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Visual stimuli modulate precise synchronous firing within the thalamus

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Abstract

The work of Mircea Steriade demonstrated that the neocortex could synchronize large regions of the thalamus within 10–100 milliseconds (for review see Steriade and Timofeev, 2003, Steriade, 2005). Unlike the synchrony generated by the cortex, the retinal afferents synchronize a restricted group of neighboring thalamic neurons with <1-millisecond precision (Alonso et al., 1996, Yeh et al., 2003). Here, we use a large sample (n=372) of simultaneous recordings from neighboring neurons in the Lateral Geniculate Nucleus (LGN) to illustrate the high specificity of the synchrony generated by retinal afferents and its dependency on sensory stimulation. First, we demonstrate that cells sharing a retinal afferent show a balanced receptive field diversity: while slight receptive field mismatches are common, the largest mismatches in a specific property (e.g. receptive field size) are restricted to cells that are precisely matched in other properties (e.g. receptive field overlap). Second, we show that these receptive field mismatches are functionally important and can lead to a 5-fold variation in the percentage of synchronous spikes driven by the shared retinal afferent under different stimulus conditions. Based on these and other findings, we speculate that the precise synchronous firing of cells sharing a retinal afferent could serve to amplify local stimuli that may be too brief and small to generate a large number of thalamic spikes.

Keywords

LGN; retinogeniculate; correlated firing; visual cortex; spike timing; thalamocortical

Introduction

The connection between a retinal cell and a thalamic cell from the Lateral Geniculate Nucleus (LGN) is somewhat unique in the visual system. Most geniculate cells receive input from, or are dominated by, just one single retinal afferent and this connection is so strong that it is usually responsible for most geniculate spikes generated (Chen and Regehr, 2000, Cleland and Lee, 1985, Cleland et al., 1971, Mastronarde, 1992, Usrey et al., 1999, Hamos et al., 1987). In addition, the timing of a retinogeniculate connection is so precise that the jitter between presynaptic and postsynaptic spikes is usually less than 1 millisecond (Cleland et al., 1971, Mastronarde, 1987, Usrey et al., 1999). These three characteristics of retinogeniculate connections—limited convergence, strength and time precision—are responsible for some important properties in information processing at this level of the visual pathway. On the one hand, they guarantee a high reliability of information transmission to the cortex by making

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retinal spikes precisely correlated with thalamic and cortical spikes (Lee et al., 1977, Kara and Reid, 1999). On the other hand, they boost thalamocortical efficiency by generating a tight correlated firing among the thalamic inputs that converge onto a common cortical target (Alonso et al., 1996, Usrey et al., 1998). Yet another important property of retinogeniculate connections could be to help processing local, transient stimuli that fail to generate a large number of spikes. Our results are consistent with this idea. Here we demonstrate that cells sharing a retinal afferent have small but significant receptive field mismatches that can cause pronounced variations in the strength of their correlated firing. Moreover, we show that, while the correlation strength due to shared retinal afferents is considerably reduced during stimulus transients, small transient stimuli are able to drive 2 times more synchronous spikes in cells that share a retinal afferent than those that do not.

Objective

The objective of this paper is to honor the outstanding work of Prof. Steriade and, at the same time, provide a detailed description of the response properties and synchronous firing of thalamic cells that share input from the same retinal afferent. We pursue these objectives by performing simultaneous recordings from multiple neighboring neurons within the visual thalamus (LGN) while identifying those that share input from the same retinal afferent by their tight correlated firing.

Methods

Surgery and electrophysiological recordings

Cats were anesthetized with ketamine (10 mg/kg, IM) and thiopental sodium (20 mg · kg⁻¹, IV, supplemented as needed) and then paralyzed with Norcuron (0.2 mg · kg⁻¹ · h⁻¹, IV). All surgical and experimental procedures followed the guidelines of the U.S. Department of Agriculture (USDA) and were approved by the Institutional Animal Care and Use Committees at the University of Connecticut and the SUNY College of Optometry. Geniculate cells were recorded from layers A and C of LGN with a multielectrode matrix of seven independently movable electrodes (Thomas Recording, Marburg, Germany, (Eckhorn and Thomas, 1993)). A glass guide tube with an inner diameter about 300 µm at the tip was attached to the shaft probe of the multi-electrode to reduce the inter-electrode distances to approximately 80–300 µm. This method allowed us to record from multiple neighboring cells with overlapping receptive fields (distance between cell pairs <300 µm). Recorded signals from all seven electrodes were amplified, filtered, and collected at 31,250 Hz by a computer running the Discovery software package (Datawave Systems, Longmont, CO). For each cell, spike waveforms were initially identified during the experiment and verified off-line for each cell by using cluster analysis software from Datawave Systems (Longmont, CO) and Plexon Inc. (Dallas, TX). See Yeh et al. (2003) for further details on surgical procedures.

Visual stimuli

Visual stimuli were generated with an AT-vista graphics card (Truevision, Indianapolis, IN) and shown on a 20-inch monitor (Nokia 445Xpro, Salo, Finland; frame rate: 128 Hz; mean luminance: 60 cd/m²). Cells were classified as X or Y based on the linearity of spatial summation measured with contrast reverse gratings (Hochstein and Shapley, 1976). We recorded from 88 pairs of tightly correlated geniculate cells (YY: 35, XX: 22, XY: 7, non-classified: 24; in non-classified pairs, one or both cells could not be unequivocally classified as X or Y with the linearity test). Thirty-seven cell pairs were recorded within layer A, 31 within layer C and 20 across layers. Nine cell pairs were obtained from Yeh et al. (2003). In 68 pairs we were able to measure the strength of the tight correlations under multiple stimulus conditions, which included white noise and at least another stimulus: sparse noise (n=44),

contrast reverse gratings ($n=39$), moving bars ($n=41$) or white noise with a smaller pixel size ($n=26$). If the duration of the recordings permitted, the moving bars were presented at different temporal frequencies (0–14 deg/sec) and the sparse noise presented at different contrasts (98% or 16%). On average, each cell pair was tested under 5 different stimulus conditions (different temporal frequencies and different contrasts were counted as different stimulus conditions). Measurements with sparse noise were also performed in 49 cell pairs that were not tightly correlated but had overlapping receptive fields of the same sign. White noise consisted of a series of 16×16 -pixel pseudo-random checkerboards (0.45–0.9 degrees/pixel) each presented for 15.5 milliseconds (Reid et al., 1997, Sutter, 1992). The sparse noise (Jones and Palmer, 1987) consisted of a series of 16×16 individual squares that could be either white or black, each square presented for 31 milliseconds 16 times (size: 1.8×1.8 degrees/pixel; separation between square positions in grid: 0.9 degrees/pixel). The contrast reverse gratings were shown at two different spatial frequencies (0.55 and 1.1 cycles/deg) and 8 spatial phases and were repeated at least 8 times for each spatial phase. Bars ($1^\circ \times 15^\circ$) were moved at different velocities (0.2 to 14 deg/sec) and could be either light or dark.

