Thalamic Burst Mode and Inattention in the Awake LGNd

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Summary

Awake mammals are often inattentive in familiar environments, but must still respond appropriately to relevant visual stimulation. Such “inattentive vision” has received little study, perhaps due to difficulties in controlling eye position in this state. In rabbits, eye position is exceedingly stable in both alert and inattentive states. Here, we exploit this stability to examine temporal filtering of visual information in LGNd neurons as rabbits alternate between EEG-defined states. Within a single second of shifting from alert to an inattentive state, both peak temporal frequency and bandwidth were sharply reduced, and burst frequency increased dramatically. However, spatial dimensions of receptive field centers showed no significant state dependence. We conclude that extremely rapid and significant changes in temporal filtering and bursting occur in the LGNd as awake subjects shift between alert and inattentive states.

Introduction

It has been known for more than three decades that dorsal thalamic nuclei serve to gate or filter sensory information passing to sensory neocortex. Seminal early studies established that a “transfer function” regulates the passage of sensory information through the dorsal thalamus and that thalamic transmission is modulated by reticular stimulation and by sleep-wake cycles (Bartlett et al., 1973; Coenen and Vendrik, 1972; Livingstone and Hubel, 1981; Maffei et al., 1965; Singer, 1977). The discovery of thalamic “burst mode” and “relay mode” and the low-threshold, voltage- and time-dependent Ca2+ conductance that controls these modes (Jahnsen and Llinas, 1984a, 1984b) provided a crucial key to understanding both the mechanism underlying the thalamic gating of sensory information and the nonlinear nature of this gating (Steriade and Llinas, 1988; McCormick, 1992; McCormick and Feeser, 1990; Sherman and Koch, 1986; Sherman and Guillery, 1996).

Although burst mode has typically been associated with anesthetic states and slow-wave sleep, considerable evidence indicates that thalamocortical “bursts” can also occur in awake subjects (Fanselow et al., 2001; Guido and Weyand, 1995; Martinez-Conde et al., 2002; Ramcharan et al., 2000; Swadlow and Gusev, 2001; Weyand et al., 2001). The prevalence of thalamic bursting in awake subjects is of interest in light of evidence that such bursts can be reliably triggered by visual stimulation (Denning and Reinariegel, 2005; Guido et al., 1995), are highly informative (Reinagel et al., 1999), are prevalent during presentation of natural scenes (Lesica and Stanley, 2004), and are very effective in activating cortical circuits (Swadlow and Gusev, 2001; Swadlow et al., 2002). Thalamic neurons display considerable bursting in awake, inattentive subjects (Swadlow and Gusev, 2001), and the current study tests the notion that visual processing in this state may differ considerably from that seen in the alert state.

Previous studies of temporal tuning during burst mode have been limited to anesthetized subjects (Mukherjee and Kaplan, 1995; Grub and Thompson, 2005). Little is known about the receptive field properties of LGNd neurons in the awake but inattentive state. This may largely be due to the fact that, in commonly studied mammals such as primates and carnivores, eye stability is closely linked to visual attention. Thus, stable and repeatable eye positions can be achieved in alert subjects that are trained to fixate, but not in inattentive subjects. In the rabbit, however, there is no linkage between eye stability and visual attention. Eye position in this species is very stable in both alert and inattentive states, often remaining within a range of $\pm 0.5^\circ$ for periods of several minutes (Fuller, 1981; Collewijn, 1971; Swadlow and Weyand, 1985, 1987). This level of stability is similar or better than that achieved by awake primates that are trained to fixate (e.g., Snodderly and Kurtz, 1985). Moreover, hippocampal EEG of the rabbit provides a reliable index of state changes: the alert state is characterized by hippocampal “theta” activity (4–8 Hz) when thalamus is in “relay mode,” and the inattentive state is characterized by high-voltage irregular (HVIR) activity when thalamus is in “burst mode” (Green and Arduini, 1954; Swadlow and Gusev, 2001).

In the present work, we have exploited the remarkable ocular stability of the awake rabbit to examine state-related variations in the temporal filtering of visual information. We conclude that extremely rapid and significant changes in temporal filtering and bursting occur in the LGNd within 1 s following the shift from the alert to the inattentive state.

