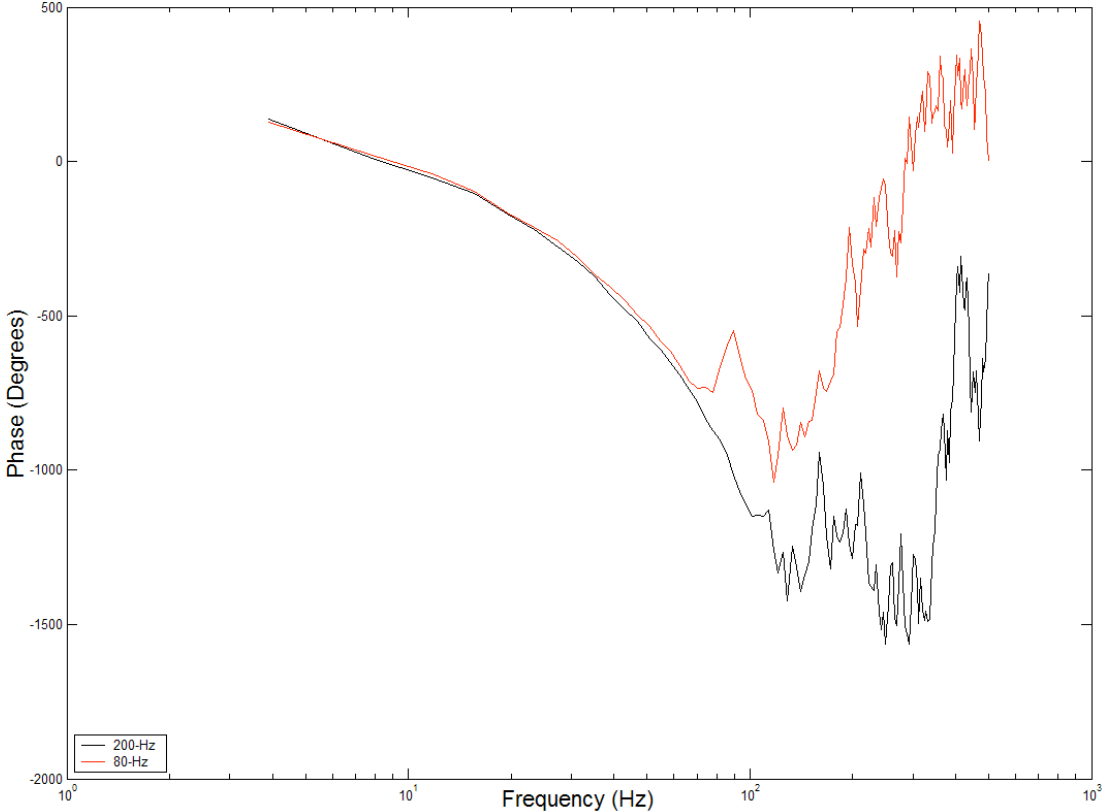


FIGURE 4. The phase of the coherence calculated between the signal and a full-field stimulus modulated at either 200 Hz (black line) or 80 Hz (red line).

