Neural response dynamics of spiking and local field potential activity depend on CRT monitor refresh-rate in the tree shrew primary visual cortex

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Abstract

Entrainment of neural activity to luminance impulses during the refresh of cathode ray tube (CRT) monitor displays has been observed in the primary visual cortex (V1) of humans and macaque monkeys. This entrainment is of interest because it tends to temporally align and thus synchronize neural responses at the millisecond timescale. Here we show that in tree shrew V1, both spiking and local field potential (LFP) activity are also entrained at CRT refresh-rates of 120, 90 and 60Hz, with weakest but still significant entrainment even at 120Hz and strongest entrainment occurring in cortical input layer IV. For both luminance increments ("white" stimuli) and decrements ("black" stimuli), refresh-rate had a strong impact on the temporal dynamics of the neural response for subsequent luminance impulses: Whereas there was rapid, strong attenuation of spikes and LFP to prolonged visual stimuli composed of luminance impulses presented at 120Hz, attenuation was nearly absent at 60Hz refresh-rate. In addition, neural onset latencies were shortest at 120Hz and substantially increased, by about 15ms, at 60Hz. In terms of neural response amplitude, "black" responses dominated "white" responses at all three refresh-rates. However, black/white differences were much larger at 60Hz than at higher refresh-rates, suggesting a mechanism that is sensitive to stimulus timing. Taken together, our findings reveal many similarities between V1 of macaque and tree shrew, while underscoring a greater temporal sensitivity of the tree shrew visual system.

Keywords: Electrophysiology, Response Latency, Spike Timing, Neural Coding
Introduction

Tree shrews are the closest living relatives of primates, and diverged from the rest of the Euarchonta clade about 85 million years ago (Liu et al., 2001). They are day active mammals with a highly developed visual system, comprising at least three distinct retinotopic visual areas in addition to a large primary visual cortex (V1) (Sesma et al., 1984; Wong and Kaas, 2009). Tree shrew V1 shares many features with that of primates, such as for example orientation columns (Bosking et al., 2002), which are not seen in rodent visual cortex (Van Hooser et al., 2005). Using CRT stimulation delivering precisely timed and predictable luminance impulses lasting about 2ms, neural responses in macaque V1 have been shown to become aligned to these luminance impulses, particularly for high contrast visual stimulation (Williams et al., 2004). This entrainment is of interest, because it tends to temporally align visual responses and will thus strongly enhance synchronization of responses among neurons. Here, we were interested in examining whether such entrainment to CRT monitor stimulation also occurs in tree shrew V1, and to compare its magnitude and extent with findings in macaques. These issues are relevant for understanding how visual functions in primates evolved from a common ancestor, and for evaluating the similarity of tree shrew and macaque V1 in terms of temporal neural response dynamics. We found that both spiking and LFP activity were strongly entrained to CRT monitor refresh-rate, and that entrainment decreased with increasing CRT refresh-rate.

During CRT monitor stimulation, each luminance impulse can be regarded as a separate, briefly flashed stimulus. By varying the refresh-rate, we could thus deliver luminance impulses separated by different time intervals. Here, we used three refresh-rates to investigate how changes in the temporal stimulus profile during a stimulation period of about 80ms affect cortical responses. Higher refresh-rates are usually preferred, because they more closely approximate the continuous nature of the visual environment. We found that at 120Hz refresh-rate, the neural response declined rapidly during the 80ms stimulus presentation period, such that each
successive luminance impulse evoked progressively less activity. At 60Hz however, responses
to subsequent luminance impulses were nearly independent of each other and decreased little
over the course of stimulus presentation. Finally, we examined temporal dynamics of neural
responses separately for visual stimuli with luminance increments (white) and decrements
(black) relative to the background. We found dominance for black stimuli in terms of latency and
response magnitude, which was most striking at low refresh-rates.

Material and Methods

Animal preparation

Experiments were performed on six adult tree shrews (Tupaia belangeri) aged 2-5 years.
Animals initially received Ketanarkon (i.m. 100mg/kg) and Atropine (i.m. 0.02mg/kg). We then
performed a tracheotomy, and administered the muscle relaxant Pancuroniumbromide (i.p.,
initial dose 0.4mg/kg, then 0.2mg/kg approximately every 45min). Animals were artificially
respirated at 100 strokes per minute (Harvard Instruments Respirator) using a mixture of 70%
N₂O and 30% Oxycarbon (95%O₂/5%CO₂) and Isoflurane (0.5%-1.5%). Animals were then
transferred to a stereotaxic device (David Kopf) that was modified to permit visual stimulation.
Animals wore contact lenses to prevent drying of eyes. An eye drop of atropine was applied to
the eye for pupil dilation. Visual stimulation was monocular, the other eye was covered. To gain
access to the primary visual cortex, the temporal muscle was removed, the bone cleaned, and a
hole (~2 to 3 mm diameter) was drilled around AP -1mm and ML +4mm. A small slit was made
in the dura using a syringe needle to permit introduction of the tetrodes into the cortex. After
tetrode placement, the cortex was covered with handwarm 2% Agarose (in 0.9% NaCl) to
prevent drying and provide stability. During the entire experiment, body temperature was
maintained at 37°C via an electric heating pad controlled by a rectal thermal probe. Heart rate
was monitored using a Tektronix differential amplifier, with typical values ranging from 380 to 420 beats per minute. All procedures were conducted according to local regulation approved by the veterinary office of the Canton of Fribourg.

