Article

Distinct Frequency Specialization for Detecting Dark Transients in Humans and Tree Shrews

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SUMMARY

Despite well-known privileged perception of dark over light stimuli, it is unknown to what extent this dark dominance is maintained when visual transients occur in rapid succession, for example, during perception of moving stimuli. Here, we address this question using dark and light transients presented at different flicker frequencies. Although both human participants and tree shrews exhibited dark dominance for temporally modulated transients, these occurred at different flicker frequencies, namely, at 11 Hz in humans and 40 Hz and higher in tree shrews. Tree shrew V1 neuronal activity confirmed that differences between light and dark flicker were maximal at 40 Hz, corresponding closely to behavioral findings. These findings suggest large differences in flicker perception between humans and tree shrews, which may be related to the lifestyle of these species. A specialization for detecting dark transients at high temporal frequencies may thus be adaptive for tree shrews, which are particularly fast-moving small mammals.

INTRODUCTION

Converging studies have demonstrated that ON and OFF dominant neurons in the early visual system, as well as the primary visual cortex (V1), exhibit an asymmetric pattern of activity in terms of response gain and response latency (Jin et al., 2011; Veit et al., 2011; Yeh et al., 2009). Behaviorally, the differential spatial visual resolution of light and dark stimuli has long been known to physicists and astronomers and has been documented by Galilei (1632), who reported the observation that a dark patch on a light background seems smaller than a same-sized light patch on a dark background. This observation, named the irradiation illusion by von Helmholtz (1867), has been the basis for many studies examining the differences in the perception of light and dark stimuli. These studies have largely focused on spatial aspects of differences between light and dark stimuli at the level of behavioral perception (Blackwell, 1946; Buchner and Baumgartner, 2007; Komban et al., 2011; Lu and Sperling, 2012) and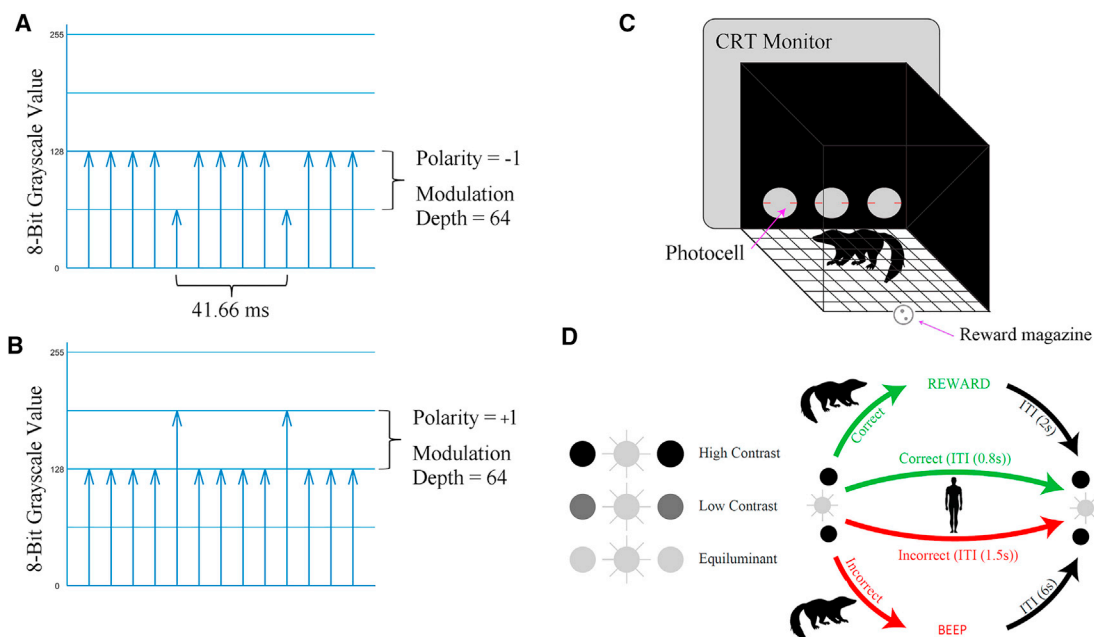 evoked neural activity (Kremkow et al., 2014; Liu and Yao, 2014; Zaghloul et al., 2003; Zemon et al., 1988) and suggested that neuronal nonlinearity is the underlying mechanism for the greater visual spatial resolution for dark stimuli than light stimuli (Kremkow et al., 2014; Ratliff et al., 2010). However, temporal aspects of the behavioral and neuronal differences in the processing of light and dark stimuli have been relatively less the focus of research. These studies have generally been limited to the dynamics and time course of behavioral (Komban et al., 2014) and neuronal responses to light and dark stimuli (Gollisch and Meister, 2008; Jin et al., 2011; Komban et al., 2014; Rekauzke et al., 2016). However, natural visual scenes comprise a range of both spatial and temporal frequency information, and the temporal resolution of ON/OFF neuronal channels of a species has direct implications for the efficiency of perception of moving targets. However, unlike differential visual spatial resolution of light and dark stimuli, whether such ON/OFF asymmetry induces differential visual perception and neuronal activity in response to temporally varying visual stimuli is unknown.

We address this question in the current study using flickering stimuli with luminance increments or decrements at different frequencies in human participants and tree shrews. Tree shrews (*Tupaia belangeri*) are close relatives of primates and are slender, small, day-active, and fast-moving animals with a well-developed visual system (Callahan and Petry, 2000; Fitzpatrick, 1996; Martin, 1968; Schafer, 1969). Sinusoidally modulated flickering stimuli have traditionally been used to investigate visual temporal resolution and to estimate temporal contrast sensitivity functions in different species (for a review, see Jarvis et al., 2003). Sinusoidally modulated stimuli span luminance increments and decrements around a mean luminance. Taking advantage of flickering stimuli to separately study behavioral and neuronal responses to temporally varying light and dark stimuli, we generated distinct light and dark flickering stimuli by implementing brief impulses of luminance deviations from an intermediate gray background. Our results demonstrate a differential visual temporal resolution for temporally varying light and dark stimuli in human participants and tree shrews, albeit at different frequencies. We also demonstrate a neural correlate for such differences in the V1 of tree shrews.

RESULTS

We used impulses of transient increments or decrements of luminance from a midgray background to generate visual

Figure 1. Examples of Visual Flicker Stimuli and Experimental Procedure

(A) Example of a visual flicker stimulus generated by transient impulses and used in our experiments. In this example, every fifth frame of a monitor with a refresh rate of 120 Hz is a transient impulse that produces a flicker frequency of 24 Hz. The transient has a polarity of −1, because it is a decrease from the midgray level. The modulation depth is 0.5 (or 64 in the intensity scale).

(B) Similar to (A), except with a positive polarity, because it shows transient increments at the 24 Hz flicker frequency.