Receptive field mapping

Receptive fields and impulse responses (e.g. Fig. 1) were measured with white noise stimuli and reverse correlation (Reid et al., 1997, Sutter, 1987, Sutter, 1992). The receptive fields were calculated in units of spike per second and normalized by the response peak, and then transformed into contour plots smoothed with a cubic spline (Matlab, Mathworks, Natick, MA). The most peripheral contour line represents 20% of the maximum response and each additional contour line represents a 20% increment in response strength (Matlab, Mathworks, Natick, MA). The 20% contour line was chosen to measure the receptive-field size and receptive field overlap (measurements below 20% would be less accurate due to the presence of surround responses and background noise). The receptive field size was measured as the number of contiguous pixels that generated more than 20% of the maximum response. The receptive field overlap was calculated as the percentage of pixels from the cell with the smaller receptive field that were superimposed with pixels from the cell with the larger receptive field (see Fig. 3). It should be emphasized that the receptive field overlap and receptive field size in this study refer exclusively to the receptive-field center and not the surround.

The impulse response was defined as the time-course of the most effective white-noise pixel within the receptive-field center (the pixel that generated the maximum response). The impulse responses were normalized by the peak amplitude and smoothed with a cubic spline (the peak is the maximum, in absolute value, of the first phase of the impulse response; it is positive for on-center cells and negative for off-center cells). The difference in response latency for each cell pair was calculated by subtracting the peak time of the impulse response with the longest latency from the peak time of the impulse response with the shortest latency. Finally, the receptive field similarity (see Fig. 5) was measured by cross-correlating the spatiotemporal receptive fields of the two cells and then expressing the correlation index as a percentage: the receptive field similarity is 100% if the two cells have identical receptive fields of the same sign and it is –100% if they have identical receptive fields of opposite sign.

Cross-correlation analysis

We measured the correlated firing from multiple pairs of neighboring geniculate cells that were simultaneously recorded and searched for correlograms that had a narrow peak with less than 1 millisecond width at half-height. If the narrow peak passed our significance test (see below), this cell pair was classified as ‘tightly correlated’. There is strong evidence indicating that tight correlations are generated by divergent retinal afferents. First, tight correlated geniculate cells are known to receive input from the same retinal afferent [see (Alonso et al., 1996) for triple recordings from an s-potential and two geniculate cells and (Usrey et al., 1998) for triple

recordings from a retinal cell and two geniculate cells]. Second, the percentage of neighboring geniculate cells that share a retinal afferent (Hamos et al., 1987) is similar to the percentage of neighboring cells that are tightly correlated (Alonso et al., 1996). Third, tightly correlated geniculate cells share many properties in common [e.g. receptive field position, sign, size, timing (Alonso et al., 1996, Yeh et al., 2003, and figures 3, 5 in this manuscript)] as would be expected from cells that share a retinal input (Cleland et al., 1971, Mastronarde, 1987, Usrey et al., 1999). And fourth, tight geniculate correlations are much faster (~0.5 milliseconds width at half-height) than the fastest synchrony measured in the cat retina [~3 milliseconds width at half-height (Mastronarde, 1989); although see (Brivanlou et al., 1998) for salamander retina].

Tight correlations were identified, as in previous studies (Alonso et al., 1996, Yeh et al., 2003), by the following criteria. First, the correlograms were filtered between 125 and 700 Hz to eliminate the slow correlations and high frequency noise [this technique is as effective as the shuffle subtraction (Perkel et al., 1967) and allows measurements of tight correlated firing under more diverse stimulus conditions]. Second, a correlogram baseline was calculated as the average value between 2 and 1 milliseconds (i.e. the immediate neighborhood of the one-millisecond peak). Finally, a tight correlation was considered significant if the maximum of the filtered correlogram within -1 and 1 milliseconds was larger than 3.1 standard deviations of the baseline. Although the entire width of the tight correlation is less than 1 millisecond, the measurements were made between -1 and 1 milliseconds (2 milliseconds total) because the peak can sometimes be slightly displaced from zero (e.g. between -0.6 milliseconds and 0.3 milliseconds). These small peak asymmetries are probably due to small differences in the conduction velocity of the axonal branches. Correlograms with less than 20 events in the central 4 bins were discarded to avoid noisy measurements. Tight correlations were first measured for significance under white noise stimulation. When a tight correlated pair passed the significance criteria with this stimulus, we searched for significant tight correlations under other stimulus conditions.

Correlation strength was measured from the raw, unfiltered correlograms (only in correlograms that passed the significance test) as:

$$\text{Correlation strength} = (\text{peak} - \text{baseline}) / \text{spike count}$$

where *peak* is the correlogram count between -1 and 1 ms, and *spike count* is the total number of spikes from each neuron (from each cell pair we obtained two values of correlation strength, one for each cell). Stimulus modulations of correlation strength were measured in 68 cell pairs that were recorded for a period of time long enough to reveal significant tight correlations under at least two different stimulus conditions. The shuffle correlograms were obtained by shuffling the repeated stimulus cycles of each spike train (Perkel et al., 1967). We experimented with different types of shuffle using both custom-made (Matlab, Maths Works, Natick, MA) and commercially available software (Nex Technologies, Littleton, MA). The shuffle correlogram was obtained by shuffling sections of 4 stimulus cycles or 1 stimulus cycle, shuffling once or averaging 5–10 different shuffles. We also obtained shuffle correlograms by cross-correlating the peristimulus time histograms [PSTH, (Brody, 1998)]. All these different approaches gave very similar shuffle correlograms (e.g. see figure 7). If not stated differently, the shuffle correlograms shown in the figures were obtained by shuffling sections of 4 stimulus cycles 5 times and averaging these shuffles. Correlograms were calculated by using a bin of 0.1 milliseconds (most figures) and 0.5 milliseconds (correlograms with 50 milliseconds time window in Figure 5a).