Electrophysiology
Tetrodes were fabricated by twisting together four 12.5 µm-diameter nickel–chromium wires (RO-800; Kanthal Precision Technology) and the impedances were reduced to 200–300 kΩ by gold plating. Two tetrodes spaced approximately 300µm apart were advanced into the primary visual cortex using a hydraulic microdrive (David Kopf Instruments). Identical penetrations were made in all experiments normal to the cortical surface in a sagittal plane, by tilting the microdrive backwards at an angle of 30°. Since we did not tilt the electrode laterally, an angle of 25° remained between the penetration path and the surface normal vector (see the projection of the penetration path onto a coronal section and the surface normal vector in Figure 1A). The cortical depth of the recorded units was computed by correcting the penetration depth using an appropriate factor (cos(25°)=0.92). For a given penetration, we recorded activity at multiple depths typically spaced around 200µm apart. The signal was amplified by a RA16PA Medusa preamplifier and then filtered and digitized by a RZ5 Bioamp Processor (Tucker-Davis Technologies, Alachua, FL). LFPs were filtered between 1 and 200Hz and sampled at 509 Hz. Spike waveforms were recorded by thresholding the signal filtered between 300Hz and 4kHz and sampled at 24.4 kHz. The spike waveforms were sorted into clusters corresponding to single neurons using the public domain software MClust 3.5 where possible. In total, we identified single neurons at 26 sites and multi unit activity at 26 additional sites. Since we observed no functional differences between single and multi neuron activity, these were grouped together for all analyses.
Histology

At the end of a recording session, electrolytic lesions were made at multiple sites using a constant current stimulator (WPI A360) passing 10µA for 10s. Animals were perfused with 0.9% NaCl followed by ice cold 4% PFA in 0.1M phosphate buffer (pH 7.4). The top of the skull was removed, and a coronal cut was made through the brain at AP +4mm in the stereotaxic frame. The brain was then removed and immersed in a mixture of 2% DMSO and first 10% and later 20% glycerol in 0.1M phosphate buffer (pH 7.4). The posterior part of the brain was then cut into 50µm sections using a freezing microtome (Microm HM440E). The lesions were located in Nissl-stained coronal sections. Because our track was not in a coronal plane the lesions were on different sections. If a lesion was visible in more than one adjacent section, we chose the section with the relatively largest lesion as the reconstructed depth of the lesion. The cortical depth of each recording site was reconstructed relative to a reference lesion at the deepest recording site, accounting for brain shrinkage due to histological processing of around 8-17%.

Stimuli

Stimuli were generated with Psychophysics Toolbox running on a Mac Mini and presented on a gamma corrected 21” diameter (~56.7° visual angle) Compaq Qvision 210 cathode ray tube monitor running at either 119.22 (120Hz), 89.73 (90Hz), or 60.31 Hz (60Hz). A single luminance impulse decayed with a time constant of about 2ms as measured with a light sensitive diode and an oscilloscope. Maximum luminance measured with a Minolta TV-color analyzer was determined as 50 cd/m² and “white”, “gray”, and “black” values were adjusted to have 100%, 50% and 0% of this value, respectively. Before recording we mapped the approximate location of the receptive field of the unit under study by manually sliding bars generated with a simple graphics program back and forth on the monitor. The stimulus was then positioned in this area at eccentricities between 7° and 17°. We employed the sparse noise stimulus, consisting of a sequence of randomly positioned white and black squares over a gray background. In most
cases, 1.3°×1.3° black or white squares were positioned on a 15×15 grid with 50% overlap between adjacent positions, spanning a total area of 10°×10°. To avoid edge effects and to obtain a homogenously sampled surface with the overlapping stimuli, we actually showed 16x16 pixel positions spanning 11.3 visual degrees and removed a 0.5 pixel wide border from each side after weighting of the pixels. Each black or white square was presented for a total duration of about 80ms (83.3 ms at 120Hz and 60Hz, 77.7ms at 90Hz), corresponding to 10, 7 and 5 video frames at refresh-rates of 120, 90 and 60Hz respectively. There was no blank period between subsequent stimuli. The sequence of all stimuli was repeated 10 times in different pseudorandom orders. Altogether the sequence usually lasted for about 7 minutes (Total of 512 stimuli (16x16 pixel positions x 2 (black and white)), each shown 10 times for ~80ms).

We also employed a white noise stimulus at every location and for all refresh-rates. The spatial extent was the same as for the sparse noise stimulus at each location but all pixel positions were filled randomly with 50% white and 50% black squares. Note that the pixel size during white noise stimulation was half the width and height of a sparse noise pixel that always filled two adjacent pixel positions. We generated 2000 individual random stimuli and each stimulus was presented for the same time as the sparse noise: 10 frames at 120Hz, 7 frames at 90Hz, 5 frames at 60Hz. The entire sequence lasted about 3 minutes for white noise (2000 stimuli, each shown for ~80ms).

**Locking ratio**

We calculated the locking ratio as the ratio of the mean Fourier components at the refresh-rate to the mean Fourier components of the surrounding 20 Hz (Williams et al., 2004).

\[
LR = \frac{\frac{1}{N} \Sigma_i |z_i|^2}{\frac{1}{M} \Sigma_i |\gamma_i|^2}
\]
Where the $z_i$ are $N$ independent estimates of the Fourier components at the refresh-rate (120, 90 or 60 Hz) and the $y_i$ are $M$ independent estimates of the Fourier components in the 20 Hz surrounding the frame rate (110-130, 80-100 or 50-70 Hz). LFPs were upsampled to 1 kHz and spike trains binned into 1ms bins to yield signals with equal temporal resolution. Both signals were cut into usually $N = 26$ (120 and 60Hz) or $N = 24$ (90Hz) non overlapping snippets of $\sim 16.4s$. Fourier components were estimated for each snippet using a rectangular window without tapering. For the power estimates of the surrounding frequencies, the component at the frame rate as well as 1.8 Hz to either side were excluded. Thus 269 estimates of power at the surrounding frequencies were gathered for each snippet of data. To determine if a unit was significantly entrained to the frame rate of the monitor we bootstrapped a distribution from these power values of the surrounding frequencies and recalculated the locking ratio exactly as described above. We repeated this procedure 1000 times and took the upper 1% value as threshold for significance. For the analyses shown in Figure 3, a running average of the locking ratio across nine neighboring recording depths was normalized to lie between values of 0 (minimal locking) and 1 (maximal locking). Normalization was performed separately for the three refresh-rates.

