Supplementary methods

Surgery and electrophysiological recordings

Two Dutch-belted rabbits were implanted with a chronic array of independently movable microelectrodes in the lateral geniculate nucleus (LGN) and chronic recording electrodes in the hippocampus. A metal bar was attached to the skull for head fixation and a search coil was inserted in the sclera to monitor eye position. The microelectrodes used to record LGN cells were constructed from quartz-insulated platinum-tungsten filaments with a stock diameter of 40 µm. The filaments were pulled to a taper under high temperature and then sharpened to a fine tip. Each multielectrode array had 7 electrodes, which were separated from each other by 150 µm and guided through fine-diameter (150 µm OD) stainless-steel tubes. Seven miniature microdrives were affixed to the skull to allow independently moving each electrode. The electroencephalographic (EEG) hippocampal electrodes were made of two platinum-iridium microwires that were placed above and below of the CA1 layer of the hippocampus (1 mm vertical separation). A Plexon data acquisition system (Plexon Corp., Dallas, Texas) was used to record single unit activity in LGN, EEG activity in the hippocampus and eye position (in some experiments we also recorded EEG neocortical activity). All surgical procedures were performed under general anesthesia and were approved by the Institutional Animal Care and Use Committee at the University of Connecticut in accordance with NIH guidelines. Details about the surgical methods have been described elsewhere.

Electrophysiological recordings were performed in awake rabbits. Rabbits were held snugly within a stocking and sat on top of a foam rubber pad with their heads restrained.
In this environment, they generally sat quietly for 4-8 hours and kept their eyes still for time blocks of several minutes. Data were collected while the eye remained within ±0.75°. However, eye movements were usually smaller than ±0.5° and in some cases smaller than ±0.25°. When eye movements exceeded ±0.75° the trial was aborted and the stimulus was repositioned at the receptive field center. These minimal eye movements combined with the remarkable stability of the recordings obtained with our miniature multielectrode arrays allowed us to repeat, with high reliability, measurements of neuronal contrast sensitivity during different brain states (Supplementary Fig. 2).

Visual stimulation and data analysis

Visual stimuli were presented with a VisionWorks stimulus generator (Vision Research Graphics, Inc) on a FlexScanF980 monitor (EIZO Nanao corporation, Japan, refresh rate: 160 Hz). We recorded from LGN cells with concentrically organized receptive fields located at different eccentricities (elevation: +15° to −5° with respect to the horizon; azimuth: 20°–70° from the midline, 0° being directly in front of the animal). Each cell was tested with a sinusoidal-drifting grating, which is a commonly used stimulus for contrast measurements in LGN. The drifting gratings were optimized in size, spatial frequency and temporal frequency. The size of the grating was ~1.2 times the diameter of the receptive field center, the spatial frequency was 0.2-0.3 cycles degree−1 and the temporal frequency was 2-3 Hz. These stimulus properties were chosen to generate the strongest responses possible in both non-alert and alert states.
As reported previously\textsuperscript{1,3}, alertness changes the temporal response properties of LGN cells. During the alert state, LGN responses become more sustained\textsuperscript{1,3}, and there is also an increase in the bandwidth and peak of the temporal frequency tuning curve\textsuperscript{1}. Using data from a previous study\textsuperscript{1}, we have found that the enhancement of visual responses with alertness is most pronounced at the optimal temporal frequency of the cell (measured during the alert state) and falls by half at frequencies that are ±15 Hz apart from the optimal. Ideally, we would have made repeated measurements of all possible combinations of temporal frequency, contrast and state but this was not technically feasible (such a stimulus protocol would be too long for testing in an awake animal). Therefore, we used a fixed temporal frequency for our contrast measurements, a strategy that is frequently adopted by researchers doing similar measurements in anesthetized animals\textsuperscript{2,4}.

LGN cells were classified as sustained or transient based on responses to small spots presented at the receptive field center during the alert state. The spot was presented for at least 0.5 seconds and its size and sign matched the LGN receptive field (e.g. light spot for an on-center LGN cell). Cells that fired more than 10 spikes per second above baseline (within 400-500 msec after the spot onset) were classified as sustained and those that fired less as transient. This criterion was based on a significant bimodal distribution of response strength to stationary stimuli (for detail see\textsuperscript{1}). To generate contrast response functions, we measured the first Fourier harmonic (F1) of the response and the mean firing rate (F0). Other response harmonics (F2-F10) were also measured but they were much weaker than F1 and F0. As reported previously\textsuperscript{3}, when stimulated with a contrast
reverse grating, both sustained and transient LGN neurons in rabbit respond at twice the frequency of the stimulus (frequency doubling). However, like LGN cells in cats\textsuperscript{5} and primates\textsuperscript{6}, rabbit LGN neurons do not show frequency doubling when the stimulus is a drifting grating (Fig. 1).