Results

Data for generation of temporal frequency tuning curves were obtained in both alert and inattentive states from 42 LGNd neurons with concentric, center-surround receptive fields. Thirty-one of these neurons responded in a sustained manner to standing contrast, while 11 responded in a transient manner. An additional 10 LGNd neurons were studied to examine the time course of changes in temporal tuning and bursting immediately following EEG-defined state transitions.
Relay Mode and Burst Mode in Awake LGNd

Figures 1A and 1B illustrate segments of two representative records obtained during visual stimulation in the alert (A) and in the inattentive (B) states. From top to bottom, the figures show the power spectral densities of these EEG periods are shown above, and a representative photograph of the left eye during these states is shown to the right (rostral is to the left). Traces indicate (1) hippocampal EEG activity, (2) eye position (horizontal, H; vertical, V), (3) the LGNd spike trains (asterisks indicate bursts), and (4) stimulus triggers, indicating stimulation at frequency 0.5 and 2 Hz. “Bursts” (illustrated in inset below [A]) are defined according to Lu et al. (1992) as a cluster of at least two spikes with interspike intervals of ≤ 4 ms, in which the first spike in the burst has a preceding interspike interval of at least 100 ms (Lu et al., 1992). Note that the eye is quite stable (< ±0.25°) during these records (27 s each).

Figure 1C shows that the eye often remained stable even as subjects switched from inattentive to alert states, or vice versa (this record was taken in the absence of visual stimulation). Note that in the alert state (A), the EEG is dominated by theta activity (~ 6 Hz), and the horizontal and vertical position of the eyes remained stable within ±0.25° (trace two in Figures 1A–1C). When eye movements did occur, the stimulus presentation was aborted. The frequency of spontaneous bursting was considerably higher in the inattentive than in the alert state (Figures 1C and 5B). Bursts (schematically illustrated in the inset in Figure 1A) are defined as clusters of at least two spikes with interspike intervals of ≤ 4 ms, in which the first spike in the burst has a preceding interspike interval of at least 100 ms (Lu et al., 1992; Guido et al., 1995; Sherman, 1996). Spontaneous firing rates were more irregular and somewhat lower in the inattentive state, but these differences in firing rates were not statistically significant (below).
Temporal Tuning in Alert and Inattentive LGNd

Temporal aspects of visual responses differed remarkably in alert versus inattentive states. Figure 2 shows responses of a sustained (A–C) and a transient (D–F) LGNd neuron during the alert (A and D) and inattentive (B and E) states. In the alert state, the sustained cell responded to frequencies as high as 25 Hz and showed a clear maintained discharge at the lowest frequencies (0.5 and 2 Hz). In the inattentive state, however, the sustained cell failed to respond at frequencies above 15 Hz and lost the sustained component of the response (e.g., compare responses at 0.5 Hz of [A] and [B]). The transient cell responded, in the alert state, to the full range of stimulus frequencies tested (up to 40 Hz). In the inattentive state, the bandwidth of the temporal frequency tuning curve became considerably narrower (the transient neuron failed to respond at frequencies above 30 Hz). Figures 2C and 2F show the tuning curves generated in the alert and inattentive states based on the data shown above. The transition from the alert to inattentive
state caused a dramatic reduction in both the peak frequency (vertical arrows) and bandwidth (horizontal arrows) of the temporal frequency tuning curves.

We quantified the state-dependent changes in temporal frequency tuning in 42 LGNd neurons that were classified as transient or sustained. The distinction between sustained and transient neurons is robust in rabbit LGNd. It is based on the duration of the response, in the alert state, to maintained stimulation of the receptive field center and displays a clear bimodal distribution. Representative examples of sustained and transient responses to a maintained stimulus are shown in Figures 3A and 3B, and the mean population responses (above pre-stimulus values) are shown in Figures 3C and 3D. Cells were classified as sustained (Figures 3A and 3C) or transient (Figures 3B and 3D) during the alert state based on responses to a maintained stimulus of appropriate contrast polarity (black or white), presented over the receptive field center and measured at a latency of 0.4–0.5 s following the stimulus onset (asterisks, see Experimental Procedures). Figure 3E shows that the response at this interval was bimodally distributed (p = 0.02, Hartigan’s dip test, Hartigan and Hartigan, 1985).