Receptive field mapping

Receptive fields were estimated for each unit by weighting each square on the grid with the average number of spikes fired in response to its onset in a window from 25 ms to 110 ms after stimulus onset. Receptive fields were calculated separately for trials with black stimuli and white stimuli. Every square for which the response exceeded a threshold of two standard deviations above the mean firing rate for all trials was considered to be within the receptive field. All units had well delimited receptive fields in the 120Hz and 90Hz conditions. At 60Hz, receptive fields could not be estimated for white stimuli for 9 units and for black stimuli for one unit due to weak
responses in this condition. These units were excluded for the latency and amplitude measurements.

Evoked responses

For the peristimulus time histograms (PSTH) and visual evoked potentials (VEP), we averaged spiking activity and local field potentials respectively of trials where a black or white stimulus was present inside the defined receptive field. We then subtracted the average response to trials, where the stimulus was far (at least three pixel positions distance) from the receptive field. In these trials, only gray background was shown in the area of the receptive field. By subtracting this background related component, we approximate the pure stimulus related part of the response (exceeding the response to the background). Because we had no inter stimulus interval there was the possibility of obscuring the “baseline” of the next trial by the still ongoing response to the preceding stimulus. To address this in our analysis we only consider trials that were preceded by an “outside” trial for both “stimulus inside” and “stimulus outside” averages.

Slopes

To estimate the slope of the attenuation of the responses we determined the overall peak of the PSTH, for both black and white stimuli, after the PSTH of the background response was subtracted. We then identified all subsequent peaks (usually 7 for 120Hz, 5 for 90Hz and 4 for 60Hz responses). Peaks were expected to occur for every screen refresh in temporal offsets corresponding to the frame rate. If no clear peak was present around the expected time – as was often the case for the later peaks at 120Hz – we took the value of the PSTH at the expected time. Linear fits were made through the first five peaks at 120Hz and all peaks at 90Hz and 60Hz. The later peaks at 120Hz had values close to zero and would have confounded the true attenuation slope. They were thus excluded from the linear fits. The slope for the decline of
the VEP was estimated in exactly the same manner, except that in this case the peaks were negative.

**Background Response**

To estimate the magnitude of the response to the redraw of the gray background, we calculated the modulation ratio of the PSTH for trials with only the gray background present in the receptive field. To this end, we identified the peaks in the background PSTH in the same way as described above for the attenuation slope analysis. We then windowed the signal, centered around each peak resulting in signal snippets with lengths depending on the refresh-rate – 8ms for 120Hz, 11ms for 90Hz and 17ms for 60Hz. These peak snippets were averaged and the modulation amplitude was determined as the difference between the maximum and the minimum of this mean background redraw evoked peak. We divided this value by the mean firing rate across the gray trials to obtain the modulation ratio. The modulation ratio for the local field potentials was calculated in the exact same manner, by averaging the negative peaks and dividing their modulation amplitude by the mean amplitude of the LFP across the gray trials.

**Latencies**

To estimate onset latency of spiking and LFP activity, we report the time point at which stimulus evoked activity (background response subtracted) first surpassed four standard deviations of the mean background response. If this threshold was already exceeded by the unit in the period before the visual delay (24ms) or if it never surpassed this threshold, the unit was excluded due to bad signal to noise ratio. At 60Hz, latencies could frequently not be estimated for white stimuli due to weak responses in this condition.
White/Black Ratio

We calculated the white/black ratio similar to Yeh (Yeh et al., 2009) as \( \log \left( \frac{FR_{\text{white}}}{FR_{\text{black}}} \right) \), the logarithmic ratio of the mean firing rates for “white” compared to “black” stimulus trials. This ratio is zero for equal firing rates and the more negative it is, the more the “black” response dominates the “white”.

Results

We present here data collected from the primary visual cortex (V1) in six tree shrews during terminal experiments under isoflurane anesthesia. We recorded single unit, multi unit and local field potential (LFP) activity from a total of 52 sites covering all six cortical layers. We confirmed the location of recording sites using electrolytic lesions at reference depths along the recording track using histological analysis (see Figure 1A). We describe robust entrainment of neural responses to the refresh-rate of the cathode ray tube monitor (CRT) used for visual stimulation, and demonstrate differences in neural activity for different refresh-rates as well as for stimulus luminance increments (“white”) and decrements (“black”) relative to the intermediate luminance background. We used a sparse noise protocol for visual stimulation, where single spots of light are presented on a 15x15 grid, which is positioned over an area that includes the receptive field of the unit under study (see Figure 1B). Receptive fields were similar in size for “white” and “black” stimuli, as shown for a single neuron and a multi-unit site in Figure 1C and D respectively. Stimuli were presented in pseudorandom order as a continuous sequence, without intervening blank periods, lasting about 7 minutes, and each single light spot was presented during a period of about 80ms corresponding to 10, 7 and 5 CRT frames for 120Hz, 90Hz and 60Hz refresh-rate respectively.
Entrainment of spiking and LFP responses to monitor refresh-rate

The luminance profile for a CRT monitor pixel is shown in Figure 2A for the three refresh-rates we employed in our study (120, 90, 60Hz). Luminance peaks correspond to the time point of excitation by the cathode ray. The screen refresh time, i.e. the time between peaks, varies with the refresh-rate and pixel luminance decays rapidly with a time constant of about 2ms, according to our measurements. As seen in Figure 2B for an example neuron, spikes occur in clusters separated by the screen refresh time, and this effect appears most prominent at low refresh-rates. Note that spikes appearing at a given time are not actually related to the immediately preceding screen refresh, but are caused by a screen refresh that is about 30ms in the past. This temporal shift is due to the neuronal response latency resulting from signal transduction in the retina and action potential transmission to V1. The monitor refresh-rate also exerted a strong influence on the local field potential (LFP) signal. In Figure 2C, we show a set of single trial LFP responses collected at the same cortical site. At 60 Hz, we observe large negative peaks in the LFP occurring at about 17ms intervals, corresponding to the time between screen refreshes. These peaks are thus evidence of robust entrainment. At 90Hz and 120Hz monitor refresh-rates, entrainment corresponds to LFP peaks separated by 11ms and 8ms respectively. As can be seen in Figure 2C, LFP entrainment appears to decrease with increasing monitor refresh-rate.