Each cell was studied at 8 different contrasts (0\%, 2\%, 5\%, 11\%, 23\%, 43\%, 73\%, 95\%), each contrast was presented 4 times and each presentation lasted 3.6 seconds. Contrast was defined as

\[
\text{Contrast} = \frac{L_{\text{max}} - L_{\text{min}}}{L_{\text{max}} + L_{\text{min}}}
\]  

(1)

where \(L_{\text{max}}\) is the maximum luminance at the crest of the sinusoidal grating and \(L_{\text{min}}\) the minimum luminance at the trough of the sinusoid (\(L_{\text{max}}\) at 95\% contrast: 108 cd m\(^{-2}\)). All cells selected for this study were tested at least twice at each state and the contrast measurements were highly repeatable (Supplementary Fig. 2).

The LGN responses to different contrast were fit with several functions. Consistent with previous studies in visual cortex\textsuperscript{7,8}, the best fits were obtained with a hyperbolic ratio function.

\[
\text{Resp} = R_{\text{max}} \left( \frac{C}{C+C_{50}} \right)^n
\]  

(2)

where \(\text{Resp}\) is the neuronal response, \(R_{\text{max}}\) is the maximum response, \(C\) is the contrast, \(C_{50}\) is the contrast that evoked the half-maximum response and \(n\) is the exponent. The hyperbolic ratio gave excellent fits, when using F1 values, in both the alert state (\(r = 0.99 \pm 0.01\) for sustained neurons; \(r = 1.00 \pm 0.00\) for transient neurons) and the non-alert state.
(r = 0.98 ± 0.01 for sustained neurons; r = 0.97 ± 0.02 for transient neurons). Moreover, the residual variance not accounted by the fit was minimal (alert_sustained: 1.66 ± 1.29 %; alert_transient: 0.82 ± 0.57 %; non-alert_sustained: 3.02 ± 2.58 %; non-alert_transient: 6.32 ± 4.25 %). The residual variance was measured as RSS*100 / TSS, where RSS is the residual squared sum and TSS the total squared sum. RSS was calculated by subtracting the response measured at each contrast from the response predicted by the fit, squaring each residual difference, and summing all the squared values. The TSS was calculated by subtracting the response measured at each contrast from the response averaged across all contrasts, squaring each difference value, and summing all the squared values. The goodness of fit was considerably better for F1 than F0 measurements in both the alert (correlation coefficients: F1 = 0.99 ± 0.01, F0 = 0.98±0.03, residual variances: F1 = 1.44 ± 1.2, F0 = 4.39 ± 5.42) and non-alert states (correlation coefficients: F1 = 0.98 ± 0.02, F0 = 0.90 ± 0.11, residual variances: F1 = 3.88 ± 3.34, F0 = 18.38 ± 18.42; see Supplementary Fig. 3).

Excellent fits were also obtained with another sigmoidal function

\[
\text{Resp} = \frac{R_{\text{max}}}{1 + e^{-\frac{(C-C_{75})}{C_{75}-C_{25}}}}
\]  

(3)

