Synaptic Mechanisms of Temporal Diversity in the Lateral Geniculate Nucleus of the Thalamus

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The lateral geniculate nucleus (LGN) contains a unique and numerous class of cells called lagged cells, which introduce a time delay into the neural signal provided to cortex. Previous studies have shown that this delay is dependent on GABA_A receptors within the LGN. Furthermore, lagged cells have distinct integrative properties with a slower rising, more sustained, and overall lower firing rates than nonlagged cells. We have recorded intracellularly from lagged cells in the cat LGN and found a unique property of their retinal inputs that underlies both their temporal and integrative visual response properties. Lagged cell EPSPs, which often derive from a single retinal input, have smaller amplitudes, repolarize more quickly, and are followed by a Cl[−]-dependent hyperpolarization compared with nonlagged cells. The Cl[−]-dependent hyperpolarization sums early in the visual response generating a powerful synaptic inhibition that coincides with the peak frequency of retinal input and delays the spike response in lagged cells. The hyperpolarization subsides rapidly over ~20–40 ms allowing for slow summation of the retinal input leading to the visual spike response. Given the tight association of single retinal EPSPs and the following inhibition, we propose that both functional properties result from the triadic circuitry prevalent in the LGN and particularly prominent in lagged X-cells. Thus, our results show for the first time a dynamic interaction of retinal excitation and fast feedforward inhibition that determines the integrative properties and the delay in firing of lagged cells.

Introduction

Intrinsic cellular properties and circuitry within the lateral geniculate nucleus (LGN) modify the neural representation of the visual scene provided by retinal ganglion cells (RGC). In addition to spatial processing, notably stronger surround suppression (Hubel and Wiesel, 1961), the LGN alters the representation in the time domain (Mastronarde, 1987a; Humphrey and Weller, 1988a; Wolfe and Palmer, 1998). In particular, Mastronarde (1987a) identified a class of LGN relay cells whose responses are delayed in time. These lagged (X_L) cells and their nonlagged (X_N) counterparts receive excitatory drive from a homogeneous population of RGCs but, whereas nonlagged cells follow the discharge pattern of the RGC input, excitation in lagged cells is delayed by a leading suppression. In lagged X-cells, this leading suppression coincides with the peak discharge rate of the RGC input. Both the delayed excitation and the leading suppression are abolished by pharmacological blockade of GABA_A receptors (Heggelund and Hartveit, 1990). X_L cells are abundant in the LGN accounting for up to 40% of the relay X-cell population (Mastronarde, 1987a) and provide a delayed signal to cortex that is thought to contribute significantly to the emergence of

direction-selective cells in layer 4 (Adelson and Bergen, 1985; Saul and Humphrey, 1990).

The rapid onset of the GABA_A-mediated leading suppression suggests involvement of local thalamic interneurons. Early electron microscopic studies of thalamic relay nuclei including the LGN identified a unique synaptic architecture where the principle afferents make excitatory contacts onto dendrites of relay neurons and also dendritic processes of local inhibitory neurons (Famiglietti and Peters, 1972; Hamos et al., 1985). The latter in turn make inhibitory contacts on the same relay cell dendrites receiving the direct afferent excitation. These triadic synapses are formed on grape-like appendages of the relay cell dendrite and the entire structure is enclosed within a glial sheath. In the LGN, Humphrey and Weller (1988a) found these grape-like appendages to be present at many branch points within the dendritic trees of all X_L cells but relatively few X_N cells. This circuitry predicts a feedforward inhibition tightly locked (~1 ms) to optic tract single inputs, which has been identified in 34% of mouse LGN neurons *in vitro* and shown to control spike number and increase spike precision (Blitz and Regehr, 2005).

Here, using intracellular recording *in vivo*, we report on the synaptic events underlying the initial spike suppression and leading inhibition in the responses of X_L cells to a same-sign contrast stimulus (bright spot for an on-center cell) limited to the receptive field (RF) center. We show for the first time that the retinal EPSPs in X_N cells have larger amplitudes and consist of a single excitatory event but that those in X_L cells have smaller amplitudes and consist of an EPSP–IPSP complex. These unique synaptic events can account for the delayed spike responses to visual stimuli and give further support to the hypothesis that the distinct responses of lagged and nonlagged X-cells follow from their ret-

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inal patterns of synaptic input: direct dendritic excitation in X_N cells and triadic excitation–inhibition in X_L cells.