Figure 4 illustrates the bandwidth and peak temporal frequency of sustained and transient LGNd cells in alert versus inattentive states. In the alert state, sustained cells had significantly narrower bandwidths than did transient cells (Figure 4A, 17.3 Hz versus 31.6 Hz, respectively, p < 0.001) and were tuned to significantly lower peak temporal frequencies (Figure 4B, 10.7 versus 24.2 Hz, p < 0.001). The transition from alert to inattentive state resulted in a very significant narrowing of bandwidth and lowering of peak temporal frequency response, in both sustained and transient neurons. Thus, both the bandwidth and the peak frequency response in the inattentive state were approximately half the values seen in the alert state (r = 0.63, r = 0.74, respectively, p < 0.001 in both cases).

Figure 4C shows the relationship between the shifts in the bandwidth and peak that are seen when subjects go from the inattentive to the alert state. The overall correlation between these two parameters is highly significant, showing that shifts in the peak frequency response (toward the higher frequencies) that occur in the alert state are accompanied by increases in the bandwidth. (r = 0.88, p < 0.001).

The Time Course of State-Dependent Shifts in Temporal Tuning and LGNd Bursting

Figure 5 illustrates the time course of the changes in bursting and temporal tuning following the state transition. We studied ten neurons as the rabbit spontaneously shifted between an alert state (marked by hippocampal theta activity and cortical desynchronization) and an inattentive state (marked by hippocampal HVIR and cortical slow-wave activity). We selected for analysis only those instances in which a clear transition occurred, that was preceded by >10 s in the alert state, and followed by >10 s in the inattentive state. For each cell, we plotted the number of bursts/s that occurred during the spontaneous LGNd activity for 10 s before and after these transition points. This transition (dashed lines) was studied at least twice for each cell and was identified by two independent observers with remarkable precision (mean interobserver discrepancy = 0.23 s ± 0.2 s).

The top traces of Figure 5A show the spontaneous activity of two LGNd cells, before and after the state transition (bursts indicated by asterisks), and both hippocampal and cortical EEG traces. Further description of burst characteristics can be found in Figure S2 in the Supplemental Data available online. Note that the transition from hippocampal theta activity to HVIR occurs concomitantly with the shift from neocortical desynchronization to lower-frequency, higher-voltage neocortical activity, and that 8–12 Hz sleep spindles, indicative of early sleep stages (stages 1 or 2) are not present. Figure 5B shows the results from all ten cells studied under conditions of “spontaneous” activity (i.e., in the absence of visual stimulation). The frequency of bursting (bursts/s) was 12.4 times higher during the 10 s following the transition than during the previous 10 s. Remarkably, during the 1 s period following the transition, the burst frequency increased by a factor of 7.3 (as compared to the previous second, p < 0.001, paired t test). Spontaneous firing rates were slightly lower in the inattentive than in the alert state (7.6 versus 9.3 spikes/s), but this difference was not significant.
Figure 5C shows the results for similar experiments in which we examined the time course of the temporal tuning shift after the state change. For the same ten neurons, we examined the responses (F1, first harmonic) to a high-frequency visual stimulus (stimulus conditions identical to those used in the above temporal tuning experiments. For these brief tests (<30 s each) we required eye position to remain very stable (±0.25º). This stimulation was presented at a temporal frequency that was near the high end of the tuning curve for each cell, as determined during the alert state (usually 20–30 Hz, filled circles). In five of these neurons, we also measured the visual response to a visual stimulus of lower temporal frequency (5–10 Hz, near the peak of the temporal frequency tuning curve, open circles). Notably, the response to high-frequency visual stimulation was significantly reduced during the initial second following the transition (p < 0.001, paired t test). However, the response to the lower-frequency visual stimulus showed only a mild attenuation following the transition point. Together, these results indicate a considerable narrowing of the temporal frequency tuning curve within the initial second of the state shift.