To quantify the entrainment of spike and LFP signals, we used an established method based on Fourier decomposition (Williams et al., 2004). Briefly, we determined a locking ratio by dividing the Fourier component at the monitor refresh-rate by the average Fourier components of the surrounding frequencies. The average locking ratios for LFP (mean ± SEM: \( LR_{120} \): 6.6 ± 0.5, \( LR_{90} \): 18.1 ± 1.1, \( LR_{60} \): 21.6 ± 1.4) and spikes (mean ± SEM: \( LR_{120} \): 2.7 ± 0.2, \( LR_{90} \): 6.2 ± 0.5, \( LR_{60} \): 10.2 ± 1.3) are shown in Figure 3A. These quantitative analyses show that entrainment was dependent on the monitor refresh-rate (One-Way ANOVA: LFPs: \( p < 0.001 \), Spikes \( p < \))
Using a bootstrap analysis (see methods), we estimate that neural signals recorded at all (n = 52) of our sites were significantly entrained to all stimulus frequencies (bootstrapping method, p < 0.01). Entrainment was strongest at 60Hz and declined with increasing monitor refresh-rate for both signals. At 120Hz, we observed weaker but still significant entrainment in both signals. Locking ratios for spikes and LFPs recorded at the same cortical site were strongly correlated (120Hz R-value: 0.65 (p < 0.001), 90Hz: R-value: 0.50 (p < 0.001), 60Hz: R-value: 0.65 (p < 0.001)). Thus, sites with strong LFP entrainment also showed strong entrainment of spikes to the monitor refresh-rate.

Previous studies (Williams et al., 2004) found the magnitude of the entrainment to be dependent on the stimulus contrast. To address this issue, we recorded neural activity for each site also during the presentation of white noise, where each grid location contains either a “white” or “black” stimulus varying randomly in time. This stimulus thus has similar overall luminance compared to sparse noise, but higher contrast. We observed two- to three-fold increases in locking ratio for white noise compared to sparse noise stimuli, which was significant across all refresh-rates for both spikes and LFPs (paired t-tests: P<<0.001), indicating that entrainment to the CRT refresh-rate is contrast dependent. Note that locking ratios are computed based on the continuous time series of recorded data, thus including stimulation conditions both inside and outside the receptive field as well as “white” and “black” stimuli, and are thus not directly comparable to the trial-averaged neural data presented further below.

We examined entrainment of spiking and LFP activity as a function of the depth at which neural activity was recorded. As in previous studies in macaque monkey (Williams et al., 2004), we found that locking tended to be strongest in intermediate layers of the visual cortex, at depths between 700 and 1000μm. For example, locking ratios obtained for spiking activity at a monitor refresh-rate of 90Hz are shown in Figure 3B as a function of recording depth. Recording locations with strong locking ratios are shaded in black, and were found exclusively at cortical
depths between about 700 and 1000μm. This depth corresponds to the granular input layer IV of the visual cortex, where signals arrive from the lateral geniculate nucleus. We computed the mean normalized locking ratio as a function of recording depth for spiking activity at the three monitor refresh-rates. As shown in Figure 3C, locking was generally strongest for layer IV units, whereas locking was weakest for supragranular layer units. Interestingly, locking for infragranular units appeared to depend on monitor refresh-rate, such that it was relatively weak for 90 and 120Hz, but elevated for 60Hz. Since the deep layers communicate with subcortical areas, this enhanced entrainment may result from a frequency dependent interaction with other prominent visual structures such as the superior colliculus or pulvinar (Grieve et al., 2000; Shipp, 2004). The variation of locking ratios with recording depth for the LFP is shown in Figure 3D. As for spiking activity, LFP locking ratios were highest in layer IV regardless of monitor refresh-rate. These results reveal substantial differences between the cortical layers in terms of temporal locking to the sensory stimulus.

Effect of background redraw on neural activity

Given the strong effects of monitor refresh-rate on timing of spiking and LFP responses, we wanted to examine what impact it had on stimulus evoked neural activity. We noticed that many units showed an entrained response when the “black” or “white” stimulus was presented at a location outside the receptive field, and only the gray background was present inside the receptive field. This “background response” occurred in response to luminance transients associated with the redrawning of the intermediate luminance gray background. In Figure 4A, we show the PSTHs for this “background response” for an example single unit at the three different refresh-rates. It is clearly visible, that at 60 Hz the unit responded to every single refresh of the background, whereas at 120 Hz this “background response” is virtually absent. The example unit had the following average “background modulation ratio” (BMR: modulation amplitude/mean firing rate): $BMR_{60}$: 0.60, $BMR_{90}$: 3.06, $BMR_{120}$: 5.49. Across the population, we observed a
similar pattern (mean ± SEM: $BMR_{120}$: 1.0 ± 0.11, $BMR_{90}$: 2.2 ± 0.13, $BMR_{60}$: 3.5 ± 0.24), and this effect was significant (One-Way ANOVA: $p << 0.001$).

The local field potentials showed the same trend (data not shown): (mean ± SEM: $BMR_{120}$: 0.14 ± 0.010, $BMR_{90}$: 0.82 ± 0.041, $BR_{60}$: 1.39 ± 0.057). A one-Way ANOVA showed this difference was significant ($p << 0.001$). These findings suggest that the redraw of the gray background is robustly reflected in V1 activity at 60 Hz but not at 120 Hz refresh-rate. Figure 4B shows a PSTH of the same single unit over all trials where a black or white dot was presented in the receptive field. At times preceding the visual response delay, the background response from the previous trial is clearly visible. For the majority of the remaining analyses, except where noted, we subtract the trial averaged background response from the PSTH and VEP (see methods) as an approximation of the pure stimulus evoked activity for both spiking and LFP. We note that the background subtraction does not affect the significance of any of the reported results, which are valid similarly for the original data without background activity subtracted. The results for the same example recording site described above are shown in Figure 5 for “white” and “black” stimuli separately. As can be seen, little neural activity is visible at times preceding the visual response latency of approximately 25ms, because of the subtraction of the background response. There are however some small amplitude fluctuations, for example at 60Hz refresh-rate, which are remnants of the background activity that were not completely removed by the subtraction procedure. Below, we continue to analyze the temporal dynamics of this evoked activity.