Synchrony generated by sparse noise stimuli

As explained above, the sparse noise stimuli consisted of a sequence of individual squares that could be either light or dark and were presented at 16×16 different positions, each one for 31 milliseconds. The synchrony generated by sparse noise stimuli was obtained by selecting the square position and contrast polarity (dark or light) that generated the largest number of synchronous spikes within a specific time window (100 milliseconds after the stimulus pulse). In a given cell pair (A–B), a synchronous spike in cell A is a spike that occurred within 1 millisecond of a spike generated by cell B, therefore, the number of synchronous spikes can be slightly different for cell A and B. For example, if cell A generates a spike at 25.5 milliseconds and cell B generates 2 spikes at 25 and 26 milliseconds after the stimulus, there will be 1 synchronous spike in cell A and 2 synchronous spikes in cell B. The one-millisecond time window was chosen to match the time precision of the synchrony generated by a retinal afferent (the width of the tight correlation). A shuffled synchrony was calculated to estimate the number of synchronous spikes that were simply due to the stimulus transience and not the tight correlation. The 16 stimulus repetitions were shuffled three times to obtain three values of shuffled-synchronous-spikes/stimulus. Then, these three values were averaged to obtain the shuffled synchrony (Figure 8). For this analysis we selected 44 tightly correlated cell pairs and 49 non-tightly correlated cell pairs, and included all the sparse noise stimulus conditions tested for each pair (i.e. different contrasts).

Results

Retinal cells and geniculate cells that are monosynaptically connected can be reliably identified *in vivo* by cross-correlation analysis (Cleland et al., 1971, Mastronarde, 1987, Usrey et al., 1999). The monosynaptic retinogeniculate correlogram is characterized by a very thin peak (~ 1 millisecond width) displaced from zero which is found in a small percentage of retino-geniculate cell pairs with overlapping receptive fields (12 out of 205 cell pairs in Usrey et al., 1999). As a consequence of this precise correlated firing, geniculate cells that share a retinal afferent can be also identified *in vivo* by a very narrow peak (< 1 millisecond width) centered at zero in the intra-geniculate correlograms, called here *tight correlation* (Usrey et al., 1998, Alonso et al., 1996, Yeh et al., 2003, see methods for detail). Figure 1a illustrates an example of two tightly correlated geniculate cells (cell A₁ and cell A₂). These two cells were recorded with exceptionally good isolation for more than five hours and they are the best studied cell pair, and one of the strongest tight correlations reported, in more than 10 years doing these extremely difficult recordings (Alonso et al., 1996, Yeh et al., 2003, Alonso et al., 2006). It is usually a standard practice to document papers with different cell examples; ours will be an exception. Here, we will use our ‘star cell pair’ to illustrate the main points of the paper and, at the same time, provide one of the most complete and detailed documentations of the responses from two geniculate cells that share a retinal afferent. This cell pair will serve as our modest tribute to the outstanding work of Prof. Steriade.

The cells A₁ and A₂ from our ‘star cell pair’ had receptive fields of the same sign (off-center), same type (Y cells), similar receptive field positions and sizes (Fig 1a, left) and similar response time-courses (Fig 1a, middle). Cross-correlation analysis between the firing patterns of cells A₁ and A₂ (obtained under white noise stimulation) revealed a strong narrow peak centered at zero characteristic of cells that share a retinal afferent (Fig 1a, right; see methods for detail). Unlike cells A₁ and A₂, most neighboring geniculate cells did not show tight correlated firing even if the receptive fields were just slightly mismatched (Figure 1b).

Only a small percentage of neighboring geniculate cells show tight correlated firing

In a simultaneous recording from multiple geniculate cells with overlapping receptive fields, the percentage of cell pairs that show significant tight correlations can range from 0–40%.

Figure 2 shows two representative examples of simultaneous recordings from neighboring cells in LGN. In the first example (Fig. 2a), 9 neighboring geniculate cells with overlapping receptive fields were simultaneously recorded, and from all possible 36 pairs no one showed a tight correlation (throughout the paper, on-center cells are shown in continuous lines and off-center cells in discontinuous lines). The second example (Fig. 2b) illustrates a simultaneous recording from 5 neighboring cells, 4 of which had off-center overlapping receptive fields. At first sight, several cell pairs from this second example could seem excellent candidates to share input from a common retinal afferent since their receptive fields are well overlapped and have the same sign (e.g. 2×5 or 1×4). However, a significant narrow peak was only found in cell pair 4×5 . It is possible that cells 4 and 5 shared other properties that were not measured in our study (e.g. center-surround antagonism, temporal frequency tuning, etc.), however, what these two examples make clear is that tightly correlated geniculate cells are a small and highly select group of neighboring geniculate cells within the thalamus.

The receptive field diversity of tightly correlated geniculate cells is carefully balanced and can be accurately described by three mathematical functions (exponential, gaussian and linear)

Geniculate cells that share a retinal afferent tend to have similar receptive fields (Alonso et al., 1996) but slight receptive field mismatches are not uncommon. Figure 3a shows 88 tightly correlated cell pairs plotted as a function of receptive field overlap and receptive field size ratio (receptive field size ratio: larger receptive field size/smaller receptive field size). The tightly correlated pairs covered a triangular region of the plot that could be accurately described by an exponential, a gaussian and a linear function. The three functions describe the distribution of receptive field overlap (exponential), receptive field size ratio (gaussian) and maximum receptive field size ratio measured within each interval of receptive field overlap (linear). In this triangular space, cell pairs that are mismatched in receptive field size tend to be well matched in receptive field overlap and cells with poor receptive field overlap (i.e. 40%) tend to be precisely matched in receptive field size.