**Bursting and Temporal Tuning in Awake LGNd**

As was the case for spontaneous bursting, visually driven bursting was much more prevalent in the inattentive than in the alert state. However, there were no significant state-related differences in the number of spikes/burst (2.94 versus 3.00 spikes/burst in alert versus inattentive states, respectively). In order to normalize for observed state-dependent differences in visually driven firing rates, we calculated a “burst fraction” for each neuron, which indicated the percentage of all spikes that were part of a burst. The “burst fraction” was calculated, during low-frequency (5 Hz or less) visual stimulation in both alert and inattentive states, and a difference of ~6-fold was seen in both sustained and transient LGNd populations (Figure 6A, overall mean = 0.36 versus 0.06, p < 0.001). We used only spike data based on low-frequency stimulation to calculate burst fraction because any wide-band neurons would, by necessity, have low burst fractions during high-frequency stimulation. This is because reliable burst responses at frequencies of >10 Hz would violate the very definition of a “burst,” which requires a preceding interval of at least 100 ms. Notably, there was a reasonably good positive correlation between the burst fractions in the two states (r = 0.54, p < 0.001), which supports the notion of intrinsic differences in burstiness among LGNd neurons. This relationship was also significant when bursts were treated as unitary events and burst fraction was expressed as the percentage of firing events that were bursts (r = 0.44, p < 0.005, the correlation between measuring burst fraction in these two ways was very high r = 0.97, n = 42).

In the inattentive state, some LGNd cells were much more bursty than others, raising the possibility that these differences could be related to the differences in temporal tuning among LGNd neurons in this state (Figure 4). However, this was not found to be the case. Figures 6B and 6C show the relationship between bandwidth and burst fraction for sustained and transient cells, respectively. In the inattentive state, wide ranges in burst fraction are seen in both populations, but this was not significantly related to either bandwidth (r = −0.03 and r = −0.05 in sustained and transient cells, respectively) or peak temporal frequency (not shown in this figure, r = 0.09 and r = −0.02 in sustained and transient cells, respectively).

**Receptive Field Size and the Inattentive State**

The sizes of receptive field centers did not differ appreciably in the two states. Figures 7A and 7B show receptive fields of two LGNd neurons plotted in the alert and
inattentive states. The power spectra of the hippocampal EEGs recorded in the two states are shown below. Receptive fields were generated by reverse correlation using sparse noise stimuli. Figure 7C shows records of the receptive field sizes measured in 15 LGNd neurons. Although the area of the receptive field centers were, on average, slightly larger in the alert state (by ~10%), this difference was not statistically significant.

**Discussion**

Our results show that both the bandwidth and the preferred temporal frequency of LGNd neurons are sharply reduced, in a correlated manner, when awake rabbits shift from the alert to the inattentive state and that this transition is accompanied by a dramatic increase in LGNd “bursts.” Remarkably, these changes in LGNd...
bursting and temporal tuning occur within less than a second following EEG-defined state shifts (Figure 5). This rapid time course supports our contention that rabbits are awake during the “inattentive” state. We have previously argued (Swadlow and Gusev, 2001) that rabbits are awake but drowsy/inattentive during hippocampal HVIR because (1) the eyes are open (as in Figure 1) and (2) they can easily shift from inattentive (HVIR) to alert (theta) states either spontaneously or when presented with an innocuous but novel visual stimulus (waving hand, spot of light on ceiling). Our present results further support these arguments by showing that these shifts in temporal frequency tuning and bursting occur within a single second of the EEG-defined state transition. It is highly improbable that rabbits enter a sleep state less than 1 s following the termination of cortical desynchronization and hippocampal theta activity, particularly since no 8–12 Hz “spindles” (the classic signs of early sleep stages) are observed in thalamus or cortex during this period.

