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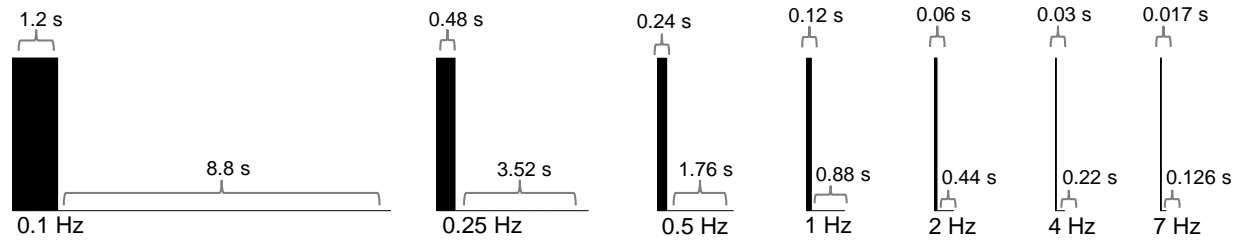
Detailed methods for statistical analysis

For the experiments shown in Figs. 3 – 5, we used linear mixed models (linear models with random effects) to make statistical comparisons between experimental conditions. Mixed models are defined in terms of a mean structure, describing the expected value of each observation, and a covariance structure, describing the relationships among the observations. For our purposes, the main interest is in the mean structure. However, the covariance structure must also be accounted for in order to make meaningful statistical inferences about the mean structure. We specified the models to have a saturated factorial mean structure, meaning that each of the 63 combinations of experimental factors (photon count, duty cycle and frequency) and each of the 11 constant-light conditions has its own mean value that varies independently of the others. Random effects were used to account for within-subject correlations. The random effects structure employed a nested sum of two terms. One of the terms applied to all values for a given subject, while the other applied only to the two replicates made in a single stimulus condition. The variance parameter for the first random effect reflects between-subject variation that appears in all stimulus conditions, whereas the variance parameter for the second random effect accounts for effects that are common to the two replicates of a stimulus condition made for a given subject.

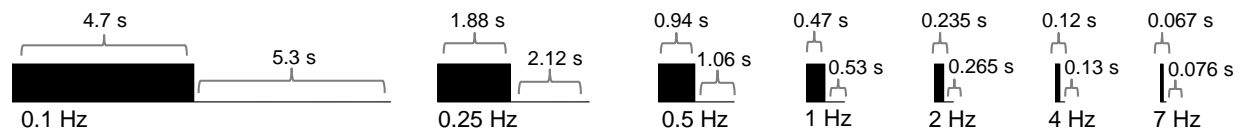
We used likelihood ratio tests to establish the presence of strong effects for each of the three experimental factors. All three factors showed highly statistically significant differences between levels ($p < 0.001$ for all 3 factors). Subsequently, we conducted post-hoc mean comparisons between the fitted curves for each condition (expressed as functions of frequency) and their corresponding constant-light conditions, and between selected trials of interest. All error estimates are S.E.M. unless stated otherwise.

We investigated the fit of the mixed model using standard residual diagnostics. Plotting the residuals against the fitted values revealed no relationships, but there were weak trends in which the dispersion of the residuals became lower with both increasing photon count and with decreasing duty cycle. To account for this heteroscedasticity, the variance was allowed to differ among the 9 combinations of two parameters, total photon count and duty cycle (i.e. 3 photon counts \times 3 duty cycles). To visualize the results, fitted means were plotted as a function of frequency for each of the 9 combinations of total photon count and duty cycle. Standard errors shown in these plots were derived from the linear effects model.

12% duty cycle:



47% duty cycle:



93% duty cycle:



Figure S1. Waveforms of the 21 stimuli that contained $13.7 \log \text{ photons cm}^{-2}$. One flicker cycle is illustrated for each stimulus. All waveforms are drawn to scale, on both time (x) and intensity (y) axes. Waveforms for the $14.7 \log \text{ photons cm}^{-2}$ and $15.7 \log \text{ photons cm}^{-2}$ stimuli (not shown) would be 10- and 100-fold taller, respectively.

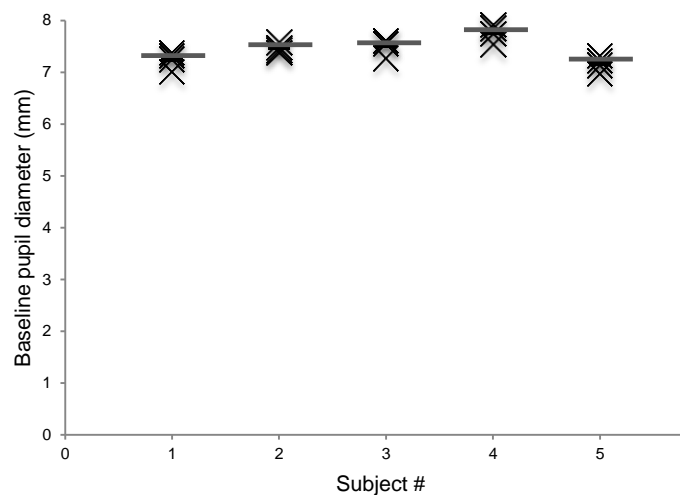


Figure S2. The baseline pupil diameters of the five subjects who were tested with the 63 flickering stimuli. For every subject, pupil diameter was measured after 1-hr dark adaptation on four separate days, with each measurement made at about the same time of day. The crosses represent individual measurements, and each horizontal bar indicates the subject's averaged baseline pupil diameter.