In contrast to the tightly correlated pairs, cell pairs that were not tightly correlated covered the entire rectangular space of the plot (Fig. 3b). Although these cells were also described by a gaussian function, this function was slightly displaced towards larger ratios of receptive field size. A comparable difference in distribution shape (triangular vs. rectangular; different gaussian distributions) was found by plotting receptive field overlap against the response-time difference for each cell pair (Fig. 3c, d; note that many cell pairs have the same x, y values and are shown superimposed). Finally, consistently with a previous study (Alonso et al., 1996), most tightly correlated cell pairs (87/88) had receptive fields of the same sign (e.g. on-superimposed with on-) but the receptive field sign was equally distributed in non-tightly correlated cell pairs (same sign: 151/284). As is illustrated in figure 3, whereas the receptive fields of tightly correlated geniculate cells tend to be similar (Alonso et al., 1996), small receptive field mismatches are frequent and carefully balanced. This balanced distribution suggests that the receptive field mismatches, rather than being random errors in retinogeniculate connectivity, may serve an important function in visual processing.

The strength of the tight correlated firing is modulated by the stimulus

It is well known that some geniculate cells receive one sole and powerful retinal input that is responsible for most geniculate spikes generated (Mastrorade, 1992, Hamos et al., 1987, Cleland et al., 1971, Chen and Regehr, 2000, Cleland and Lee, 1985). Therefore, if two geniculate cells were to receive their only retinal input from the same afferent, most of their spikes should be tightly correlated and their receptive fields should be nearly identical. Interestingly, after having recorded from more than 300 pairs of neighboring geniculate neurons (Fig. 3), we have not yet found two identical receptive fields. The frequency and neat

distribution of the receptive field mismatches in tightly correlated cells suggest a possible function: to cause variations in the percentage of synchronous spikes generated under different stimulus conditions. Figure 4a shows examples of the tight correlated firing from cells A_1 and A_2 (same as Fig. 1a) measured with different stimuli: a stationary white screen, a stationary black screen and light bars moved at different velocities. As shown in this figure, the widths of the correlogram peaks were similar under all stimulus conditions, however the peak amplitude was very different. While the narrow peak was the most salient feature in the correlograms obtained with a moving bar, the peak was barely visible when using a stationary white-screen. To quantify these changes in correlated firing, we measured the tight correlation strength by calculating the percentage of spikes contained within the narrow peak after subtracting the baseline (see methods for more detail). As shown in Figure 4b, the strength of the tight correlation (large squares) was strongly modulated by the stimulus. It was lowest when the monitor was fully illuminated (probably because the two cells were off-center) and it was highest when the bar was moved at intermediate velocities. Increments in correlation strength were not always accompanied by increments in firing rate (small squares). In this specific example, the firing rate (both mean and per stimulus cycle) peaked at the highest velocities (Orban et al., 1985), while the tight correlation was strongest at intermediate stimulus velocities. Notice that these changes in correlation strength are very significant and much more pronounced than what could be expected from repeated measurements of correlation strength under the same stimulus conditions (shown as the standard deviations on the left of Fig. 4b).

Most pairs of tightly correlated cells could not be tested with the same level of detail as cell pair A_1 – A_2 . These recordings are difficult to obtain in the first place, and they are also difficult to maintain for long periods of time. In spite of these difficulties, in 68 cell pairs we were able to measure significant tight correlations under at least two different stimulus conditions (average: 5 different stimulus conditions per cell pair). These cell pairs were tested with white noise and at least another stimulus: sparse noise ($n=44$), contrast reverse gratings ($n=39$), moving bars ($n=41$) or white noise with a smaller pixel size ($n=26$). If the duration of the recordings permitted, some stimuli were tested at different temporal frequencies or contrasts (each temporal frequency or contrast was considered a different stimulus condition). Figure 5a illustrates the fluctuations in tight-correlation strength measured in the 68 cell pairs. Each cell pair is shown as a vertical line linking the maximum and minimum strength measured; the x-axis shows the strength averaged across all stimuli. As illustrated in this figure, correlation strength was modulated by the stimulus sometimes by as much as 5-fold and the greatest modulations were found in the cell pairs that were most strongly correlated (Fig. 5b demonstrates a linear relation between the average strength and the difference between the maximum and minimum strength measured, $r = 0.71$, $p < 0.001$). The correlation strength spanned from 0.5 to 52%, consistently with measurements in retinogeniculate connections (the percentage of geniculate spikes preceded by retinal spikes can range from 1% to >80% across cells, Usrey et al., 1999, Cleland et al., 1971, Mastronarde, 1992). Moreover, because the strongest tight correlations were found in cells with the most similar receptive fields (Fig. 5c, see also Alonso et al., 1996), the fluctuations in correlation strength increased as a function of receptive field similarity (Fig. 5d).

The simplest explanation for these results is that the pronounced fluctuations in correlation strength are caused by the receptive field mismatches of cells that share a retinal afferent. In weakly correlated cells, the receptive field mismatches can be very pronounced but the weakness of the shared retinal connection limits the variations in correlation strength. However, in strongly correlated cells, the correlation strength can greatly vary because the geniculate receptive fields are far from being identical, and some stimuli can exploit the receptive field differences more than others. Cell pair A_1 – A_2 provides a good example of how slight receptive field differences can lead to variations in correlation strength. A vertical bar, moving through the receptive fields, generated strong, transient responses followed by weaker, maintained

responses (Fig. 6a). When the bar moved slowly (0.4 deg/sec), the two visual responses occurred simultaneously and the cells generated many synchronous spikes (Fig. 6a, left). However, when the stimulus velocity increased (3.5 deg/sec), the slight difference in receptive field positions (Fig. 1a) made the visual responses to separate in time, reducing the number of synchronous spikes. [Notice that the transient parts of these responses are completely misaligned in time, probably because the receptive field centers were slightly displaced in the horizontal axis (Fig. 1)].