The pronounced increases in both spontaneous and visually driven cell burstiness in the inattentive state suggest that the corresponding changes in temporal tuning are due to factors associated with the “burst mode” of thalamic function (Jahnsen and Llinas, 1984b; Lu et al., 1992; McCormick and Feeser, 1990). Although we cannot be certain that all of our “burst events” were mediated by low-threshold calcium currents associated with burst mode, we think it very likely that the great majority were. Lu et al. (1992) showed that most LGNd bursts identified in extracellular recordings (with the criteria used here) were indeed generated by this mechanism. However, the association of burst mode in awake, inattentive subjects with changes in temporal tuning does not imply that these changes are caused by the low-threshold calcium current that underlies thalamocortical bursts. Indeed, Castro-Alamancos (2002) has shown that reticular stimulation or direct application of acetylcholine to somatosensory thalamic neurons eliminates the suppression to high-frequency stimulation that is seen in anesthetized subjects and that simple depolarization of the thalamic neurons is sufficient to account for this effect. Similarly, Carandini et al. (1997) have shown that conductance-based changes in gain can shift both the peak and bandwidth of temporal frequency tuning curves in a manner very similar to that which we have observed (also see Alitto and Usrey, 2004). Interestingly, preliminary observations show that changes in contrast sensitivity of LGNd neurons accompany the transition from alert to inattentive state (M.C. et al., 2005, Soc. Neurosci., abstract) and that these state-dependent changes are best described as a change in response gain (e.g., Reynolds et al., 2000).

In the alert state, LGNd neurons are clearly segregated into sustained and transient concentric populations, based on their response to standing contrast. Transient cells had significantly higher peak frequencies and broader bandwidths than did sustained neurons, but temporal filtering of both populations become strongly band-pass filtered in the inattentive state, with attenuation at both high and low frequencies. Attenuation of low frequencies is especially obvious in sustained cells, which loose the sustained component of their response to standing contrast when in the inattentive state (Figures 2A and 2B; also see Swadlow and Weyand, 1985; Funke and Eysel, 1992). Despite the loss of maintained responding in sustained LGNd cells when subjects are inattentive, the peak of the temporal frequency tuning curve of these cells (and of transient cells) shifts to a lower peak frequency, implying very powerful attenuation of high frequencies in the inattentive state (Castro-Alamancos, 2002, 2004).

Previous studies aimed at defining the relationship between thalamic “bursting” and temporal tuning of
LGNd neurons have been performed in anesthetized subjects and used two markedly different methodologies. One approach has been to keep “state” constant by maintaining constant anesthetic levels and to ask how the frequency response characteristics of single LGNd spikes differ from those of bursts. Using this approach in mouse LGNd, Grubb and Thompson (2005) found that single LGNd spikes were much more broadly tuned to frequency than were bursts. This approach has also been used to examine spatial receptive field properties (Alitto et al., 2005; Rivadulla et al., 2003) and to examine bursts from an information theoretic perspective (Reinagel et al., 1999). A second approach has been to vary the level of anesthesia (the state) and to thereby control the degree of bursting in LGNd neurons. Using this strategy, Mukherjee and Kaplan (1995) showed an inverse relationship between burstiness and bandwidth of the temporal frequency tuning curve. They concluded that the “temporal tuning of a cell was directly related to the degree of burstiness of its spike train,” and that the LGNd can act as a “tunable temporal filter” of visual information.

Our strategy, although applied in the awake state, was very similar to that of Mukherjee and Kaplan (1995). Indeed, we did not compare the sensory responses of bursts with single spikes, as was done by Grubb and Thompson (2005). In our study, however, we controlled the state of alertness/inattention, rather than anesthetic level. Moreover, our results are consistent with those of Mukherjee and Kaplan (1995) in finding that increases in bursting are associated with decreases in bandwidth. However, our results do not imply (as theirs do) that LGNd burstiness and temporal tuning covary continuously over a wide range. Our results do not support this notion, at least within the inattentive state. We did find that different LGNd neurons displayed markedly different degrees of state-related bursting and widely different temporal tuning properties (Figures 4 and 6). Our results, however, suggest that this is due to heterogeneity among LGNd neurons, not to a continuum of burstiness and temporal tuning within the inattentive state. Thus, we found that the temporal frequency bandwidth seen in different LGNd neurons in the inattentive state was not related to the degree of bursting (the “burst fraction,” Figures 6B and 6C) in that state. The burst fraction in the inattentive state was, however, strongly related to its value in the alert state (Figure 6A). Moreover, the values of both the bandwidth and peak of the temporal tuning curves in the inattentive state were highly correlated with their values in the alert state (Figures 4A and 4B). Together, these findings imply that the differences in burst fraction and temporal tuning characteristics among different LGNd neurons within the inattentive state signify heterogeneity among LGNd neurons. However, these results do not imply that the state of the thalamus in inattentive subjects is equivalent to that during deep anesthesia or even sleep. Under such conditions, the level of bursting is likely to be even more extreme, and the bandwidth of LGNd neurons could be reduced even further.