(C) Experimental setup used for tree shrews. Visual stimuli were presented on a cathode ray tube (CRT) monitor. Tree shrews reported their response by poking their nose in one of the three holes. Photocells positioned in each nose hole detected the animals' responses.
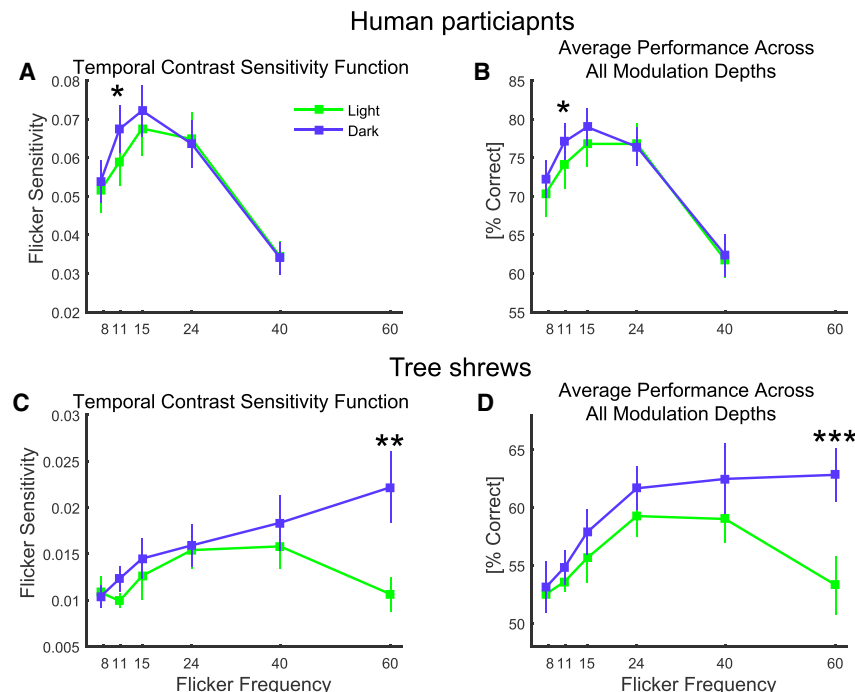
(D) Type of stimuli that were used during the training of tree shrews (left panel). Middle column shows the target flickering stimulus, and flanking columns show the change of distractor contrast from very high contrast to zero contrast at the end of training. The sequence of a trial for tree shrews and human participants is illustrated in the right panel.

flickering stimuli (range tested, 7.5 to 60 Hz) (Figure 1). This approach allowed us to estimate behavioral and neuronal visual temporal resolution separately for both contrast change polarities (increments and decrements) at different flicker frequencies.

Polarity-Dependent Temporal Characteristics of Contrast Sensitivity in Human Participants

Participants performed a 3-alternative forced-choice (3AFC) flicker detection task. On every trial, a flickering stimulus, with particular values of three independent variables (flicker frequency, modulation depth, and polarity), was presented at a randomly selected location (the target location) and two equi-luminant distractors were presented at the remaining two locations. The thresholds at 67% correct performance were estimated from psychometric fits (Figure S1A). The estimated thresholds of individual participants were used for statistical testing. We observed lower thresholds and therefore greater sensitivity for luminance decrements than luminance increments, particularly in the lower range of flicker frequencies. Human participants were most sensitive (indicated by the inverse of threshold) to flicker around 15 Hz for both dark and light stimuli (Figure 2A), which is in the range of previously reported values of about 10–15 Hz for sinusoidal flicker (Jarvis et al., 2003). Two-way repeated-measures ANOVA revealed a significant main effect of frequency ($F_{4, 32} = 31.53$; $p < 0.0001$) but not po-

larity ($p > 0.1$) on participants' thresholds. However, the same analysis revealed a significant frequency-polarity interaction ($F_{4, 32} = 2.85$; $p < 0.05$), and post hoc tests revealed that the only significant difference between the two polarities occurred at the flicker frequency of 10.9 Hz. As an alternative analysis, bootstrapping estimates of thresholds from psychometric fits on the averaged data confirmed a significant difference ($p < 0.05$) solely at the flicker frequency of 10.9 Hz. Threshold is a temporal sensitivity measure representing the midway of a critical range of modulation depth in which the sharpest changes of perception take place. We also analyzed an alternative measure by summing average performance at each of six modulation depths tested. This measure is independent of fitting psychometric functions and produces values that are equivalent to the area under the performance versus modulation depth curve. As shown in Figure 2B, the temporal sensitivity patterns estimated by this measure were similar to those drawn from the psychometric functions, exhibiting main effects of frequency ($F_{4, 32} = 30.20$; $p < 0.0001$) and polarity ($F_{1, 8} = 4.64$; $p < 0.05$), as well as a significant difference between two polarities exclusively at the flicker frequency of 10.9 Hz. Furthermore, we conducted two-way repeated-measures ANOVAs on supra-threshold performances (three, two, and one highest modulation depths) (Figure S2) and showed a significant main effect of frequency ($p < 0.001$) but no significant difference between dark

Human particiapnts

Figure 2. Temporal Modulation Sensitivity for Light and Dark Flicker in the Human Participants and Tree Shrews

(A) Temporal contrast sensitivity function based on the thresholds estimated from the psychometric functions fitted for individual subjects. The sensitivities to two polarities are significantly different only at 11 Hz.

(B) Average performance across all modulation depths showing a qualitatively similar pattern to that drawn from the psychometric function estimation.

(C and D) Analogous graphs illustrating a temporal sensitivity pattern for light and dark flicker in tree shrews. The temporal sensitivity curves based on the thresholds (C) and mean performance across modulation depths (D) show intermediate peak sensitivity from 24 to 40 Hz for the light flicker and peak sensitivity at 60 Hz for the dark flicker.

Error bars indicate SEM. *p < 0.05; **p < 0.01; ***p < 0.001.

and light stimuli (p > 0.35) and no significant polarity × frequency interaction (p > 0.1).

We then examined reaction times (RTs) (Figure 3). RT data are usually not normally distributed, as was the case for our data, so we employed non-parametric statistical tests. First, we used the aligned rank transform for non-parametric factorial analyses (Wobbrock et al., 2011), which revealed significant main effects of frequency and modulation, as well as frequency × polarity, modulation × polarity, and frequency × modulation interactions (p < 0.001 and p < 0.001, as well as p < 0.05, p < 0.01, and p < 0.01, respectively). This test was complemented by non-parametric Wilcoxson signed rank tests on average RTs of light and dark stimuli at different frequencies and modulation levels. As expected, there is a clear reduction in response time as the modulation depth increases (Figure 3A). Our results suggest that participants could detect dark flicker faster than light flicker across tested flicker frequencies except 10.9 Hz (Figures 3A and 3B). This was exactly the flicker frequency for which there was a significant difference between two polarities in response accuracy. This suggests a general advantage of the human visual system in detecting dark transients across flicker frequencies, with a trade-off between response speed and accuracy.