Materials and Methods

Surgical procedures. Experiments were conducted in accordance with the ethical guidelines of the National Institutes of Health and with the approval of the Institutional Animal Care and Use Committee of the University of Pennsylvania. Adult male cats (2.5–3.5 kg) were anesthetized with an intraperitoneal injection of Nembutal (25 mg/kg) followed by supplemental isoflurane (2–4% in 70% N_2O and 30% O_2). The animal was paralyzed with gallamine triethiodide (Flaxedil) and artificially ventilated (end tidal CO_2 held at 3.8–4.0%). Anesthesia was maintained by continuous infusion of Nembutal (3–10 mg/kg-h) throughout the experiment (12–16 h). Heart rate, blood pressure, and electroencephalogram were continuously monitored. Rectal temperature was maintained at 37–38°C.

A craniotomy and durotomy were made at Horsley–Clarke coordinates A6 and L9 and a warm solution of agar (3.5%) was injected between the dura and the brain. The stability of the recordings was further improved by a bilateral pneumothorax, drainage of the cisterna magna, and hip suspension. Intracellular recordings from laminae A and A1 of the LGN were made with glass pipettes (40–80 M Ω) filled with 3M potassium acetate (except as noted in the text). In some experiments, a bipolar stimulating electrode was placed straddling the optic chiasm.

Visual stimulation. The corneas were protected with neutral contact lenses after dilating the pupil with 1% atropine and retraction of the nictitating membrane with 1% phenylephrine HCl (Neo-Synephrine). Spectacle lenses were chosen by the tapetal reflection technique (Fernald and Chase, 1971) to optimize the focus of stimuli on the retina. The position of the monitor was adjusted so as to center the area centralis on the screen.

Stimuli were generated by writing to the framebuffer portion of a Cambridge Research Systems visual stimulus generation card mounted in a conventional PC and presented on an Image Systems model M09LV monochrome monitor operating at 125 frames/s, a spatial resolution of 1024 × 786 and mean luminance of 47 cd/m². The screen subtends 36 by 27° (28.7 pixels per degree), and lookup tables were linearized for a contrast range of $\pm 100\%$. Custom software allowed for interleaving of visual stimulus protocols with injection of currents through the recording electrode and on-line display of acquired signals. Computer-assisted hand-plotting routines were used with every cell to provide initial estimates of critical parameters, especially the location and size of a region of interest spanning the RF in space. All final analysis was performed off-line in MATLAB from records stored on a Nicolet Vision (LDS), which included V_m , injected current, and stimulus marks, all sampled at 10 kHz and 16 bits.

RF structure was estimated by forward averaging of V_m and spikes with several thousand frames of precomputed lowpass filtered white noise. Spikes were removed from the V_m numerically before averaging. The noise was 16 × 16 in space, covering 2 × 2° to 5 × 5°, and frame duration was 16 ms. The resulting estimate of the 3D spatiotemporal weighting function was displayed as the average bright correlation minus the average dark in steps of 10 ms out to 320 ms. The main purpose of this measurement was to precisely center other stimuli on the RF and to rigorously choose stimuli that were limited to either the center or the surround. In addition, we often obtained area summation curves with flashed rings or disks to further assure that stimuli were appropriately sized.

Cell classification. Cells were classified as X or Y based on several tests including RF size and their responses to counterphased sinusoidal gratings. Only X-cells are reported here since no clear lagged Y-cells were observed. Cells were classified as on-center if dark stimuli in the center suppressed spike output and bright in the center elevated spike output for a prolonged period. Off-center cells were defined reciprocally.

Two-thirds (66%) of our population of X-cells were classified as single-input cells (Mastrorade, 1987b), meaning that they received a single excitatory input from the retina. This was established based on the unitary nature of the EPSP amplitudes observed during spontaneous activity and the fact that these EPSPs exhibited reasonable refractory

periods (>1.8 ms) as seen in interspike interval histograms. EPSPs were detected as threshold crossings of the first derivative of the V_m and were also checked by eye, especially during presentation of optimal visual stimuli where firing rates are high, and depression of the EPSP amplitude was significant.