where C75 and C25 are the contrasts that evoked 75% and 25% of the maximum response and Resp and R_{\text{max}} have the same meaning as for the hyperbolic ratio. The correlation indices and residual variances obtained with this sigmodal fit (F1 values) were excellent (r = 0.99 ± 0.01, res. var. = 1.77 ± 1.23% for sustained cells in alert state; r = 0.99 ± 0.01, res. var. = 2.77 ± 1.38% for transient cells in alert state; r = 0.98 ± 0.01, res. var. = 2.99
± 1.95 for sustained cells in non-alert state; \( r = 0.97 \pm 0.02, \) res. var. = 5.64 ± 3.52% for transient cells in non-alert state). However, since the residual variance was smallest for the hyperbolic ratio, we used this function (equation 2) to extract all parameters. The \( R_{\text{max}} \) was obtained by calculating Resp when \( C = 95\% \) (the maximum contrast used in our experiments). The \( C_{50} \) was obtained by calculating C when Resp = \( R_{\text{max}}/2 \). The \( C_{75}-C_{25} \) was obtained by calculating C when Resp = \( 3R_{\text{max}}/4 \) (\( C_{75} \)) and C when Resp = \( R_{\text{max}}/4 \) (\( C_{25} \)) and then subtracting both values. The exponent (n), a measurement of the steepness of the contrast response function, was the only parameter extracted directly from equation 2. As rabbits transitioned from non-alert to alert states, the \( R_{\text{max}} \) increased by a factor of ~2 while the \( C_{50}, C_{75}-C_{25} \) and n remained relatively constant. This basic result (doubling the Rmax with minor changes in the other parameters) was the same for measurements of F1 and F0. However, the F1 measurements revealed a small but significant difference in the \( C_{50} \) while the measurements of F0 revealed a small but significant difference in the exponent. The average parameter values for the F0 measurements were as follows: \( R_{\text{max}} \) (alert: 28.32 ± 11.63, non alert: 12.20 ± 5.65; \( P < 0.0001 \)); \( C_{50} \) (alert: 29.18 ± 15.78, non-alert: 23.40 ± 16.46, not significant); \( C_{75}-C_{25} \) (alert: 33.88 ± 14.19%, non alert: 35.36 ± 14.93%, not significant); exponent (alert: 1.35 ± 0.63; non alert: 0.92 ± 0.56, \( P < 0.002 \)).

The contrast response functions obtained at different brain states were compared with a non-parametric Wilcoxon test and the differences between transient and sustained cells were compared with a non-parametric Mann-Whitney test.

The peristimulus time histograms obtained at each stimulus contrast (e.g. Fig. 1) were fit with a sinusoidal function, \( f(t) \), followed by rectification, \( R(t) \):
\[ f(t) = B + A \sin(\phi + \rho) \]  \hspace{1cm} (4)
\[ R(t) = \max(f(t),0) \]  \hspace{1cm} (5)

where \( t \) is time, \( B \) is the baseline of the rectified sinusoid, \( A \) is the amplitude, \( \phi \) is the temporal phase and \( \rho \) is the phase offset\(^6\). Bursts were quantified separately for each stimulus contrast using the same criteria from a previous study\(^1\) (a cluster of 2 or more spikes with interspike intervals of \( \leq 4 \) ms, the initial spike having a preceding interspike interval of \( \geq 100 \) ms). Each burst was counted as a single event, independently of the number of spikes contained within the burst.

**Monitoring changes in brain state: alert vs. non alert**

We measured LGN neuronal responses in two different brain states, alert and non-alert. These two states were identified in chronic recordings from hippocampal EEG. As shown previously\(^1\), these hippocampal recordings can be reliably separated into theta (4–8 Hz) and high-voltage, irregular activity (HVIR). Theta hippocampal activity has been associated with an aroused, alert state and HVIR with a non-alert/drowsy state. For the sake of simplicity, we refer to these two states as alert and non-alert states through the entire text. We prefer the term ‘non alert’ to ‘drowsy’ because it emphasizes that the animals are not asleep. During the recording session, the eyes are always open and rabbits often transition from one state to another, in the absence of any apparent stimulation, with remarkable speed (within less than a second\(^1\)). It is important to emphasize that rabbits are not required to attend selectively to any specific stimulus during the alert state. The term ‘non alert’ refers here to an awake state but with a very low level of arousal\(^9\). Rabbits often alternate spontaneously between alert and non-alert states but, once the...
laboratory environment becomes familiar, they tend to spend more time in the non-alert state. In the laboratory environment, any unexpected stimuli often elicit a switch from HVIR to theta activity. However, our repetitive, uninteresting visual stimuli were ineffective in this regard (otherwise, measuring contrast response functions during HVIR would not have been possible). Measurements of contrast response functions were usually more difficult during theta activity. In order to maintain near-continuous theta activity for these measurements, the hippocampal EEG was closely monitored and novel auditory stimuli were generated when necessary. Following criteria adopted in a previous study\(^1\), we used an automated fast Fourier transform analysis to choose records that were either strongly dominated by hippocampal theta activity (alert state) or showed little or no theta activity (non-alert/drowsy state). The state-related changes in the contrast response function cannot be attributed to variations in the optics of the eye. The rabbit eye shows no measurable accommodative changes and the ciliary body is poorly developed. Indeed, studies using behavioral methods have concluded that ‘no accommodation mechanism exists in the rabbit’ and our own studies show that spatial acuity and LGN receptive field size are similar in non-alert and alert states (see discussion in \(^1\)).