**Monitor refresh-rate dependent response dynamics of neural activity**

We observed striking differences in neural response dynamics for the different monitor refresh-rates. The PSTH of an example single unit, shown in Figure 5A, reveals that spikes were indeed strongly entrained to the screen refresh, particularly at 60 and 90Hz, consistent with the above quantitative analyses. Entrainment occurred for both “white” and “black” stimuli. At 60Hz,
individual CRT luminance impulses elicited neural responses that were almost completely separable from each other in time. By contrast, at 120 Hz there was considerable overlap between neural activation peaks elicited by subsequent screen refreshes. Interestingly, these overlapping peaks add up in a highly non-linear fashion: Responses to subsequent screen refreshes at 120Hz are strongly attenuated, unlike in the 60Hz condition, where every frame elicits responses of approximately equal magnitude. To quantify this response attenuation, we computed the slope $m$ of a fit through the individual response peaks in a time window from about 50ms to 110ms following stimulus onset. For the example neuron from Figure 5A, we obtained the following values: $m_{120,\text{white}} = -2.9$, $m_{120,\text{black}} = -7.4$, $m_{90,\text{white}} = -3.0$, $m_{90,\text{black}} = -5.2$, $m_{60,\text{white}} = -0.1$, $m_{60,\text{black}} = -1.7$ Hz/ms. These values were similar to the average values across all recorded units, shown in Figure 6A. A two-Way ANOVA using white/black and refresh-rates as factors revealed significant effects of refresh-rate ($p << 0.001$) and of black/white ($p < 0.01$) on neuronal response attenuation.

The above analyses indicate that neural activity remained relatively constant for subsequent screen refreshes at 60Hz, but declined rapidly at higher refresh-rates. Indeed, at the end of the visual response period around 110ms after stimulus onset, only a fraction of the maximum response to the luminance impulse at the beginning of the visual stimulation period remained ($RR_{120,\text{white}} = 5\%$, $RR_{120,\text{black}} = 9\%$, $RR_{90,\text{white}} = 16\%$, $RR_{90,\text{black}} = 34\%$, $RR_{60,\text{white}} = 55\%$ and $RR_{60,\text{black}} = 67\%$) compared to the activity of the highest peak for the example neuron (Figure 5A). These values were similar to the average values across all recorded units, shown in Figure 6B. According to a two-Way ANOVA using white/black and refresh-rate as factors, the effect of refresh-rate was significant ($p << 0.001$) but there was no main effect of black/white ($p > 0.1$). Together, these findings suggest that there was minimal neural response decline at 60Hz refresh-rate, indicative of near independence of neural responses to subsequent screen refreshes. On the contrary, the strong decline observed at 90 and 120Hz suggests a strong
nonlinear dependence among neural responses to luminance impulses generated by subsequent screen refreshes.

Given these strong effects of refresh-rate on temporal dynamics of spiking responses, we proceeded to examine whether a similar effect was visible in LFP signals. We found that this was indeed the case: LFP signals from the same cortical site as the spiking activity analyzed above are shown in Figure 5B as a function of monitor refresh-rate. Similar to what we observed for spiking activity, there was relatively little overlap between LFP activations elicited by subsequent screen refreshes at 60Hz, where each refresh elicits a clearly separate signal peak. At 120Hz, peaks are not clearly discernible, but merged together into a single nearly continuous response. We quantified refresh-rate dependent LFP attenuation by computing the attenuation slope $m$ in a time window from about 50ms to 110ms following stimulus onset, similar to our analysis of spiking attenuation above. Note that attenuation in LFP signals is associated with positive values of the parameter $m$. For the recording site in Figure 5B, we obtained the following values: $m_{120,white} = 0.085$, $m_{120,black} = 0.111$, $m_{90,white} = 0.027$, $m_{90,black} = 0.041$, $m_{60,white} = -0.003$, $m_{60,black} = 0.010$ ms$^{-1}$. These values were similar to the average values across all LFP sites, as shown in Figure 6C. A two-Way ANOVA using white/black and refresh-rates as factors revealed a significant effect of both refresh-rate ($p << 0.001$) and black/white ($p = 0.02$). At the end of the visual response period around 110ms after stimulus onset, the remaining response to a single screen refresh was $RR_{120,white} = 11\%$, $RR_{120,black} = 1\%$, $RR_{90,white} = 8\%$, $RR_{90,black} = 7\%$, $RR_{60,white} = 89\%$, $RR_{60,black} = 67\%$ compared to the activity to the initial peak activity for the example LFP site (Figure 5B). These values were similar to the average values across all LFP sites, as shown in Figure 6D. According to a two-Way ANOVA using white/black and refresh-rate as factors, the effect of refresh-rate was significant ($p << 0.001$) but there was no main effect of black/white ($p > 0.1$).
Effect of monitor refresh-rate on average response magnitude and latency

In addition to the effects on temporal dynamics, we also found that CRT refresh-rate had a large impact on firing rate and visual response latency. The mean firing rate, analyzed without background response subtraction, during the visual response period (25 to 110ms after stimulus onset) averaged across all recorded units, is shown in Figure 7A, as a function of refresh-rate and black/white. The magnitude of the mean background response is shown in gray bars as a function of refresh rate for comparison. Since mean firing rate is positively correlated with refresh-rate, V1 acts like a high-pass filter for sparse noise stimuli as a function of refresh-rate. Interestingly, the opposite is true for activity during stimulation with the uniform gray background. Here, the mean firing rate significantly decreases with increasing refresh-rate (one-Way ANOVAs, \( p < 0.01 \)), consistent with a low-pass filtering property of V1 in this case. A two-Way ANOVA revealed significant effects of both refresh-rate and black/white (\( p << 0.001 \)) on mean firing rate, with “white” stimuli evoking less activity than “black” stimuli. These reductions in activity for “white” compared to “black” stimuli were most pronounced at 60 Hz (\( R_{120}: 29\% \), \( R_{90}: 26\% \), \( R_{60}: 60\% \)).