FIGURE 5. Coherence during 40-Hz Delta Stimulus

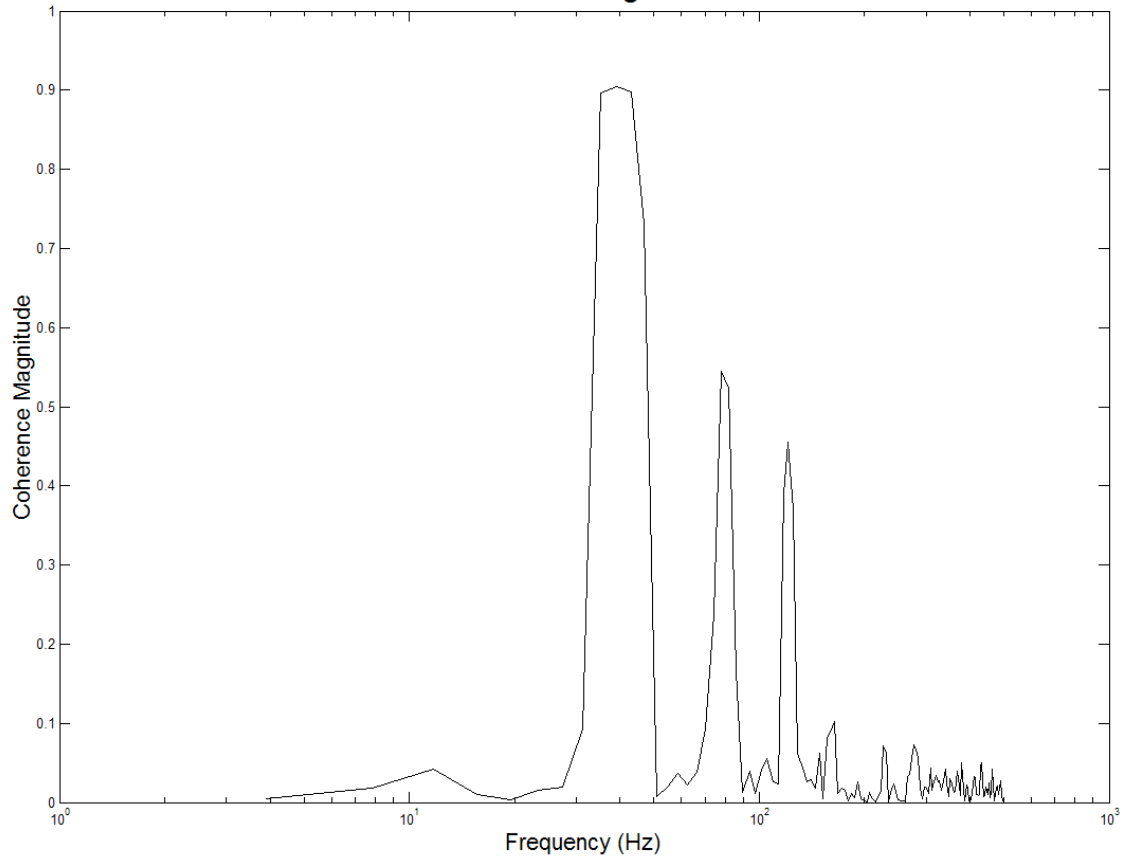


FIGURE 5. The coherence between the signal and a full-field stimulus that was flashed on and off every 25 ms.

FIGURE 6. Low-Frequency Coherence versus Time from Stimulus Onset

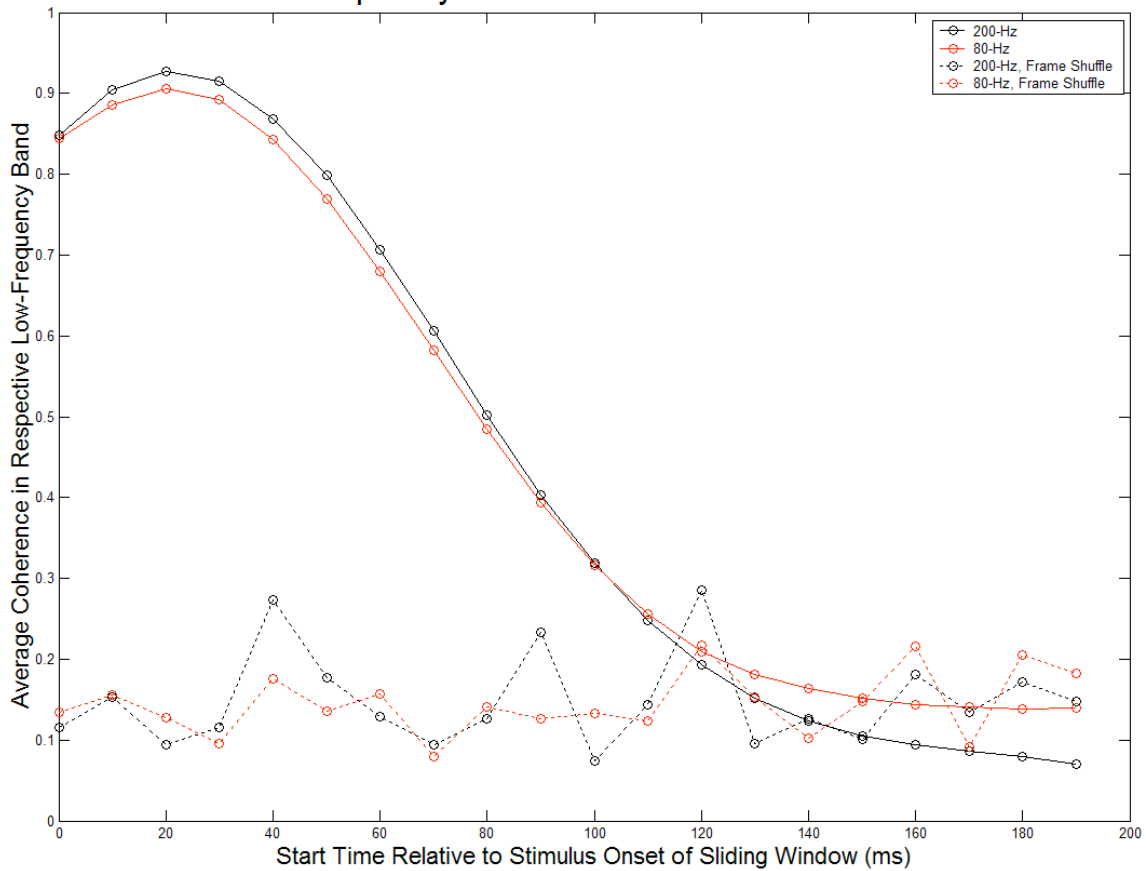


FIGURE 6. The coherence was calculated as a function of time by calculating the coherence magnitude between the signal and the full-field temporally modulated stimulus within a two-second, sliding window. The sliding window moved in 10-ms increments. These values were calculated for both the 200- (black line) and 80-Hz (red line) stimuli. They were compared to the control coherence values calculated between the signals and a shuffled stimulus obtained by shuffling the luminance values over frames (dotted lines).