The reduction of synchronous spikes was most pronounced during the transient than the maintained part of the response (Fig. 6a, right). Consequently, when the A_1 – A_2 correlogram was calculated separately for the different response parts, the narrow peak was only observed in the maintained part (Fig. 6b; the correlograms from this figure complement the series shown in Fig. 4a). The transient part generated either a broad hump above the shuffle correlogram (Fig. 6b, middle, left) or a copy of the shuffle correlogram (Fig. 6b, middle, right). [The shuffle simulates the correlogram that would be obtained if the two cells had the same receptive fields as A_1 – A_2 but did not share a retinal afferent].

Transient responses, as their name indicate, are short in duration and have a lower chance to overlap in time when compared with maintained responses [e.g. a difference of 30 milliseconds in response latency will be enough to prevent synchronous firing if the each cell response lasts 30 milliseconds (transient response in Fig. 6a, right), but this will not be the case if each response lasts much longer (maintained response in Fig. 6a, right)]. On the other hand, when they do coincide in time, transient responses tend to generate a large number of synchronous spikes because they have short interspike intervals. Figure 7a illustrates the best-synchronized transient responses that we have encountered in our recordings. In this example, cells A_1 and A_2 were stimulated with a full-field, low-spatial-frequency, grating that was briefly presented (stimulus duration: 50ms) on the monitor screen. With this stimulus, 90% of the spikes from cell A_1 occurred within 1-millisecond synchrony of the spikes from cell A_2 (the quality of spike isolation is shown at the bottom of Fig. 7a). The timing precision illustrated in this figure is remarkable not only because of the high percentage of synchronous spikes but also because of the tight coupling in response latency. Although the latency of the first spike varied by more than 5 milliseconds (which is half duration of the transient response), the latency variability was nearly identical in the two cells (e.g. the first-spike latency for the first four stimulus trials is increasingly longer for the 4th, 2nd, 1st and 3rd trials in both cells, starting the count from the top of the raster)

While the percentage of A_1 – A_2 synchronous spikes reached its highest value with this stimulus, the correlation strength was among the lowest measured in A_1 – A_2 . The short transient duration, together with the large spike time variability, made the correlogram peak broad and small in amplitude. As a consequence, although cells A_1 and A_2 had an additional source of synchrony (the shared retinal afferent), the correlogram calculated from the response transient was similar in width, and only slightly larger in amplitude, than the shuffle correlogram (see Fig. 6b, left-middle for another example). In other words, the interspike intervals were so short in this response transient that, just by chance, without much need of additional synchrony, the number of synchronous spikes was large.

Cells that share a retinal afferent generate ~2 times more synchronous spikes during response transients than other neighboring cells with overlapping receptive fields

Geniculate cells that share a retinal afferent should be able to send more synchronous spikes to the cortex than other neighboring geniculate cells, both because they have better matched receptive fields (Fig. 3) and because of the tight correlation generated by the shared retinal input. However, as shown in Figure 6 (right side), some transient responses are not synchronous even in cells with very similar receptive fields. Moreover, as shown in Figure 7, the additional

synchrony from cells that share a retinal afferent may be sometimes barely higher than what it would be expected by chance during a response transient (if the interspike intervals are short).

While full-field stimuli can generate strong transient synchrony in a large number of thalamic neurons, as the stimulus size is reduced, the transient synchrony becomes weaker and the number of synchronized cells is reduced. Therefore, brief, small stimuli may require the additional synchrony from cells that share a retinal afferent to activate the cortex. To quantify the significance of this additional synchrony we used sparse noise stimuli. The sparse noise consisted of a series of small light and dark squares (1.8×1.8 deg) briefly presented (31 milliseconds per square) at 16×16 different positions in visual space. Measurements were obtained in 44 cell pairs that shared a retinal afferent and 49 that did not but had overlapping receptive fields. In these experiments, we first identified cells that shared a retinal afferent based on the presence of a significant narrow peak in the correlogram obtained with white noise (see methods). Then, we stimulated the cells with sparse noise and selected the square position and polarity (i.e. black or white) that generated the largest number of synchronous spikes. Notice that in these experiments we count all the synchronous spikes, including those simply due to stimulus-dependent correlations (these measurements are different from the measurements of correlation strength presented above). Figure 8a illustrates an example of a strong transient synchrony measured in a cell pair that shared a retinal afferent (C_1 and C_2) at two different spatial positions (the strongest synchrony is shown at the top). For comparison, Figure 8b shows an example of a weak transient synchrony from two other cells (D_1 and D_2) that had overlapping receptive fields but did not share retinal input. Synchronous spikes are shown in red and non-synchronous spikes in black; each line represents a stimulus trial. To quantify the synchrony that could be attributed to the shared retinal afferent, we shuffled the spikes obtained in different stimulus trials and plotted this shuffled synchrony against the measured synchrony for each cell (Fig. 8c; the shuffle illustrates the synchrony that would be expected if we removed the shared retinal). As expected, the shuffled synchrony was similar to the measured synchrony in cells that did not share a retinal afferent (open squares). In contrast, cells that shared a retinal afferent (filled squares) were displaced from the diagonal line, indicating the presence of a substantial additional synchrony that could not be explained simply based on receptive field similarity. On average, cells that shared a retinal afferent generated ~2 times more synchronous spikes than those that did not, but had overlapping receptive fields (Fig 8d, 1.9 vs. 0.9; $p < 0.001$ Mann-Whitney test).

Conclusions

- Geniculate cells that share a retinal afferent have similar but not identical receptive fields. This receptive field diversity involves mismatches in receptive field overlap (range: 40%–100%), receptive field size (range of receptive field size ratio: 1–2) and response latency (range of difference in response latency: 0 to >10 milliseconds) whose distributions can be accurately described with mathematical functions (ranges are given based on these mathematical functions, Fig. 3).
- The receptive field mismatches are carefully balanced: cells with receptive fields mismatched in one property (e.g. receptive field overlap) tend to be well matched in other property (e.g. receptive field size) and vice versa.
- The strength of the tight correlated firing generated by a shared retinal afferent can fluctuate by as much as five times under different stimulus conditions and the greatest fluctuations are found in the cells that are most strongly correlated and have most similar receptive fields.

- When driven by transient, small stimuli, cells that share a retinal afferent generate, on average, ~2 times more synchronous spikes than other neighboring cells with overlapping receptive fields.