Conductance and reversal potential estimations. The membrane conductance and reversal potential at each point during a synaptic response was calculated as in Higley and Contreras (2003). Synaptic responses were elicited by flashed stimuli at multiple V_m levels by varying the amount and sign of injected current. The total conductance was taken as the inverse slope of the resulting $V-I$ relationship (G_{tot}). Subtracting G_{tot} calculated during a preceding 50 ms baseline period (resting conductance) from the G_{tot} calculated during the synaptic response to a visual stimulus gives a measure of the total evoked synaptic conductance, G_{syn} . To calculate E_{rev} , we fit a linear function to a plot of ΔV_m , calculated as the difference between the V_m during the synaptic response and the resting V_m , versus resting V_m . The E_{rev} of the synaptic response equals the x-intercept of this curve.

Statistical analysis. Population data are reported as mean $\pm$ SD. Significance was calculated using two-tailed unpaired t tests.

Results

Database and cell identification

We obtained intracellular recordings from 89 neurons from the dorsal laminae of the LGN of barbiturate anesthetized cats. Fifty-five cells were included in the database and had a stable membrane potential (V_m) more negative than -60 mV (-65 ± 7 mV) and input resistance > 15 M Ω . All recorded cells showed the electrophysiological characteristics of thalamic relay cells, notably, a rebound spike-burst riding on a low-threshold spike (Jahnsen and Llinás, 1984) at the offset of hyperpolarizing current pulses from resting V_m . We classified our cells as X-cells ($n = 27$) or Y-cells ($n = 21$) according to established criteria (Enroth-Cugell and Robson, 1966; Hoffmann et al., 1972). In addition, seven LGN cells remained unclassified. From the X-cell population, 11 neurons were classified as lagged cells (X_L) and the remainder ($n = 16$) were nonlagged cells (X_N) (Mastrorade, 1987a). There were no lagged cells among our Y-cell population.

Lagged cell classification

We distinguished X_L from X_N cells by their distinct responses to a same-sign contrast stimulus limited to their RF center (defined by forward correlation of V_m with spatiotemporal white noise; Wolfe and Palmer, 1998; Niell and Stryker, 2008). Figure 1 compares the spiking and synaptic behaviors of four X_L (Fig. 1A) and four X_N (Fig. 1B) cells in response to a same-sign contrast spot. We recorded synaptic responses while injecting depolarizing and hyperpolarizing current pulses and the analysis of voltage versus injected current will be presented below. In these eight example cells, peristimulus time histogram (PSTH) and spike rasters were plotted from depolarized current levels to emphasize the differences in response properties. All X_N cells demonstrated an early increase in firing rate whereas X_L cells demonstrated an initial suppression of firing with respect to baseline, which was followed by a delayed increase in firing rate. Furthermore, X_L cells showed characteristically lower and more sustained firing rates than X_N cells. Underlying the spike output of the X_N cells, the stimulus locked average V_m showed an early depolarization followed by a small repolarization that paralleled the accommodation in firing rate (Fig. 1B, V_m , dashed lines represent approximate onset of synaptic response). When measured with depolarizing current, the X_L cells showed a leading hyperpolarization underlying the cessation of firing, which slowly gave way to a modest depolarization leading to an increase in firing rate (Fig. 1A, V_m). Thus, the underlying V_m responses explain the spiking behavior of the

Figure 1. Examples of responses to same-sign contrast stimuli in X_L and X_N cells. In all cells, a stimulus of same-sign contrast was presented in the RF center at the time indicated by the gray rectangle shown below V_m traces. Averaged synaptic responses to

two cell classes, X_L cells show an early inhibition followed by a slower rising, more sustained and smaller amplitude depolarization, while X_N cells show a nondelayed, faster rising and larger depolarization with various degrees of accommodation. We classified all cells that responded to a same-sign contrast stimulus with an initial inhibition, followed by depolarization and an increase in firing rate as X_L cells.