To keep our subjects in an alert state, it was usually necessary to vary the level of nonvisual sensory stimulation in the immediate environment. This raises the possibility that changes in temporal tuning/bursting may have been due to arousal mechanisms related to the stimulation, not simply to the alert state. We found, however, that shifts in temporal tuning and bursting were associated with state changes and not with the presence or absence of such nonvisual sensory stimulation. Thus, subjects in an alert EEG state showed broad temporal tuning and little bursting with or without added nonvisual sensory stimulation. Indeed, in the experiments described in Figure 5, the level of nonvisual sensory stimulation was identical during the several seconds prior to and following the shift from the alert to the inattentive state, but bursting and temporal tuning differed dramatically.

By contrast with the clear state-dependent changes in the temporal tuning curves of LGNd neurons, we saw little state-dependent differences in the size of the receptive field centers. Receptive fields were an average of ~10% larger in the alert state, but this difference was not statistically significant. Similarly, Livingstone and Hubel (1981) found no obvious differences in receptive field sizes of LGNd neurons studied during slow-wave sleep versus aroused conditions, and Uhlich et al. (1995) reported little effect of brain stem cholinergic activation on receptive field center size. In addition, Grubb
and Thompson (2005) found no differences in the spatial frequency tuning of bursts versus nonbursts of mouse LGNd neurons, and Alitto et al. (2005) found no difference between bursts and single spikes in the size of receptive field centers. Rivadulla et al. (2003) did report a reduction in the receptive field centers that were generated by burst spikes. However, as discussed by Alitto et al. (2005), these differing results of Rivadulla et al. (2003) concerning receptive field size could be due to the different methods used to generate the receptive field centers.

Many mammals appear to spend considerable periods of time in the inattentive state, especially in a familiar and nonthreatening environment. Nevertheless, they must be able to respond appropriately to biologically relevant stimuli when inattentive. One would expect that the marked changes in neuronal response properties observed here would be associated with equally distinct changes in sensory capacities, but there have been few psychophysical studies of inattentive subjects that are not confounded by pharmacological manipulations. Thus, Sharma et al. (2002) report a reduction in critical flicker fusion and a concomitant increase in drowsiness in human subjects given clinically relevant oral doses of the antipsychotic procyclidine. There is a literature in human subjects relating level of vigilance to psychophysical measures, and reduced vigilance is reported to result in a shrinking of the useful visual field (Roge et al., 2002) and a lowering of the critical flicker fusion and a concomitant increase in the size of the squares was either 0.8º or 1.6º, which was appropriate given that the center diameter of most LGNd receptive fields studied was 2º–4º (below). Receptive field maps were plotted separately for white and black squares. The receptive filed size was calculated as the number of grid spaces included within the 30% contour line of the maximum response.

Visual Stimulation and Receptive Field Measures
Visual stimuli were presented on a FlexScanF980 (EIZO Nanao corporation, Japan) computer monitor, with 160 Hz refresh rate. Visual stimuli were created with a VisionWorks stimulus generator (Vision Research Graphics, Inc). Reverse-correlation analysis was performed for quantitative description of the receptive field of a neuron in both alert and inattentive states. Sparse noise stimulation consisted of a 16 × 16 grid array of randomly presented white squares on a black background or black squares on a white background. Each square lasted 18.75 or 25 ms (three or four frames) and was presented ten times. The size of each grid space was 0.8º, and the size of the squares was either 0.8º or 1.6º, which was appropriate given that the center diameter of most LGNd receptive fields studied was 2º–4º (below). Receptive field maps were plotted separately for white and black squares. The receptive field size was calculated as the number of grid spaces included within the 30% contour line of the maximum response.