Polarity-Dependent Temporal Characteristics of Contrast Sensitivity in Tree Shrews
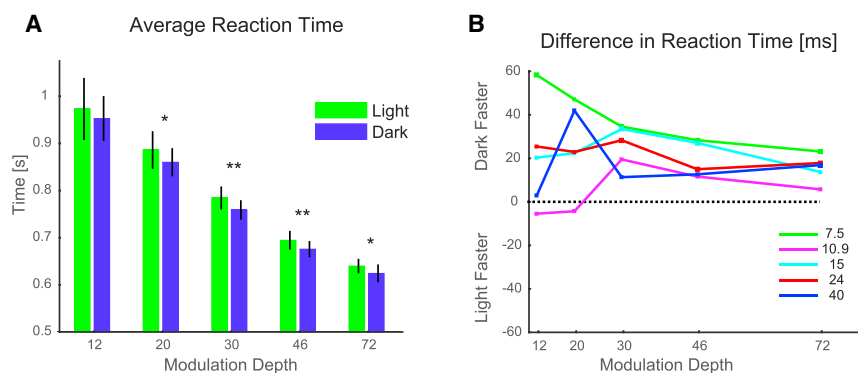
We trained tree shrews extensively on an analogous flicker detection task (Figures 1C and 1D). Due to the higher flicker fusion frequency of tree shrews, an additional level of frequency (60 Hz) was added to the five levels of frequency tested in human participants. The performance pattern of tree shrews resembled an initial sharp rise followed by a plateau or a slow increase. After extensive testing, we found that linear regression of the initial

part of the data, anchored to the chance level, produced the most reliable fits (Figure S1B). We estimated thresholds using this method and generated average temporal sensitivity functions separately for light and dark stimuli. For the light flicker, temporal sensitivity was highest at the intermediate frequencies (between 24 and 40 Hz), while the sensitivity remained at peak values at 60 Hz for the dark flicker (Figure 2C). This was the maximum frequency we could test with our experimental apparatus and is markedly higher than the sensitivity peak obtained previously for tree shrews using sinusoidal flicker (Callahan and Petry, 2000). A two-way repeated-measures ANOVA revealed a significant main effect of frequency ($F_{5, 25} = 4.35$; $p < 0.001$), a significant main effect of polarity ($F_{1, 5} = 7.54$; $p < 0.05$), and a significant frequency × polarity interaction ($F_{5, 25} = 2.61$; $p < 0.05$), with post hoc tests demonstrating a significant difference between the two polarities at 60 Hz ($p < 0.01$).

To obtain a model-free measure of temporal modulation sensitivity, we also analyzed sums of average performances at each of the six modulation depths tested. This was especially important as a corroborative analysis, because the linear fits for tree shrews were generally not as high quality as the psychometric fits for human participants. Figure 2D shows that the result of this analysis closely resembled the threshold-based estimates. Two-way repeated-measures ANOVA revealed a significant main effect of frequency ($F_{5, 25} = 6.05$; $p < 0.001$), a significant main effect of polarity ($F_{1, 5} = 9.39$; $p < 0.01$), and a significant interaction ($F_{5, 25} = 2.80$; $p < 0.05$). Post hoc analyses revealed a significant difference between two polarities at a flicker frequency of 60 Hz ($p < 0.0001$).

Neural Responses to Polarity-Dependent Temporally Modulated Flicker

Neuronal activity results reported here are based on 91 units recorded from the V1 of six tree shrews. Receptive fields (RFs) were mapped (see Supplemental Information for details) using

A Average Reaction Time

B Difference in Reaction Time [ms]

Figure 3. Reaction Time of Human Subjects in a 3AFC Flicker Detection Task
(A) Mean reaction time of human participants at different modulation depths. Error bars indicate SEM.
(B) Curves represent the mean difference in reaction time [light flicker − dark flicker]. Bigger square symbols represent statistical significance between two polarities.

the sparse noise paradigm. Figure S3A shows an example RF mapped using this paradigm. Figures S3B–S3F and S4 display RF properties and transient-sustained index distribution of the population of neurons, respectively. We have recorded mostly ON-OFF neurons, because most of our neurons were from supragranular layers (51) and infragranular layers (23). We had also 13 neurons recorded from the layer IV and 2 neurons with uncertainty about their histologic localization. During RF mapping, we classified the neurons as OFF-dominated and ON-dominated neurons based on the polarity dominance measure (see Supplemental Information for details). As shown in Figure S3C, most neurons (∼86%) were OFF dominated, consistent with the previous findings from our lab (Veit et al., 2014) demonstrating that tree shrew striate cortex is dominated by OFF neurons.

Faster Temporal Changes in Flickering Stimulus Induce Larger Differences between Neuronal Responses to Dark and Light Stimuli
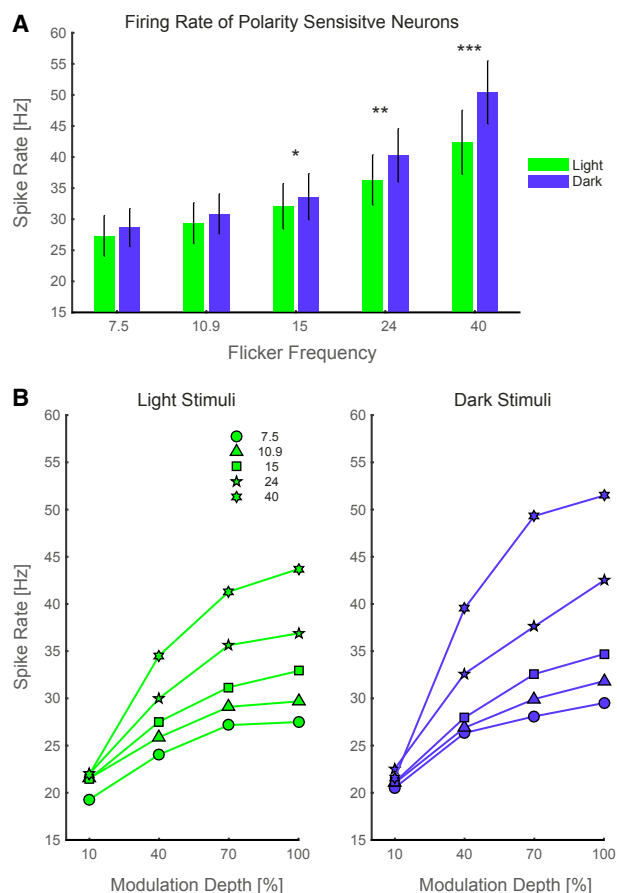Spikes of individual neurons during each trial were converted to firing rate, and repetitions of trials with the two highest modulation depths (i.e., 70% and 100%) were taken and tested for statistical differences. Two-way repeated-measures ANOVAs revealed a significant main effect of frequency in 66 (72.5%) neurons, a significant main effect of polarity in 24 (26.4%) neurons and a significant frequency × polarity interaction in 24 (26.4%) neurons. The union of neurons with significant main effect of polarity and/or significant interaction (i.e., polarity-sensitive neurons) composed 44% (40) of the neurons. Figure 4 shows the average firing rate of polarity-sensitive neurons. As evident in this figure, the difference between the two polarities becomes larger as the stimulus frequency increases. Although the statistical analyses at the level of individual neurons demonstrate that a sizable number of neurons respond to stimulus frequency and polarity, the population response average might cancel out significant effects if the neurons have opposing preferences. Two-way repeated-measures ANOVA on mean spike rate averaged across the population showed a significant main effect of frequency ($F_{4, 360} = 59.8$; $p < 0.001$), a significant main effect of polarity ($F_{1, 90} = 25.5$; $p < 0.001$), and a significant frequency × polarity interaction ($F_{4, 360} = 4.4$; $p < 0.002$). Therefore, both at the level of individual neurons and total variance explained, these neurons show significant differences in response to different fre-

quencies and polarities. Post hoc analyses showed that the significant differences between two polarities exist at frequencies higher than 15 Hz (i.e., 15, 24, and 40 Hz), and the most robust difference is at 40 Hz. Subsequently, to see the effect of modulation depth in conjunction with frequency and polarity, we averaged repetitions of firing rates separately for all stimulus conditions including all modulation depths using a three-factor within-subject ANOVA. The analysis revealed significant main effects of all three factors (frequency, $p < 0.001$; modulation, $p < 0.001$; and polarity, $p < 0.001$) and significant two-way interactions among them (frequency × modulation, $p < 0.001$; frequency × polarity, $p < 0.01$; and modulation × polarity, $p < 0.001$). Figure 4B depicts how the interaction of all three factors influences the average neuronal firing rate.