We observed a similar pattern of results in the LFP, as shown in Figure 7B. A two-Way ANOVA revealed significant effects for both refresh-rate and black/white (\( p < 0.001 \)), with “black” LFP responses dominating “white” responses. Activity reductions for “white” stimuli were most notable at 60Hz (\( R_{120}: 24\% \), \( R_{90}: 23\% \), \( R_{60}: 79\% \)). The mean background activity was similar for the different refresh-rates (one-Way ANOVA, \( p = 0.1 \)).

Examining how cortical layer affected “black” dominance in spiking activity, we calculated a white/black ratio (WBR) for each unit (see Methods), and compared its average value between supragranular (n=23) and granular (n=19) layers. We found significantly greater “black” dominance in supragranular layers than in granular layer IV (averages: WBR\(_{120,\text{supragranular}}\): -0.53 \( \pm 0.08 \), WBR\(_{120,\text{granular}}\): -0.23 \( \pm 0.06 \), WBR\(_{90,\text{supragranular}}\): -0.53 \( \pm 0.07 \), WBR\(_{90,\text{granular}}\): -0.26 \( \pm 0.05 \),
WBR_{60, supragranular} = -1.61 ± 0.15, WBR_{60, granular} = -0.77 ± 0.08) consistently for all three monitor refresh-rates (unpaired t-tests, p < 0.01).

In addition to eliciting stronger responses, “black” stimuli also activated V1 neurons earlier than “white” stimuli. To quantify this effect, we computed the response latency for each unit and LFP site (see Methods). The resulting mean response latencies are shown in Figure 7C for spiking activity (Number of units with computable latency: n_{120, white} = 51, n_{120, black} = 47, n_{90, white} = 48, n_{90, black} = 48, n_{60, white} = 25, n_{60, black} = 45 of 52). “Black” responses had shorter latencies than “white” responses at all refresh-rates. Importantly, response latencies were negatively correlated with refresh-rate. At 60Hz refresh-rate, responses occurred around 15ms later than at 120Hz. A two-way ANOVA revealed the significance of both effects of black/white and refresh-rate (p << 0.001). The LFP latency results, shown in Figure 7D, followed exactly the same pattern (Two-way ANOVA: effects of refresh-rate and black/white, p << 0.001). (Number of locations with computable latency: n_{120, white} = 50, n_{120, black} = 51, n_{90, white} = 52, n_{90, black} = 52, n_{60, white} = 17, n_{60, black} = 50 of 52). As for spiking, “black” stimuli evoked LFP responses faster than “white” stimuli, and LFP response latency was negatively correlated with refresh-rate.

**Discussion**

We have demonstrated that spiking responses in tree shrew V1 become entrained to the CRT monitor screen refresh. Entrainment was strongest at 60Hz refresh-rate, and progressively declined with increasing refresh-rate, although we still observed significant entrainment even at 120Hz. Our results parallel observations in cats, monkeys and humans, which have reported entrainment of neural responses at refresh-rates up to 100Hz (Wollman and Palmer, 1995; Gur and Snodderly, 1997; Lyskov et al., 1998; Krolak-Salmon et al., 2003; Williams et al., 2004). The presence of entrainment in these diverse species suggests that it is probably inherited from a common ancestor, which must have lived at least 85 million years ago (Liu et al., 2001;
Murphy et al., 2001; Janecka et al., 2007). Entrainment is a reflection of the high temporal precision of the visual system, which allows precise speed and distance judgments, and was probably conserved by evolution for these reasons. We extend the previously observed temporal precision results by showing that the LFP, like spiking activity, is also strongly locked to the monitor refresh. LFP locking was strongly correlated with locking of spiking activity at V1 sites. The LFP is a mass signal resulting from addition of electrical dipoles caused by synaptic inputs onto the dendrites of pyramidal cells. By contrast, spiking activity is related to action potential generation within V1 itself, and thus reflects local computations in V1 neural circuits. Highly correlated and robust locking of both LFP and spiking signals is consistent with recent reports describing close correspondence of spiking and LFP in macaque V1 in terms of receptive field characteristics (Xing et al., 2009). The generally larger locking ratios of LFPs could be due to input from the visual thalamus, which has been shown to exhibit even stronger locking to visual transients than visual cortex (Wollman and Palmer, 1995). We cannot, however, exclude methodological reasons for this quantitative difference because of the different nature of the two signals: continuous LFPs and binary spike trains.

Since these strongly entrained sensory afferent responses arrive in cortex in the granular layer IV, one might also expect stronger entrainment in this layer. This is exactly what we found, also in line with previous findings in macaque monkeys (Williams et al., 2004): Entrainment was indeed weaker in supra- and infragranular layers, consistent with the idea that cortical processing tends to temporally broaden responses by inhibitory, horizontal and feedback circuitry (Hawken et al., 1996; Oram, 2010).

The high degree of entrainment even at 120Hz suggests that the tree shrew visual system may be especially sensitive to high temporal frequencies, which would fit well with the lifestyle of this fast moving, arboreal animal. Indeed, tree shrews are able to discern visual temporal modulation (luminance flicker) at frequencies up to about 70Hz, with an optimal frequency of
about 15Hz (Callahan and Petry, 2000). These values exceed those obtained in human
subjects, where optimal frequency for luminance flicker detection is 10Hz and flicker becomes
undetectable above about 40Hz (Pantle, 1971). In contrast to these psychophysical findings,
novel signals in the human visual cortex encode temporal stimulus modulations at frequencies
exceeding 70Hz (Williams et al., 2004). Thus, consistently in both species, the frequency where
temporal modulations can no longer be perceived is substantially lower than the frequency at
which neural entrainment in V1 becomes undetectable. Similarly, visual cortex in both monkeys
and humans responds to chromatic flicker at frequencies above about 25Hz, but the two
flickering colors are perceived as a monochromatic stimulus (Gur and Snodderly, 1997; Jiang et
al., 2007). Accordingly, visual stimulation at high temporal frequencies causes significant
entrainment in cortical responses that appears to remain outside of conscious awareness.
Note, that even though such fast changes remain imperceptible, they can still influence the
perception of subsequently presented visual patterns (Falconbridge et al., 2010).
Taken together, the sensitivity for temporal visual stimulus modulations appears to be greater in
tree shrew than in humans or macaque monkeys. Apart from this however, there exist close
parallels between tree shrew and primate species in terms of perception and neural entrainment
to CRT-generated sequences of luminance transients like the contrast and layer dependence.