Discussion

The finding that the precise synchronous firing in LGN is modulated by visual stimuli is consistent with a role for neural synchrony in encoding visual information (e.g. Dan et al., 1996, Schnitzer and Meister, 2003, Reich et al., 2001, Abeles and Gerstein, 1988, Singer, 1999, Gray, 1999). Our results suggest that although most geniculate cells are dominated by a retinal input (Hamos et al., 1987, Cleland et al., 1971, Mastronarde, 1987, Usrey et al., 1999, Cleland and Lee, 1985, Chen and Regehr, 2000), other weaker and non-shared retinal inputs play an important role by creating receptive field mismatches that lead to fluctuations in correlated firing (Figs. 3–5). Moreover, although some geniculate cells receive one sole retinal input (Cleland et al., 1971, Cleland and Lee, 1985, Hamos et al., 1987, Mastronarde, 1992, Chen and Regehr, 2000), our results suggest that these 1-input cells are a minority in the LGN. An illustrative example supporting this idea is our cell pair A₁–A₂. This cell pair had a very strong tight correlation and very similar receptive fields indicating that their responses were probably dominated by the same retinal afferent. And yet, cell A₂ fired more than cell A₁ under almost all the stimulus conditions, suggesting that cell A₂ had an additional source of excitation that was not shared with cell A₁ (see Figs. 4, 6 and 7).

The tight correlated firing generated by shared retinal afferents could be important to activate the cortex when small transient stimuli fail to generate a large number of spikes (2.2 spikes/trial for cell C₁ in Figure 8). Thalamocortical efficacy is almost twice as large for synchronous spikes originating in different thalamic axons than for spikes originating within the same axon (Usrey et al., 1998). Moreover, the impact of spikes generated within the same axon is reduced by synaptic depression (Swadlow and Gusev, 2001, Chung et al., 2002, Castro-Alamancos, 2002). Finally, in the somatosensory system, it has been recently shown that most thalamocortical neurons are not strong enough to drive the cortex unless they fire in synchrony [(Bruno and Sakmann, 2006, see commentary by Alonso, 2006), although see (Swadlow and Gusev, 2002, see also Swadlow chapter in this issue) for evidence supporting the existence of exceptionally strong thalamocortical neurons].

The precise thalamic synchrony would not be required to activate the cortex if each cortical cell were to receive input from a large number of thalamic afferents. However, the available data indicate that the number of afferents converging onto a layer-4 cell is relatively small. An average layer 4 simple cell with 2.5 length/width ratio and 2–3 subregions is likely to receive input from about 30 geniculate afferents (Alonso et al., 2001), therefore, a small stimulus covering 1/3 of a simple cell subregion may activate no more than 5 afferents (other authors claim that this number is even smaller, Ringach, 2004). As would be expected from such low number of afferents, when driven with small transient stimuli most layer 4 cortical cells generate few spikes (Hirsch et al., 1998, Hirsch et al., 2002, Lampl et al., 2001).

Even if a small transient stimulus activates 5 geniculate afferents targeting the same cortical cell, the 5 afferents may not always generate responses with similar time-courses and strengths. In several years recording from neighboring geniculate cells we could not find yet two cells with identical receptive fields. Although some geniculate cells do receive all their retinal synapses from a single axon (8% according to Cleland et al., 1971, see also Hamos et al., 1987), this does not imply that two 1-input-geniculate neurons will share the same afferent. Instead, each retinal afferent may provide 100% of the retinal input to only one geniculate target and share the rest of the targets with other retinal afferents. Such circuitry design would

be ideal to provide a balanced diversity of geniculate receptive fields like the one reported here (Fig. 3).

As there are no two geniculate cells with identical receptive fields, there are no two stimuli that generate the same thalamic synchrony. The receptive fields of neighboring geniculate cells differ in subtle ways. Stimuli that exploit the receptive field similarities will generate strong synchrony while stimuli that exploit the differences will generate low synchrony even in cells dominated by the same retinal afferent. This principle is illustrated by the responses of cells A_1 and A_2 in this paper. When driven with a high-velocity moving bar, cells A_1 and A_2 generated transient responses that were not synchronous (Fig. 6, right side). However, when driven with a brief low spatial frequency grating, they responded with the most precise synchrony that we ever encountered (Fig. 7). This strong synchrony was accompanied by a tight coupling in spike-time variability that should help to reduce noise in thalamocortical transmission: even if a given stimulus generates variable spike latencies (Rieke et al., 1997), the temporal integration of the cortical cell should not be affected if the variability in response latency among the geniculate inputs is nearly identical.

In summary, our results show that a retinal afferent synchronizes a group of neighboring geniculate cells with similar but not identical receptive fields. These small receptive field differences are likely to play an important role in adjusting the amount of synchronous firing that the cortex receives under different stimulus conditions. Specifically, when stimuli are too small and too brief to generate a large number of spikes, this precise synchrony could help to activate the cortex more effectively. Our visual system may be able to detect a few photons, however, it is still not clear how many geniculate spikes are necessary to make this detection possible (Hecht et al., 1942, Sterling, 2004). The design of the retinogeniculocortical circuitry could provide a very effective mechanism to drive cortical cells with a limited number of spikes. By doing so, spike timing could be used effectively when there is a limited time to process a visual stimulus.

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Figure 1. Geniculate cells that share a retinal afferent show tight correlated firing

a Two tightly correlated Y cells with overlapping receptive fields of the same sign (off-center, left) and similar response time-courses (middle, illustrated as impulse responses calculated by reverse correlation with white noise). The correlogram (right) shows a tight correlation (narrow peak marked by star, bin width = 0.1 milliseconds). Throughout the paper, off-center receptive fields are shown in dotted lines and on-center receptive fields in continuous lines. As illustrated by the cartoon on the top-left-side of the correlogram, cells that are tightly correlated share input from the same retinal afferent. **b**. Two off-center Y cells that were not tightly correlated.