Neuronal Responses to a Single Transient of Increment or Decrement in Luminance at Different Temporal Frequencies
The larger differences in neuronal firing rate between dark and light flicker at higher temporal frequencies might result from the higher number of luminance impulses at these frequencies. Therefore, we wanted to see whether there are differences in neural responses (peri-stimulus time histograms [PSTHs]) to a single impulse of transient change in luminance as a function of flicker frequency and polarity. Figure 5A shows raster plots and spike histograms of a representative neuron at three frequencies and both polarities (see Figure S5 for other example units). To quantify the response of neurons to each transient impulse, we selected a segment of response starting at the timestamp of the respective transient impulse (Figure 5B) and identified the first two consecutive time bins in which neural activity surpassed the threshold. Subsequently, we found the closest peak or peaks near these two points. Within a 13 ms segment encompassing the peak or peaks, any timestamp that passed the threshold was considered a response to the respective transient impulse of the visual flicker. These points were summed, excluding the baseline value, and were subjected for statistical analyses. The 13 ms segment is approximately the half-duration of the shortest cycle among the frequencies tested. Rayleigh test for circular uniformity on spike times of individual units at different frequencies and polarities confirmed that the peaks selected through the previously mentioned procedure for further analyses are specific responses to transient impulses of luminance rather than general response fluctuations. Figure 5C depicts circular distribution of spike times of the same example neuron as in Figures 5A and 5B at the flicker frequency of

A

Firing Rate of Polarity Sensisitve Neurons

B

Figure 4. Average Firing Rate of a Population of Polarity-Sensitive Neurons

(A) Firing rate of neurons at the two highest modulation depths (i.e., 0.7 and 1) at different frequencies (8–40 Hz) and polarities. The firing rate increases, and the difference between the firing rate in response to the light flicker and that in response to the dark flicker becomes larger as the temporal frequency of visual flicker increases. Statistical significances depicted on the graph represent multiple comparison differences on the firing rate of the population of all neurons. Error bars indicate SEM.

(B) Population average of the firing rate of the neurons separated by the frequency, modulation depth, and polarity. Repeated-measures three-way repeated-measures ANOVA revealed a significant main effect of all factors, as well as significant two-way interactions. The error bars are not shown for clarity.

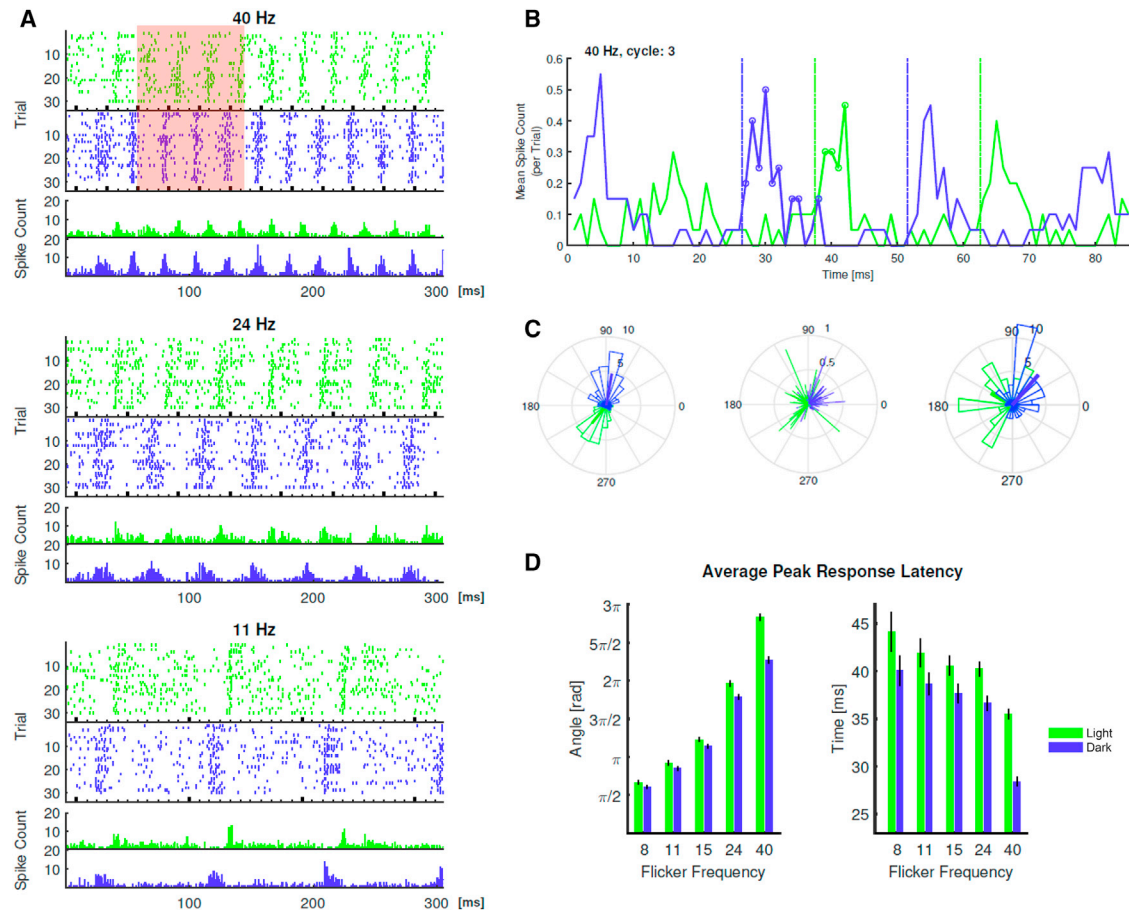*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

40 Hz. Differential directions (angles) of light and dark flicker emanate from distinct response latencies. Circular mean resultant vectors of all units (middle panel) and their Rayleigh distribution (right panel) are also shown in Figure 5C. Because different frequencies have different periods, mean directions of units (Figure 5D, left panel) were re-converted to time (Figure 5D, right panel) and were subjected for two-way repeated-measures ANOVA. These values represent the time lag from the stimulus onset until the peak response. The statistics revealed significant main effects of both frequency ($p < 0.001$) and polarity ($p < 0.002$) and a significant frequency × polarity interaction ($p < 0.05$). Table S1 summarizes the statistical analyses on different measures of

neuronal signals and shows the contribution of each factor to the total variance explained.