We used no corrective lenses during receptive field testing. Hughes and Vaney concluded, based on measures taken using a calibrated fundus camera, that the effective image shell of the rabbit eye is within 0.6 diopters of emmetropia. This conclusion was supported by Meyer et al. (1972), who investigated refractive state using electrophysiologic measures of single-line acuity. Meyer et al. also studied refractive state in the presence and absence of astigmatism with no evidence of accommodative changes (Meyer et al., 1972). This is consistent with a poorly developed ciliary body in the rabbit, with few muscle fibers and no circular fibers (Prince, 1964). De Graauw and Van Hof (1977), using behavioral methods, also concluded that “no accommodation mechanism exists in the rabbit,” but reported that their subjects were somewhat hypermetropic in the region of visual field studied here. This latter finding may be due to their use of retinoscopy to measure refractive state, in which, in an eye the size of the rabbit’s, can generate a false measure of -2 diopters of hypermetropia (Glickstein and Milrodot, 1970; discussion in Hughes and Vaney, 1978). In order to be sure that state-dependent changes in the plane of focus were not an issue in our experiments, we examined single-line acuity of multifuntion activity in the superficial superior colliculus (where the response is dominated by w-cell input from the optic tract). At a distance of 70 cm, the visual response was clearly modulated by a low-contrast dark line that subtended ~2 min of arc, but no response was generated by a stimulus of 1 min of arc. No differences were seen in this threshold measure between the aroused versus inattentive states.

Only concentrically organized cells with receptive fields at an elevation of 15º to 25º (with respect to the horizon) and from 20º–70º from the midline (0º being directly in front of the animal) are included in this study. For each recorded cell, we defined the optimal parameters of receptive field position, receptive field size, and preferred spatial frequency. These optimal parameters were used for the temporal frequency test. This test consisted of sine-wave gratings with high contrast-reverse phases, and temporal frequencies from 0.5–40 Hz in ten steps. Contrast was between 0.95 and 0.98, defined as (Lmax – Lmin)/(Lmax + Lmin), with Lmax being ~108 cd/m². The size of the grating was adjusted to ~1.2 times the diameter of the receptive field center, and the spatial frequency was optimal for the cell under study (usually 0.2–0.4 cycles/degree). In initial
experiments, we used drifting gratings for this test, but we later rejected these stimuli because it is not possible to generate smoothly drifting gratings at high temporal frequencies when the stimulus refresh rate is limited to 160 Hz.

Each of the ten temporal frequencies was presented for 15 s in the alert and in the inattentive states. Trials in the alert and inattentive states were analyzed only when two conditions were met: (1) when the appropriate hippocampal EEG activity (theta or HVIR, see section below) was seen in at least 70% of the record), (2) when eye position remained within a range of ±0.75° for the entire trial (usually a range of <±0.5° was achieved, and in some cases <±0.25°). If the eye moved more than this, the stimulation was stopped and the stimulus was repositioned over the receptive field center before repeating the test. In most cases, the full test was repeated, in both states (meeting the above conditions), at least twice.

The Distinction between Alert and Inattentive States

In one set of experiments (42 of the 52 neurons studied), hippocampal EEG activity provided the index of alert versus inattentive states, while in a second set of experiments (ten cells, Figure 5), we recorded EEG from the hippocampus and from both deep and superficial cortical layers. Hippocampal activity in the rabbit can generally be separated into theta activity (4–8 Hz) and high-voltage, irregular activity (HVIR). Theta hippocampal activity has been associated with an aroused, alert, attentive state, and HVIR has been associated with a nonaroused, inattentive state (Green and Arduini, 1954). We have previously shown that during theta activity, ventrobasal thalamocortical neurons are in tonic firing mode with few bursts in the spike train (Swadlow and Gusev, 2001). During HVIR, however, cells fire with less regularity, and many more bursts are seen in the spike train. During our recordings, rabbits often alternate spontaneously between theta and HVIR activity. However, once they are adapted to the recording situation, they generally spend more time in HVIR than in theta, and maintaining HVIR during our receptive field testing was usually not difficult. Although a looming visual stimulus close to the rabbit will often elicit a switch from HVIR to theta activity, our computer-generated stimuli were ineffective in this regard (otherwise, testing during HVIR would not have been possible). Maintaining theta activity for the duration of our tests was sometimes difficult. In order to maintain near-continuous theta activity, the EEG was closely monitored, and novel auditory stimuli were generated when necessary. In some cases, this required a near-continuous stream of novel sounds (clicks, whistles, hisses), while in other cases, less frequent sounds were required to maintain theta activity.