We found substantial differences in onset latency of visual responses as a function of monitor
refresh-rate: Shortest onset latencies were observed for 120Hz refresh-rate, and reductions in
refresh-rate were associated with increases in onset latency. These effects were large in
magnitude: At 60Hz, onset latencies were about 15ms greater than at 120Hz, corresponding to
an increase of about 30%. Previous work has demonstrated that stimulus attributes such as
size, contrast and spatial frequency have an impact on onset latencies in macaque and human
visual cortex (Gawne et al., 1996; Mazer et al., 2002). We show that, maintaining identical visual
stimulus attributes, variation of the refresh-rate had a large impact on the onset-latencies of V1
neural responses. To our knowledge, effects of stimulation frequency on onset latency have not been systematically investigated in monkeys or humans. However, we suggest that they may be related to psychophysical findings in human subjects: A recent study has described a 9ms increase in reaction times to stimuli flickering at 70Hz compared to identical stimuli flickering at 140Hz (van Diepen et al., 2010). We suggest that slower arrival of neural activity in V1 at 70Hz refresh-rate, as evidenced by increased onset latencies, might be the reason for this behavioral slowing in reaction time. This emphasizes that refresh-rate is an important parameter with large impact on neural response dynamics and probably also behavioral response times.

We observed that on a gray background of intermediate luminance, light decrements (“black” stimuli) elicited faster responses than light increments (“white” stimuli). One possible reason for this could be that, technically, “black” stimuli could be considered to begin with the decay of the preceding luminance impulse, and thus almost one screen refresh earlier than “white” stimuli. We consider this unlikely, because black responses are also highly transient in nature, and appear to be evoked by the local contrast generated by the continuous “black” stimulus embedded in the surrounding luminance impulses corresponding to the gray background that are also present in the receptive field. The local contrast induced by the “black” stimulus thus occurs at exactly the same time as the “white” stimulus, suggesting that the observed latency differences are not artifacts of stimulus delivery.

In addition to these effects on latency, we have also demonstrated that “black” stimuli elicit larger responses than “white” stimuli, paralleling observations in macaque monkeys, where a similar dominance of “black” responses has been reported for both spiking and LFP (Yeh et al., 2009; Xing et al., 2010). These findings are thought to be related to the improved performance for “black” compared to “white” stimuli of human subjects in various psychophysical tasks (Short, 1966; Krauskopf, 1980; Whittle, 1986; Bowen et al., 1989; Buchner and Baumgartner, 2007). Interestingly, our results show that black/white differences are strongly dependent on
refresh-rate and cortical layer. V1 input layer activity is already biased towards stronger responses to black stimuli, and this difference is enhanced in the supragranular layers that mediate cortico-cortical communication. This enhancement is largely independent of refresh-rate, and approximately doubles the black preference. It is likely to be the result of a cortical mechanism (Yeh et al., 2009; Xing et al., 2010). The black stimulus bias in the input layer IV however is dependent on refresh-rate, and is about three times larger at 60Hz than at 120Hz. This input layer bias could result from local layer IV processing or be inherited from the retina or visual thalamus. The LFP results are consistent with these ideas: At 60Hz, black dominance in the LFP is greatly enhanced, suggesting that the black dominance may already be present in the inputs to V1. In addition, weaker black preference in the LFP compared to spikes at higher refresh rates is expected for a local cortical mechanism, since LFPs are thought to largely reflect synaptic inputs (Rainer, 2008).

A striking aspect of our results is the large difference in response attenuation we observed at the different refresh-rates. At 120Hz, both spiking and LFP responses were rapidly and strongly attenuated during visual stimulation with luminance impulses during a period of 80ms duration. Each subsequent screen refresh evoked less activity than the preceding one, until at the end of the 80ms stimulation period the response had declined by about 75%. This response attenuation may have an important functional role in contributing to adaptation, which dampens responses to static elements in the visual input, and assigns brain resources to moving or changing elements of the visual environment that are likely to have behavioral relevance (Tolias et al., 2005; Kohn, 2007). At 120Hz, the visual stimulus delivery elicits strong attenuation, as would be the case for real visual stimulation outside the laboratory, which is of continuous nature and not composed of luminance transients. By contrast, at 60Hz refresh-rate we observed very little attenuation, and responses to subsequent luminance impulses were nearly independent. The different attenuation regimes at 60Hz and 120Hz refresh-rate might in fact explain why the “background response” was present at 60Hz but not 120Hz. At 120Hz, neural
activity has completely attenuated to the luminance impulses that make up the gray
background, whereas at 60Hz each luminance impulse is reflected in neural activity because
there is very little attenuation. Our results have potential implications for fMRI studies, where
adaptation is employed as a tool for probing cortical representations (Grill-Spector and Malach,
2001). We suggest that the importance of monitor refresh-rate on fMRI responses and their
adaptation may have been severely underestimated, and might have a substantial impact on
observed fMRI responses in visual cortex. In particular, we suggest that at least in V1,
adaptation will play a minor role at 60Hz and a prominent role at 120Hz. Parametric variation of
refresh-rates could provide substantial new insights into visual representations in the human
visual cortex. However, adaptation is only one possible candidate mechanism that might explain
the non-linear response attenuation we have observed. For example, conceptualizing this
response attenuation as a non-linear interaction between the flash and background responses
might be a good alternative model to capture the observed dynamics in neural activity.
Taken together, we have demonstrated that CRT monitor refresh-rate has a strong influence on
neural response dynamics in tree shrew V1. Neural response parameters such as onset
latency, temporal profile and temporal alignment of spikes were all strongly affected by the
refresh-rate. Variation of these timing parameters due to factors such as stimulus type, contrast
or spatial frequency have led to the idea that they might play an important role in neural coding
(Optican and Richmond, 1987; Richmond et al., 1987; Richmond and Optican, 1987, 1990;
Victor and Purpura, 1996; Reich et al., 2001; Oram et al., 2002). The large variation in these
parameters caused by changes in refresh-rate for otherwise identical stimuli casts some doubt
on the importance of the above neural response parameters for neural coding and
representation of visual stimuli. Our findings rather suggest that visual representations may in
fact be relatively invariant to changes in many aspects of temporal neural response dynamics.
We have documented a number of close similarities between V1 of tree shrew and that of
primates such as macaque monkeys and humans. This suggests that mechanisms of
information processing in tree shrew resemble those of primates in several important aspects
concerning the temporal precision to visual stimulation, highlighting the usefulness of this small
mammal for understanding visual function.