Subsequently, we examined neuronal responses to individual transients measured through the previously mentioned procedure (Figure 5B). We ran separate two-way repeated-measures ANOVAs on neural responses calculated from the PSTH of cycles 1 and 3 (first and third transient impulses). The analyses demonstrated that in neural responses to the first cycle of visual flicker (Figure 6A), there is neither a main effect of frequency or polarity nor a significant interaction between two factors. However, the statistical analysis on the neural responses to the third cycle (Figure 6B) revealed a significant main effect of frequency ($F_{4, 236} = 16.00$; $p < 0.001$) and a significant main effect of polarity ($F_{1, 59} = 7.37$; $p < 0.01$). As shown in Figure 6B, there is a clear advantage for dark visual flicker in the strength of spiking activity at the frequency of 40 Hz ($p < 0.001$). The results of similar analysis separately for OFF- and ON-dominated units are shown in Figures S6A and S6B. As seen in raster plots (Figures 5A and S5) and demonstrated by circular statistics (Figures 5C and 5D), neurons respond to transients stronger than the baseline. We compared pre-trial baseline activity and showed that there is no significant main effect of either polarity or frequency (Figure S5C). Because pre-trial baseline follows a random preceding trial, we also compared the last 25 ms of the interstimulus intervals (ISIs) based on the trials they follow. This analysis did not reveal any significant main effect of polarity or frequency, suggesting that differential light adaptation across conditions did not play a main role in our design (Figure S5D). To have a better picture of the spiking activity in response to single transients over time, we calculated the neuronal activity for all cycles throughout the trial duration. Figures 6C and 6D depict the development of spiking activity over time at different frequencies for light and dark visual flicker. This analysis showed that all frequencies start the activity at a similar level and then the amount of activity to subsequent transients decreases in all frequencies except 40 Hz, which exhibits an initial activity buildup during early cycles followed by a decrease in activity in subsequent cycles.

Time Courses of Neural Responses to Light and Dark Flicker

We used four time points as measures of neural response latency to address the way temporally varying visual stimulus influences the latency. In both PSTHs and visual evoked potentials (VEPs), the timestamp of the first point of two consecutive points that passed the threshold was considered the response onset latency. In addition, in VEPs, the timestamp of the peak activity was considered a separate measure of the response latency (data not shown). The latency of peak spiking activity was also calculated through circular statistics (Figure 5D). Figure 7 shows response onset latencies of the PSTHs and VEPs for the first and third cycles of visual flicker. The graphs show that in both PSTHs and VEPs, the neural response to dark flicker emerges faster. Two-way repeated-measures ANOVAs revealed only a significant main effect of polarity for the first cycle (PSTH, $p < 0.01$; VEP, $p < 0.001$), but not a significant frequency or interaction effect. The frequency will have meaning over time, and during the first cycle, there is no presence of temporal frequency modulation. This has appropriately been reflected in the lack of

Figure 5. Spike Times of an Example Neuron and Calculation of the Response to an Individual Transient Impulse from the PSTH

(A) Raster plots and spike time histograms of a representative neuron at the frequencies of 40, 24, and 11 Hz. Each tick in the raster plots represents one frame of monitor refreshing at 120 Hz. The bold ticks signify the start of one flicker cycle that corresponds to a transient increment or decrement in luminance. The firing of the neurons is entrained to the temporal frequency of the visual flicker. The histograms show stronger response to the dark flicker than to the light flicker, and the rasters show that the response to the dark flicker leads the response to light flicker. See also Figures S5A and S5B for two other example units. The trials have been ordered such that first, second, and third 10 trials represent the modulation depths of 100%, 70%, and 40%, respectively.
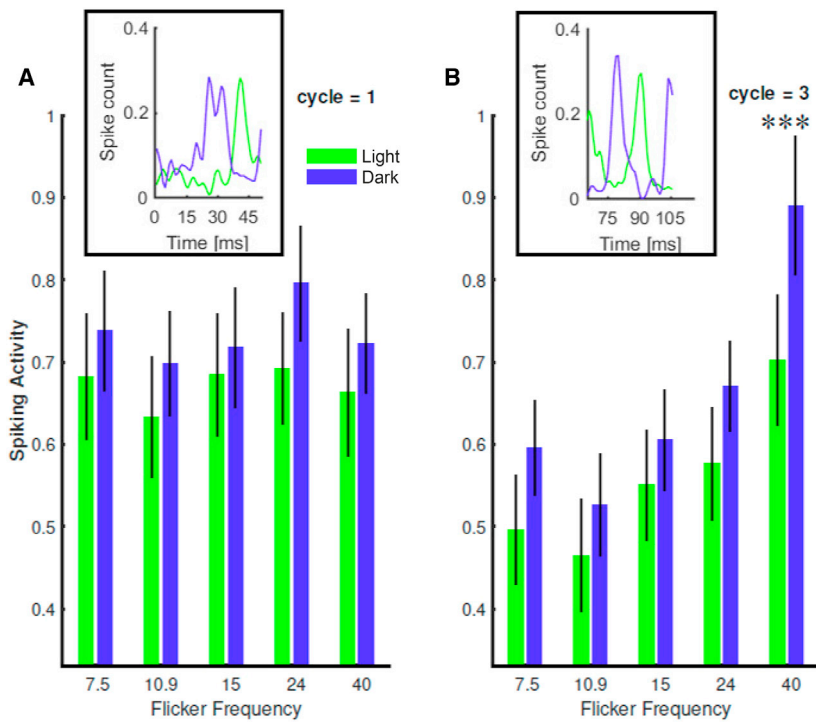
(B) Segment of the neuronal response (highlighted in Figure 6A, top panel) illustrating the method we used to calculate response to single transients (see Supplemental Information for details). Blue and green dashed lines show the time window corresponding to the neuronal spike response to one cycle of flicker. The superimposed circles on the spike time histogram (mean per trial) in this figure symbolize the points that passed the threshold and were thus considered the neuronal response to the flicker. The baseline was subtracted from these points, and the resulting values were summed to yield a measure representing the neuronal spike response to the respective transient.

(C) Left: Rayleigh statistics illustrate non-uniform circular distribution of spikes, confirming specific neural activity in response to transient impulses of luminance decrement or increment. The circular statistics were performed on a circle with a size equal to the period of each frequency. The example unit in this figure is the same unit as in (A) at 40 Hz (top). Middle: resultant vectors from the circular mean of all neuron were superimposed. Right: circular distribution and resultant vector of mean direction of all units (population Rayleigh).

(D) Mean directions of the population of the units (left) were back-converted to time and represented as the latency to the peak spiking response (right). Error bars indicate SEM.