Figure 2. Receptive fields and correlograms obtained in two separate simultaneous recordings from neighboring geniculate cells

a The receptive fields from 9 simultaneously recorded cells are shown at the center of the figure. For clarity, the receptive fields are represented by the 20% contour line only (the most peripheral contour line of the receptive field plots calculated as in Figure 1). On-center cells are represented in continuous lines and off-center cells in discontinuous lines. Representative correlograms obtained with white noise stimulation are shown surrounding the receptive field plots. The correlograms were calculated with a 50 milliseconds time window (bin = 0.5 milliseconds) to emphasize the shape of the stimulus-dependent correlations (which are several times slower than the narrow peak shown in Figure 1). Cells with overlapping receptive fields

of the same sign (on- superimposed with on-) show positive correlations that are centered or displaced from zero, depending on the relative response latency of the cells (e.g. $3a \times 5$ vs. 4×7). Cells with overlapping receptive fields of different sign (on- superimposed with off-) show a wide valley in the correlogram (e.g. 6×7). Cells with receptive fields that are partially overlapped show flat correlograms. Notice that none of the possible 36 cell pairs was tightly correlated. **b.** Receptive fields and correlograms of 5 neighboring geniculate cells that were simultaneously recorded, 4 of which had overlapping receptive fields of the same sign. One of the cell pairs was tightly correlated (4×5 , marked by a star).

Figure 3. The receptive field diversity of tightly correlated cells is carefully balanced and can be accurately described by an exponential, a gaussian and a linear function

a. Tightly correlated cell pairs plotted as a function of receptive field overlap and receptive field size ratio. Receptive field size ratio = larger receptive field size/smaller receptive field size; cell pairs with the same receptive field size have a ratio of 1. Note the triangular shape of the distribution that can be accurately described by combining an exponential, a gaussian and a linear function ($r = 0.99$ for each of the functions). The equations for the functions are as follows. The exponential function for receptive field overlap is $y = y_0 + ab^x$, where $y_0 = -16.11$, $a = 10.73$ and $b = 1.01$. The gaussian function for receptive field size ratio is

$y = a * e^{\left[-0.5\left(\frac{x-b}{c}\right)^2\right]}$, where $a = 22$, $b = 1.2$ and $c = 0.4$. The linear function for maximum receptive field size ratios is $y = 0.06x - 1.2$. **b.** Cell pairs that were not tightly correlated but had overlapping receptive field centers, plotted as a function of receptive field overlap and receptive field size ratio. The distribution of receptive field size ratios is described by a gaussian function

($r = 0.99$). The equation for this function is $y = a * e^{\left[-0.5\left(\frac{x-b}{c}\right)^2\right]}$, where $a = 119.79$, $b = 1.63$ and $c = 0.43$. Non-tightly correlated cell pairs that were recorded across layers are not included in this sample. **c.** Tightly correlated cell pairs plotted as a function of receptive field overlap and response-latency difference (difference between the peak times of the impulse responses, see methods). Notice again the triangular shape of the distribution that can be accurately described by an exponential (same as in a), a gaussian and a linear function ($r = 0.99$ for the gaussian function and $r = 0.96$ for the linear function). The equation for the gaussian function is

$y = a * e^{\left[-0.5\left(\frac{x-b}{c}\right)^2\right]}$, where $a = 38.45$, $b = 0.91$ and $c = 2.55$. The equation for the linear function is $y = 0.21x - 4.7$. Notice that many dots are shown superimposed (also in panel d) because many cell pairs had the same values of response latency difference. **d.** Cell pairs that were not tightly correlated plotted as a function of receptive field overlap and response-latency difference. The distribution of response latency differences is described by a gaussian function.

The equation for this function is $y = a * e^{\left[-0.5\left(\frac{x-b}{c}\right)^2\right]}$, where $a = 70.48$, $b = 5.62$ and $c = 3.83$

Figure 4. The strength of a tight correlation is modulated by the stimulus

a Tight correlations between cells A₁ and A₂ measured under different stimulus conditions: monitor on (white screen), monitor off (black screen) and a light, vertical bar moved at different velocities. From left to right, similar spike counts were obtained with progressively shorter times of spike collection (T) indicating that the firing rate increased with stimulus velocity. The narrow peak in the correlogram had its highest amplitude at intermediate bar velocities. The correlograms are shown, at the top, with a 10 milliseconds time window to emphasize the precision of the synchrony (narrow peak with star), and at the bottom, with a wider window to reveal the underlying slower correlations. Notice that because both cells were off-center (Fig. 1), the firing rate was lower when the monitor was fully illuminated (white screen) than when

it was dark (black screen). The red lines are shuffle correlograms. Number of spikes of cell A_1 /cell A_2 for white screen: 1945/2506; black screen: 2492/3329; moving bar at 0.2 deg/sec: 1576/2462; 0.9 deg/sec: 1944/3758; 14 deg/sec: 2712/3646. **b.** Mean firing rate (small squares) steadily increases with stimulus velocity but correlation strength (large squares) does not. Standard deviations are shown on the left of the graph for the white screen (W: white screen, B: black screen, the numbers are velocity in degrees/sec). Firing rate per stimulus cycle also increased with stimulus velocity and was highest for a bar moved at 3.5 deg/sec (not shown).

Figure 5. The cell pairs that were most strongly correlated showed the largest fluctuations in correlation strength

a Tight correlation strength measured in 68 cell pairs with different stimuli. Each cell pair is represented with two circles linked with a line; each circle represents the maximum and minimum strength measured. 'Average strength' was calculated across different stimuli. 'Strength range' was calculated as the interval between the maximum and minimum correlation strength measured in each cell pair. **b.** Linear relation between the average correlation strength and the correlation strength difference: the greatest variation in correlation strength was found in the cell pairs that were most strongly correlated. **c.** Consistently with a previous study (Alonso et al., 1996), tight correlations were strongest in cell pairs with the most similar receptive fields. The strength of the tight correlation increased as a function of receptive field

similarity ($y = e^{0.0353x}$, $r = 0.65$, $p < 0.0001$, $n = 88$). A similar but weaker exponential relation was found by plotting receptive field similarity against the average correlation strength obtained in the 66 cells that were studied with multiple stimuli ($y = e^{0.035x}$, $r = 0.61$, $p < 0.0001$, $n = 66$). Receptive field similarity was quantified by cross-correlating the two spatiotemporal receptive fields. Notice that we did not find any pair with a receptive field similarity of 100%, as would be expected from cells with identical receptive fields. The two pairs with receptive field similarity $< 20\%$ were a cell pair with partially overlapping receptive fields and a cell pair with receptive fields of different sign. This sample includes all tightly correlated cell pairs studied with white noise (same as Fig. 3). Correlation strength was measured, under white noise stimuli, independently for each cell within a cell pair, and then the two values were averaged. **d.** The largest fluctuations in correlation strength were found in cells with the most similar receptive fields. 'Correlation strength difference' is shown as averages obtained at different intervals of receptive field similarity (same data from Fig. 5b). Notice that the value point at $x = 100\%$ include receptive field similarities ranging from $>80\%$ to 91% (same data from Fig. 5c). The relation between receptive field similarity and correlation strength difference

was fit with a sigmoidal function $y = \frac{a}{1 + e^{-(x-b)/c}}$, where $a = 9.76$, $b = 40.47$ and $c = 8.37$ ($r = 0.998$, $p < 0.0001$).