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References


**Figure Legends:**

**Figure 1.** Depth reconstruction and paradigm. 

**A:** Projection of an electrode track is shown onto a 50 μm thick Nissl-stained coronal section of the primary visual cortex, with Layer IV clearly visible in dark staining. One electrolytic lesion, marked by the dotted circle, is visible in this section.

**B:** Sparse noise consists of dark or bright squares presented in pseudorandom order on a 15×15 position rectangular grid spanning 10×10 visual degrees over an intermediate luminance background shown for ~80ms with no inter stimulus time.

**C:** Receptive fields for white and black stimuli are shown in the left and right panel for an example single unit. The inner white lines determine the area that is considered to be within the receptive field. Every trial that showed a stimulus outside the outer white line was considered a background trial as there was only gray inside the receptive field and its immediate surround.

**D:** Same as C for an example multi unit from the same animal but at a different recording location.

**Figure 2.** Entrainment of neural responses to CRT monitor refresh. 

**A:** Luminance profile of the CRT monitor at the three examined refresh-rates. Brief luminance peaks occur every 8.3, 11.1 and 16.6 ms for refresh-rates of 120, 90 and 60Hz, respectively.

**B:** Raster plot of 20 trials of spikes recorded from one example V1 neuron in response to sparse-noise stimulation at the various refresh-rates. Every line corresponds to one trial and every tick to one spike.

**C:** Ten single trial LFP amplitude traces from the same recording site.

**Figure 3.** Quantitative analysis of entrainment to monitor refresh. 

**A:** Bar plots of the mean locking ratio of spiking and LFP activity at the 52 recorded sites at the three different refresh-rates. The dotted line signifies the 99% significance level as determined by a bootstrapping method. Error bars: SEM.

**B:** The Locking ratios of individual units plotted against cortical depth for the 90 Hz refresh-rate. Units with locking ratios exceeding a criterion value of η=9 are
highlighted in black. The dotted lines represent the approximate borders of layer IV. **C:** The normalized running averages (of 9 neighboring units) of the locking ratios of spiking activity are plotted against cortical depth for the three refresh-rates. The dotted lines represent the approximate borders of layer IV. **D:** Same as (C) for LFP activity.

**Figure 4.** Effects of CRT refresh-rate on neural responses. **A:** Average spiking responses of one example neuron to trials showing only gray background in the receptive field at all refresh-rates. Dotted lines represent stimulus on- and offset. **B:** Average spiking responses of the same example neuron to “black” stimuli (black traces) and “white” sparse noise stimuli (gray traces) shown in the receptive field. Dotted lines represent stimulus on- and offset.

**Figure 5.** Effects of CRT refresh-rate on time course and attenuation of stimulus related neural responses. **A:** Average spiking response time courses of one example neuron at 1ms resolution plotted separately for “black” stimuli (black traces) and “white” stimuli (gray traces) with the average background response subtracted to approximate pure stimulus-related activity. The thick black and gray bars are linear fits through the response peaks to determine the slope of the decline of neural activity as a measure of attenuation. Dotted lines represent stimulus on- and offset. **B:** Same as (A) for trial averaged LFP activity at the same cortical location as (A).

**Figure 6.** Attenuation: Population Analysis. **A:** Average values for the slope of a linear fit through the individual peaks of the PSTH for each of the 52 recorded units, separately for “black” (black bars) and “white” (white bars) responses at all refresh-rates. Error bars: SEM. **B:** Ratio of the value for a peak at the end of the response (around 110ms) to the value of the peak at the maximum of the response (around 40ms). Low values indicate high attenuation because the response declines to repeated frames of the stimulus. High values indicate near
independence of the peaks evoked by repeated monitor refreshes. Error bars: SEM. C: Same as A for a fit through the peaks of the visual evoked potential. D: Same as (B) for the LFP.

Figure 7. Latency and Amplitude of neural responses to “white” and “black” stimuli depend on refresh-rate. A: Average firing rate in a window from 25ms to 110ms after stimulus onset for the entire population of 52 units is shown for the three refresh-rates separately for “background” (gray bars), “white” (white bars) and “black” (black bars) stimuli. Error bars: SEM. B: Same as (A) for the average LFP amplitude. C: Population averaged response latencies of spiking activity shown for “black” and “white” stimuli separately at the three different refresh-rates. (n = 51, 47, 48, 48, 25, 35 of 52 from left to right) Error bars: SEM. D: Same as (C) for LFP latencies (n = 50, 51, 52, 52, 17, 50 of 52 from left to right).
A

Cortical Depth

Projection of Penetration

25°

Lesion Depth [μm]

Reconstructed Depth [μm]

Cortical Depth [μm]

B

Time

~80 ms

~10°

D

Single Unit @ ~820μm

White RF

Black RF

Multi Unit @ ~910μm

White RF

Black RF
Spikes
LFPs

0
5
10
15
20
25

Locking Ratio

120 Hz
90 Hz
60 Hz

A  Locking Ratios

Spikes  LFPs

0
5
10
15

Locking Ratio

500
1000
1500

Cortical Depth [μm]

B  Locking Ratio in Depth for 90Hz

0  5  10  15

Locking Ratio

500
1000
1500

Cortical Depth [μm]

C  Spikes: Normalized Running Average

D  LFPs: Normalized Running Average

0  0.2  0.4  0.6  0.8  1

Normalized Locking Ratio

Normalized Locking Ratio

Cortical Depth [μm]