significant effect of frequency on neural responses to and response latencies of the first cycle. However, the third cycle is when we expect main effects of both temporal frequency and polarity if they influence the timing of neural responses. Repeated-measures ANOVAs prove that both temporal frequency and polarity of temporal modulation influences the timing of neural responses. These tests demonstrated significant main effects of both frequency (PSTH, $p < 0.05$; VEP, $p < 0.001$) and polarity (PSTH, $p < 0.002$; VEP, $p < 0.001$), as well as significant interactions (PSTH, $p < 0.05$; VEP, $p < 0.001$). Post hoc analyses revealed that in the VEP, there is a significant difference between pairs of polarities at all frequencies. In PSTHs, the polarities had significantly different latencies at the frequencies of 7.5, 15, and 40 Hz. The comparison of response latencies of PSTHs separately for OFF- and ON-dominated units is shown in Figures S6C and S6D. The analysis on the latency of peak response in the VEPs showed similar results with an additional time offset.
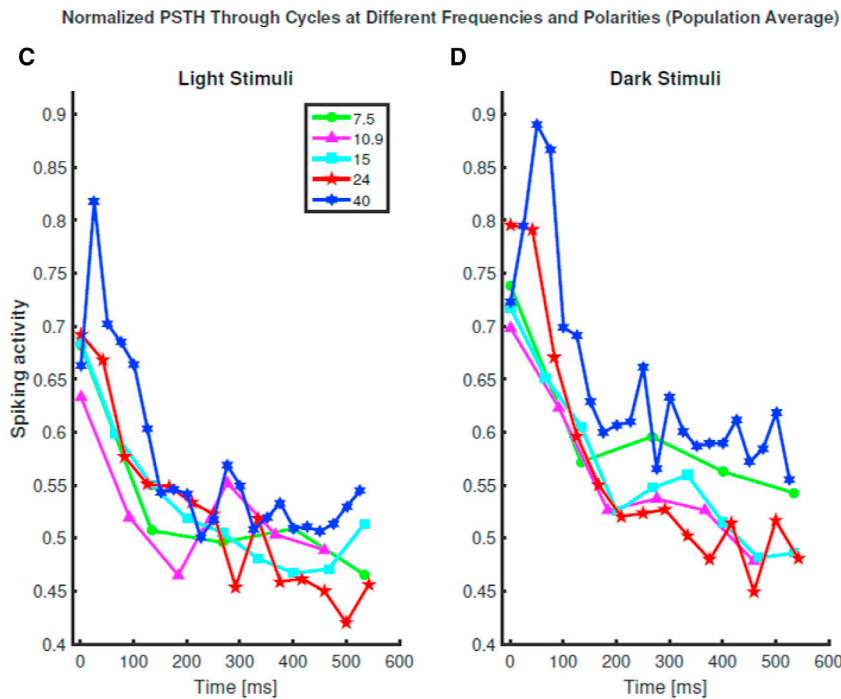
Figure 6. Neuronal Spike Response to Individual Flicker Transients

(A and B) Neuronal spike responses to the first (A) and third (B) cycles of the visual flicker. The response magnitude was calculated as described in the Supplemental Information and as illustrated in Figure 5B. The insets show the corresponding segment of the neuronal signal considered the response to the respective cycle. The response pattern changes over time as a function of frequency and polarity such that there are main effects of both factors (frequency and polarity) in the neuronal spike response to the third, but not the first, cycle of the flicker. Among the frequency levels, there is a large and significant difference between two polarities only at the frequency of 40 Hz. Error bars indicate SEM.

(C and D) Neuronal spike response to individual transient impulses at different frequencies for light flicker (C) and dark flicker (D). Different frequencies have different data points in these graphs, because the total trial duration for different frequencies was the same. The error bars are not shown for the clarity. Note initial activity buildup at 40 Hz and overall smaller spike response to the light flicker compared to the dark flicker.
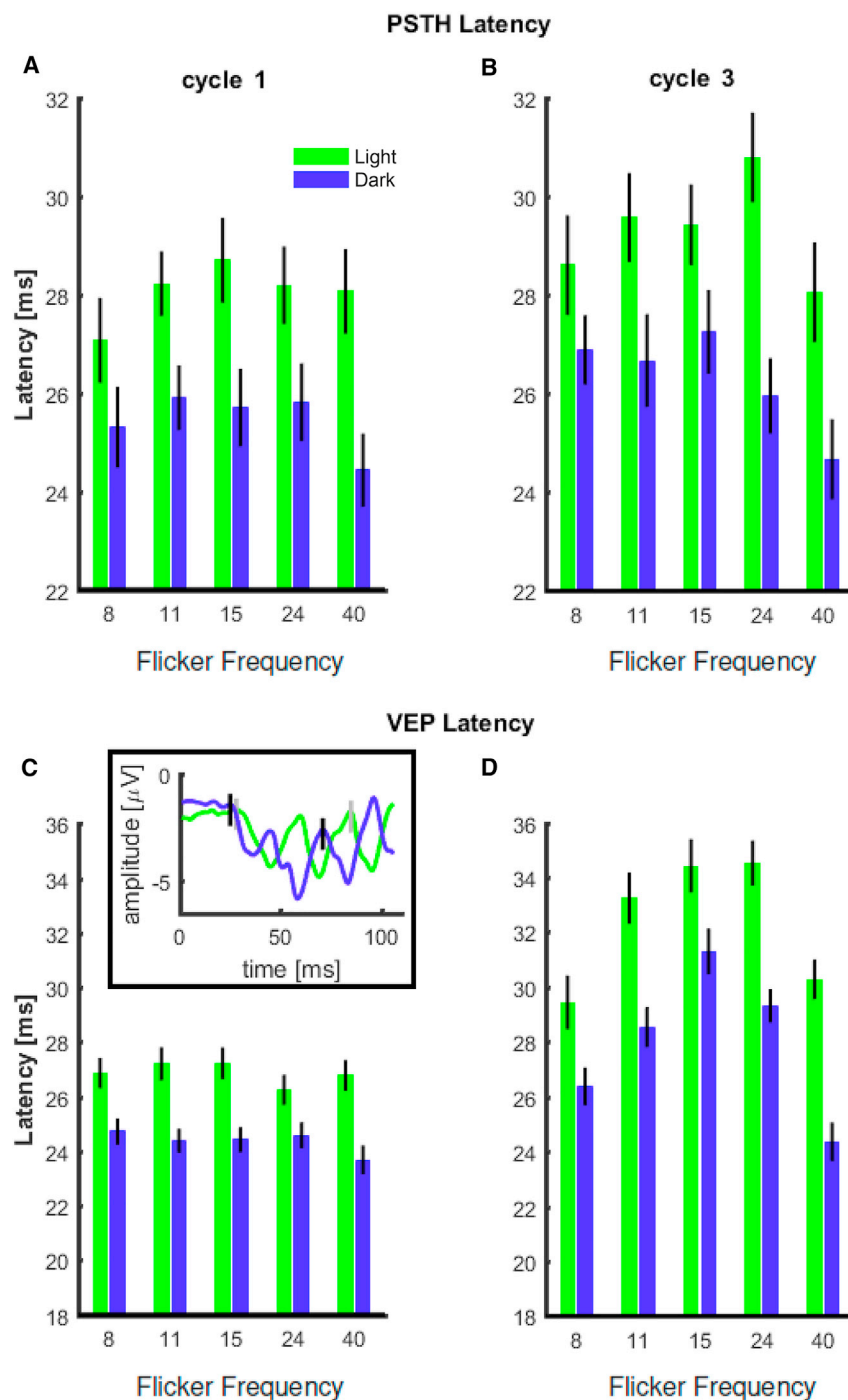
***$p < 0.001$.

DISCUSSION

Our results demonstrate significant differences in the temporal visual resolution of temporally varying light and dark stimuli in human participants and tree shrews. Tree shrews exhibited a pronounced difference in the temporal resolution of light and dark stimuli at higher frequencies (∼60 Hz), whereas the difference was less pronounced in human participants and was manifested at lower frequencies (∼11 Hz). We also demonstrate a neural correlate for such differences in the V1 of tree shrews. V1 neurons fired more strongly in response to dark stimuli than light stimuli, and the difference was larger at higher frequencies than lower frequencies. Brief transients in flicker stimulus are reflected in brief response increases in neurons, which can form a temporal pattern in downstream areas. If a neuron merely showed elevated activity continuously, this would not be detected downstream as flicker. As shown in Figures 5C and 5D, we demonstrate that the responses are entrained to the flicker frequency and show a clear non-stationarity, suggesting that the responses are temporally patterned with reference to the temporal dynamics of transients in the stimulus and are not the result of random or irregular fluctuations in the response. Each unit exhibited stimulus-locked non-stationarity, and the population of neurons demonstrated a preferred phase for the response following stimulus onset (Figure 5C, right panel). More importantly, the responses of neurons to single brief luminance impulses from the midgray background depended on frequency and polarity such that the dark transients induced stronger neural activity than

PSTH Latency

VEP Latency

Figure 7. Latency of Neural Responses to the Visual Flicker Stimuli

(A and B) Spike response latencies to the first (A) and third (B) cycles of flicker.