Figure 6. Tightly correlated cells with very similar receptive fields can fail to generate synchronous response transients

a Responses of cells A₁ and A₂ to a vertical, light bar moved at two different velocities. The first row shows the responses of cell A₁, the second row the responses of cell A₂ and the third row the synchronous spikes (within 1 millisecond). The responses are shown as rasters (20 trials) and peristimulus time histograms (PSTHs; 78 trials for 0.4 deg/sec and 284 trials for 3.5 deg/sec; bin size= 8 ms). Each column (raster and PSTH) shows one stimulus cycle (note the different time scale on the X axis). Transient responses can be seen at the entrance of the bar in the receptive field. The transient responses generate many synchronous spikes (shown in red) when the bar is moved at 0.4 deg/s but not when moved at 3.5 deg/s. Transient responses

were defined as the first second of the PSTH on the left, and as the first 200 milliseconds of the PSTH on the right. Maintained responses were defined as the response within 1–3 seconds of the PSTH on the left and as the response within 200–800 milliseconds of the PSTH on the right. **b.** Correlograms of cells A_1 and A_2 calculated using all the spikes recorded (top), the spikes from the transient part of response (middle) and the spikes from the maintained part of the response (bottom). The top left of each correlogram shows the number of spikes from A_1 and A_2 used to calculate each correlogram. (The sum of transient and maintained spikes does not equal the number of ‘all spikes’ because ‘all spikes’ includes spontaneous spikes recorded between stimuli). Total number of spikes collected from cell A_1 /cell A_2 for bar moved at 0.4 deg/sec: 2143/3934 (time of spike collection: 384 sec); for bar moved at 3.5 deg/sec: 2030/3388 (time of spike collection: 176 sec). The red lines are the shuffle correlograms.

Figure 7. The highest percentage of synchronous spikes measured in our experiments
 90% of the spikes from cell A₁ occurred in precise synchrony with spikes from cell A₂ when both cells were stimulated with a low spatial frequency grating (0.14 cycles/deg) presented for just 50 ms. **a.** Responses of cells A₁ and A₂ (same as figure 1) shown as rasters. Each raster line is a stimulus presentation. A₁& A₂ spikes that occurred within one millisecond of each other are shown in red. Synchronous spikes [A₁: 130/143; A₂: 147/207]. Synchronous spikes after shuffle [A₁: 93/143; A₂: 99/207]. The square plots below the rasters show the spike waveforms and the quality of spike isolation. Each spike is represented by a dot plotted as a function of the first (PC1) and second (PC2) eigenvectors obtained by principal component analysis (black: noise level; blue: isolated cell; lines show 0 values for PC1 and PC2). The

scatter plots show all the spikes from cells A_1 and A_2 collected during 301 seconds **b.** Correlogram obtained from the response transients shown above. The central peak is broadened by the short interspike intervals and variability in spike latency of the response transient. Red line: shuffle correlogram obtained by shuffling 5 times sections of 1 stimulus cycle and averaging these shuffles. Green line: correlogram obtained by cross-correlating the peristimulus time histograms.

Figure 8. Small, transient stimuli generate ~2 times more synchronous spikes in cells that shared a retinal afferent than in cells that did not

a Responses to sparse noise stimuli from two on-center Y cells (C₁ and C₂) that shared a retinal afferent (illustrated by cartoon on the top, left). The top of the figure shows also the receptive fields mapped by reverse correlation with white noise. Each raster line shows the response of cells C₁ and C₂ to sixteen randomized stimulus trials. The stimulus was a small dark square. Red dots are spikes that occurred within one millisecond of each other and black dots are spikes that were not precisely synchronized. Rasters at the top and bottom show responses at two slightly different positions in visual space (center of the monitor: 0°, 0°) and (−0.9°, 0°). Because the two cells were on-center there is no response when the black square is turned on (first 31 ms); the response starts when the black square is turned off. Synchronous spikes [top, C₁: 32/36; C₂: 34/51; bottom, C₁: 30/35; C₂: 30/50]. Synchronous spikes after shuffle [top, C₁: 25/36; C₂: 27/51; bottom, C₁: 23/35; C₂: 25/50]. **b**. Responses of two on-center X cells with overlapping receptive fields that did not share a retinal afferent (D₁ and D₂). The stimulus

was a brief (31 ms), light square presented at two slightly different positions [top, (center of the monitor: 0° , 0°) and bottom (0° , -0.9°)]. Synchronous spikes [top, D_1 : 20/104; D_2 : 19/40; bottom, D_1 : 11/93; D_2 : 11/37]. Synchronous spikes after shuffle [top, D_1 : 19/104; D_2 : 17/40; bottom, D_1 : 16/93; D_2 : 15/37]. **c.** Scatter plots showing the average number of synchronous spikes before (X axis) and after (Y axis) shuffling the 16 stimulus trials. Each square represents the number of synchronous spikes measured in each cell (there are two squares per cell pair). Cells that shared a retinal afferent (black squares) are farther from the unity line than those that did not (white squares), indicating that the shared retinal afferents are able to synchronize geniculate cells above chance level during stimulus transients. **d.** Cells that shared a retinal afferent generated ~ 2 times more synchronous spikes per stimulus transient than those that did not (1.9 vs. 0.9 synchronous spikes/stimulus transient, Mann-Whitney, $p < 0.001$).