Records included in the analysis of temporal frequency were either strongly dominated (>70%) by hippocampal theta activity (the alert state) or showed little (<20%) or no theta activity (the inattentive state). This judgment of “high-theta” and “low-theta” states was initially made subjectively, by agreement of two investigators after visual inspection of the records. Subsequently, these chosen records were analyzed more quantitatively by two investigators (one of the original investigators and one new investigator) who, by visual inspection, divided each data file into “theta” segments and “non-theta” segments. Inter-rater agreement was very good (Figure S1a), and only a few of the tentative high-theta files were eliminated because one or both observers rated them as <70% theta. Next, each of the files that remained (those rated by both observers as consisting of either <20% or >70% theta) were subjected to FFT analysis, and the power in the theta range (5–7 Hz) was compared to the power in the range of 2–4 Hz. Notably, this FFT analysis agreed well with the ratings of the two observers and revealed that the putative high-theta and low-theta files had nonoverlapping distributions (Figure S1b).

One set of experiments was aimed at characterizing the time course of changes in bursting and temporal tuning following the shift in EEG state (Figure 5). In these experiments, we recorded cortical EEG from both deep and superficial layers, as well as hippocampal EEG. In these experiments, we examined the transition point between theta activity (and cortical desynchronization) and HVIR (and cortical slow-wave, high-voltage activity). Two experimenters made independent estimations of this transition point (by visual inspection of the records). These estimates showed a mean interobserver disagreement of only 0.23 s (SD = 0.2 s).

The Distinction between Sustained and Transient LGNd Neurons

LGNd neurons were presented with a high-contrast, light or dark spot over the receptive field center and were classified as “sustained” or “transient” based on the resulting maintained discharge. The stimulus was presented for at least 0.5 s, the size of the spot was approximately the size of the receptive field center, and the contrast polarity (light or dark) was appropriate for the sign of the receptive field center (on or off). This test was always applied in the alert state because the maintained discharge of “sustained” LGNd neurons is sensitive to EEG state (Livingstone and Hubel, 1981; Swadlow and Weyand, 1985; Funke and Eysel, 1992; Worhgottet et al., 1998). We examined the response that occurred from 400–500 ms after stimulus onset and classified all cells that displayed an enhanced maintained discharge (above the pre-stimulus baseline) of more than ten spikes/s as sustained, and less than ten spikes/s as transient. This measure was based on the bimodal distribution of firing rate at this interval (Figure 3 in Results). All but four neurons were classified in this manner. The remaining cells were classified by the presence or absence of a maintained response 400–500 ms following the reversal of the contrast grating (presented at 0.5 Hz) that was used for the temporal frequency tests.

Data Analyses

Responses to different temporal frequencies were measured by calculating the first Fourier harmonic (F1) of the response (Smith et al., 2000). Tuning curves were fitted with Gaussian functions. From this function we extracted three different parameters: peak frequency, peak amplitude, and bandwidth at half-amplitude. T tests (paired or unpaired) were used to compare population means that were normally distributed. The Lilliefors modification of the Kolmogorov-Smirnov test was used as a normality test.

We used the Wilcoxon matched-pairs test to compare populations that were not normally distributed and the Mann-Whitney U test to compare populations with unequal number of observations. The significance of bimodality was tested with a Hartigan’s DIP test (Hartigan and Hartigan, 1985) using a Matlab algorithm of F. Mechler (available at http://manuelita.psych.ucla.edu/).

Bursts were identified according to the criteria of Sherman and colleagues (Lu et al., 1992; Guido et al., 1995; Sherman, 1996), where a burst is defined as a cluster of two or more spikes with interspike intervals of <4 ms, in which the first spike in the burst has a preceding interspike interval of at least 100 ms. We also calculated a “burst fraction” as the ratio between spikes within burst to all recorded spikes. The spikes used for calculating the burst fraction in the alert state all occurred during clear theta activity (any short periods of HVIR were not included). Similarly, burst fraction in the inattentive state was calculated during clear HVIR activity.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/49/3/10/DC1/.

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References

Inattentive Vision and Thalamic Burst Mode