(C and D) Filed potential response latencies to the first (C) and third (D) cycles of the flicker. The inset in (C) shows how the latencies were estimated in the VEPs for the first and third cycles of the flicker. Gray and black markers depict estimated latencies for the first and third cycles. Error bars indicate SEM.

lished differences of light-dark spatial visual resolution (Blackwell, 1946; Komban et al., 2011), the possible differences in the light-dark temporal visual resolution have not been addressed. Although sinusoidally modulated visual flicker (Callahan and Petry, 2000; De Lange Dzn, 1958; Jarvis et al., 2003; Kelly, 1961) has been used extensively to estimate temporal sensitivities, this does not allow the dissociation between specific aspects related to processing lights and darks. In our experiments, human participants exhibited peak sensitivity at the temporal frequency of 15 Hz for both light and dark flashes, which is in accord with the previously reported frequencies of about 10–15 Hz (De Lange Dzn, 1958; Hartmann and Banks, 1992; Keesey, 1972; Kelly, 1961, 1971). Our findings fall in the high range of this previous literature, which could be due to the transient and therefore more salient flicker resulting from brief impulses used in the current study. Although the difference was modest, human participants showed a higher sensitivity to the dark flicker than to the light flicker at 11 Hz. We also showed that at suprathreshold modulation depths, there is no significant difference between decrements and increments (Figure S2). This contrasts previously reported larger suprathreshold differences between light and dark stimuli (Zemon and Gordon, 2006). However, the paradigms are different in these two studies. Zemon and Gordon (2006) used sinusoidally varying isolated checks that vary in both polarities of an average luminance, while we employed brief temporally structured transients of luminance change. The differences in the response time were more prominent, such that human participants detected the dark flicker significantly faster than the light flicker. Our results are in general accordance with previous work that has demonstrated enhanced spatial visual resolution for the darks compared to the lights (Blackwell, 1946; Buchner and Baumgartner, 2007; Galilei, 1632; Komban et al., 2011).

the light transients; the difference was particularly prominent at 40 Hz. Moreover, a similar advantage for dark stimuli was seen in the response latency, such that neurons responded faster to the dark flicker than to the light flicker, and this difference was frequency dependent.

In general, the temporal characteristics of visual perception are multifaceted and remain less well understood than the spatial aspects of visual perception (Holcombe, 2009). Unlike the estab-

Corresponding closely to the human psychophysical findings, tree shrews also exhibited greater temporal sensitivity to the dark flicker than to the light flicker. However, the response pattern of tree shrews differed from that of the humans in two major ways: The difference in sensitivity was more pronounced in tree shrews than in humans, and unlike human participants, who showed a peak sensitivity for both polarities at 15 Hz, the temporal sensitivity of tree shrews diverged at higher frequencies such that the sensitivity to the light flicker reached a peak at a temporal frequency range of 24–40 Hz and started to descend afterward, whereas the sensitivity to the dark flicker remained at peak values at 60 Hz. The light flicker results resemble previous psychophysical findings in tree shrews obtained using sinusoidal flicker (Callahan and Petry, 2000) more closely than the dark flicker findings, which may represent a specific adaptation of fast-moving tree shrews for detection of dark elements in their surroundings. Despite distinct between-species differences in flicker discrimination, human participants exhibited higher sensitivity compared to tree shrews at all frequencies. This is unlikely to be due to better vision in humans compared to tree shrews. Rather, their overall lower performance is related to decision-making tasks in experimental settings tending to be more challenging for experimental animals compared to humans, who are used to making decisions based on sensory evidence presented on video monitors.

In our design, tree shrews and humans could not solve the task based on the differences in luminance in a given trial. However, given the impact of transients on average luminance, a limitation of this design is that the differences in luminance across trials, and thereby differential light adaptation, might contribute to behavioral performance. Polarity and luminance are associated concepts, and the effects of luminance are embedded within the polarity factor. Therefore, we cannot rule out that an interaction of luminance with polarity and rate of the transients contributed to our findings. In addition, the effects cannot solely be driven by luminance. First, we keep the animals and the units adapted to the background luminance. We compared the pre-trial, post-trial (Figures S5C and S5D), and pre-transient baseline neuronal activity between light and dark flicker and showed that polarity has no significant effect ($p > 0.5$) on the baseline activity, mitigating the possibility of differential light adaption confounding the results. Second, the Michelson contrast is similar for light and dark stimuli at modulation depths close to the threshold, where we observe a significant difference and a clear divergence. Third, human participants show larger differences between light and dark stimuli at 11 Hz, whereas the biggest differences in average luminance of the two stimuli is at 40 Hz, which suggests interaction of polarity with the temporal dynamics as a major player in the response profile of human participants. Finally, there is a trade-off between baseline and average luminance when considering both polarities. Setting the same baseline leads to a difference in time-averaged luminance between two polarities (not between target and distractors), and having equal time-averaged luminance leads to having different baselines for two polarities. What we show is detection of brief changes in polarity from a midlevel baseline and our control stimuli, and different baseline comparisons make sure that it is a genuine detection of flicker. Nevertheless, future studies should

vary the level of time-averaged luminance as an independent variable to explicitly address the potential contribution of luminance in the results.

Although accumulating evidence suggests asymmetric neural processing of light and dark visual stimuli at the level of retinal ganglion cells (Ratliff et al., 2010), thalamus (Jin et al., 2011), and V1 neurons (Kremkow et al., 2014; Veit et al., 2011; Yeh et al., 2009), the temporal differences have been studied in the context of temporal neuronal dynamics, leaving light-dark differences in response to temporally varying stimuli largely unexamined. Our results show notable differences in the processing of temporally varying light and dark stimuli in terms of both the response magnitude and the dynamics of the response. We demonstrate a temporal frequency dependence of dark-light differences and show that a sizable population of neurons responds more strongly to darks than lights, particularly at higher frequencies. Light-dark asymmetry has been shown in much higher frequencies in studies that investigated the entrainment of the activity of V1 neurons to monitor refresh rate rather than explicitly studying the neural responses to temporally varying stimuli (e.g., Veit et al., 2011). Although the larger differences in firing rate between light and dark flicker at higher frequencies might be expected in our experiments based on more visual transients being delivered at high flicker frequencies, we show, by analyzing responses to individual transients, that it is the temporal structure of visual stimulation transients, not their overall number, that underlies the frequency dependence of dark advantage.

Traditionally, sinusoidal flicker (Callahan and Petry, 2000; De Lange Dzn, 1958; Hartmann and Banks, 1992) has been used to estimate temporal resolution of observers or sensory neurons. Experiments that use sinusoidal flicker assume that the same magnitude of contrast deviation (increase or decrease) from an intermediate background luminance generates the same magnitude of neuronal response and produces a similar perceptual temporal resolution. Our results contradict this assumption. By taking advantage of the independent light and dark visual flickers generated from the brief impulses of transient increments or decrements, we demonstrate that light-dark differences are flicker frequency dependent and are apparent in response to the individual transients. Consistent with this, we show that the responses to the initial transient are similar for all flicker frequencies and that the amplitude of neural responses to subsequent transients strongly depends on flicker frequency. Examining the development of the neuronal responses over time (Figures 6C and 6D), we note an early divergence in the activity level among different frequencies and a late convergence around 200 ms after the trial onset, which reflects a steady-state response that lasts until the end of the trial. Neural responses generally, and the dark advantage in particular, are largest during the initial transients, when neural responses to 40 Hz flicker are additive for the first few cycles before falling off toward the steady-state response. This pattern of activity supports the hypothesis of two time constants acting together to regulate neuronal response dynamics. The first mechanism has an adaptation time constant of around 200 ms that is responsible for the late convergence of neuronal activity at different frequencies to a similar plateau level and operates similarly for light and dark

responses. A second mechanism with a markedly shorter time constant induces neuronal response facilitation, with a time constant of around 20–50 ms that appears to differ between light and dark responses. The apparent existence of early and steady-state response regimes raises the question, which of these regimes do animals use to guide their behavior? Because tree shrews were freely moving in our experiments, we suggest that eye, head, and body movements made in the setup bring about frequent shifts in the retinal position of visual stimulus, allowing animals to take advantage of early responses throughout their visual decision-making process. Consistent with this idea, it has been shown that the sensitivity of human observers to sinusoidal flicker was attenuated following flicker adaptation (Pantle, 1971), even at invisible flicker frequencies (Shady et al., 2004), whereas free eye movement facilitated change detection (Hollingworth et al., 2001).

In addition to differences in neuronal activity pattern, we show a difference in neuronal response latencies such that neurons respond faster to darks than to lights. This difference is thought to originate in retina (Baylor and Fettiplace, 1977; Gollisch and Meister, 2008; Nichols et al., 2013), an effect that is related to slow metabotropic glutamate receptors (mGluR6) and fast ionotropic receptors in ON and OFF bipolar cells, respectively. This retinal dark advantage ascends through the visual hierarchy and has been observed in visual thalamus (Jin et al., 2011) and V1 (Komban et al., 2014; Rekauzke et al., 2016). Consistent with previous reports (Jin et al., 2011; Komban et al., 2014; Nichols et al., 2013; Rekauzke et al., 2016), our results support the existence of a faster OFF neuronal pathway and extend this difference to temporally varying visual stimuli. Furthermore, we show temporal frequency of visual stimuli as an additional important factor that influences neuronal response latency. As shown in Figure 7, while the light-dark difference is similar among different frequencies during the first transient, there is a significant modulation of neuronal response latency as a function of temporal frequency. In addition to the neuronal activity pattern, the frequency dependence of differences in response latency confers another efficient coding scheme for neurons to resolve the temporal structure of a changing environment.

Altogether, our findings demonstrate asymmetries in neuronal activity and response latency following temporally varying light and dark visual stimuli and provide evidence that both effects strongly depend on the temporal structure of the visual stimuli. Considering our results in conjunction with previous reports (Gollisch and Meister, 2008; Jin et al., 2011; Komban et al., 2014; Kremkow et al., 2014; Ratliff et al., 2010), we support the hypothesis that light-dark asymmetry is an element of evolutionary adaptation during natural selection to optimize processing of dark-rich natural stimuli. Furthermore, given the remarkable differences between human participants and tree shrews in the processing light and dark flicker at different frequencies of temporally varying visual stimuli, we suggest that differential frequency specialization, in conjunction with light-dark asymmetries during evolution, has been tailored to match the specific needs of each species. For example, tree shrews have a substantially different lifestyle than that of humans. They are fast-moving animals and generally have to deal with faster events; therefore, light-dark differences in response magnitude

and temporal dynamics within their visual system have to be adjusted to optimize perception of fast-moving prey and avoid blurred vision when the animal moves quickly.

EXPERIMENTAL PROCEDURES

All experimental procedures with tree shrews were conducted in accordance with the applicable Swiss and European regulations and were approved by the veterinary office of the canton of Fribourg. Human participants gave informed consent before participation in the experiments, which were performed in accordance with the ethical standards specified by the 1964 Declaration of Helsinki and approved by the Ethics Committee of the University of Fribourg.

Behavioral Experiments

Nine human participants (aged 25–35; 5 males), including two authors (A.K. and F.M.), and six adult female tree shrews (*Tupaia belangeri*) participated in behavioral experiments. Both species detected a flickering stimulus among two equi-luminant distractors. Equi-luminant distractors had the same time-averaged luminance as the target flickering stimulus. The visual stimuli were generated by introducing transient impulses of light increment or decrement from a midgray background at different frequencies and different modulation depths (Figure 1).

Tree shrews were extensively trained to detect a flickering stimulus by poking their nose in the correct hole (Figure 1) in a custom-made box. Tree shrews were free to navigate within the box; they were connected to a smaller nesting box through a tube and therefore could leave the experimental box to the nesting box whenever they decided to end the session's trials. The correct detection resulted in the delivery of two reward pellets in a reward magazine opposite the stimulus-presentation side. Incorrect responses were alerted by a beep and a longer intertrial interval (6 s). The reward pellets were 45 mg raspberry-flavored food reinforcement pellets (P.J. Noyes). We fitted psychometric functions (cumulative Gaussian) (Figure S1A) for human participants and used linear regression (Figure S1B) for the initial segment of the tree shrew data, because the response pattern showed an initial sharp rise followed by a plateau.

Electrophysiological Recording and Data Analyses

Before the experiments, adult tree shrews (n = 7) were anesthetized. All vital signs were carefully monitored during the experiment. A small craniotomy removed part of the skull over V1, and two tungsten electrodes (500 μm apart; impedance, 1 MΩ) were inserted into the brain using a remotely controlled hydraulic wheel. The recording sites were histologically confirmed by inducing a lesion at multiple sites at the end of recording sessions. Electrophysiological signals were sampled at 24.4 kHz and were concurrently high-pass filtered at 300 Hz and low-pass filtered at 100 Hz. Action potential waveforms were recorded by thresholding the high-pass-filtered signal and were sorted offline. The low-pass-filtered signal was down-sampled to 1 kHz and contained local field potentials (LFPs). The stimulus presentation screen was placed 30 cm from the animal, subtending ~60° of visual field. The RF of each site was mapped using the sparse noise paradigm (see Supplemental Information for details). Visual stimuli identical to those used in behavioral experiments were presented to the minimal RF of each recording site in a randomized order. All frequencies were presented to all neurons. PSTH was estimated from the spike times by time locking the signal to the onset of each stimulus. See Supplemental Information for a more detailed version of the experimental procedures.

Statistical Analysis

In this study, we used repeated-measures ANOVA and post hoc tests, the non-parametric Wilcoxson signed rank test, bootstrap analysis, the Rayleigh test for circular uniformity, and aligned rank transform for non-parametric factorial analyses. The latter test is a non-parametric analysis for multifactor data and resembles ANOVA procedures (Wobbrock et al., 2011). This test was conducted using the open-source statistics software R. MATLAB functions were used for all other statistical analyses.

AUTHOR CONTRIBUTIONS

A.K. and G.R. conceived the study, A.K. and F.M. performed the experiments, A.K. analyzed the data, and A.K. and G.R. wrote the paper.

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