As opposed to the distinct responses to same-sign contrast stimuli described above, the responses to opposite-sign contrast stimuli were similar in X_L and X_N cells. Figure 2 shows the response of an X_L and an X_N cell to a flashed spot in the RF center of same-sign (A,B, top) and opposite-sign (A,B, bottom) contrasts. As in the examples in Figure 1, visual stimuli were presented during depolarizing or hyperpolarizing current injection to reveal the nature of the synaptic response, i.e., synaptic inhibition is increased in amplitude by depolarization and reversed or reduced in amplitude by hyperpolarization. Both the X_N and X_L cells were on-center cells and showed characteristic V_m and spike responses to a bright contrast stimulus as described above. To illustrate the delaying effect of the early inhibition in the X_L cell, we chose two example cells with a similar latency to the onset of the synaptic response (vertical dashed line, latency of X_N = 36 ms, X_L = 39 ms, measured from the most hyperpolarized V_m). While the spike output of the X_N cell started with the same latency as the synaptic response, the X_L cell spike output was delayed by 20 ms with respect to the onset of the synaptic response. This comparison shows unequivocally that for two LGN neurons receiving retinal input with the same latency, the X_L cell produces a delayed output. This delay, shown for the first time here to be caused by an actual IPSP, is of similar duration as that reported by Mastronarde (1987b) using dual simultaneous extracellular recordings between RGCs and LGN X_L cells. In contrast, in response to a dark center stimulus, the spontaneous firing rate (evoked by depolarizing current) was completely suppressed in both cells 30–40 ms after stimulus onset and for the whole duration of the

multiple spot presentations are shown while depolarizing or hyperpolarizing the cell with multiple levels of current injection [I_{inj} (nA)]. PSTHs (FR, firing rate) and spike rasters are taken from depolarized current levels. **A**, X_L cells show an initial hyperpolarization and suppression of firing followed by depolarization and an increase in firing rate. **B**, X_N cells show initial depolarization along with an increase in firing rate.

stimulus (Fig. 2*A,B*, bottom). Thus, the opposite-sign contrast stimulus suppressed firing in all X_L cells, while the same-sign contrast evoked an initial suppression followed by an increase in firing rate.

X_L cells are defined in extracellular recordings by the delay between stimulus onset and the visually driven increase in firing rate (Mastrorade, 1987a; Humphrey and Weller, 1988a; Saul, 2008). We quantified this delay in our intracellular recordings by measuring the latency to the half-maximal firing rate (measured from the PSTH generated from resting and depolarized current levels for X_L and X_N cells; Fig. 3*A,B*) in response to a spot of same-sign contrast limited to the RF center. The distribution of half-rise latencies (Fig. 3*C*) showed a small overlap but all X_N cell half-rise times were shorter than 70 ms (56 ± 13 ms, $n = 16$) while all but one X_L cell half-rise times were equal to or >70 ms (96 ± 34 ms, $n = 11$; Fig. 3*B*). To further separate our populations, we looked to incorporate a measurement of the retinal inputs since a delay in LGN firing could be due to a delay in its retinal input.

We measured the latency of the retinal input for each cell as the time when the stimulus-locked average V_m deviates 2 SD above the baseline noise (Fig. 3*A,B*, bottom, V_m). This measurement was made while injecting hyperpolarizing current so that all synaptic responses were depolarizing. This time point represents the abrupt onset of the barrage of visually driven EPSPs of retinal origin (rEPSPs; Fig. 3*A,B*, bottom, Single Trials). Surprisingly, the latency of retinal input showed a large variability among neurons, spanning 30–70 ms after the onset of the visual stimulus (Fig. 3*D*, rEPSP Onset Time), a larger range than previously observed (Mastrorade, 1987a; Jin et al., 2011). The distribution of retinal input latencies among X_L and X_N cells was in part responsible for the overlap seen in our spike output latency distribution (Fig. 3*C*). When the PSTH half-rise times are plotted against retinal input latency (Fig. 3*D*, left), all X_L cells (gray dots) had longer delays than X_N cells (black dots) for each given retinal input latency. This can be seen more clearly by subtracting the retinal input latency from the half-rise time for each cell (Fig. 3*D*, right). In the subtraction plot it is clear that spike output of X_L cells is effectively delayed (>20 ms) from their retinal input in comparison with X_N cells (<20 ms). Therefore, and as already clearly illustrated by the example in Figure 2, the leading inhibition of the visual response in X_L cells serves to further delay the spiking response.

Synaptic substrates of the leading inhibition in lagged cells

To characterize the postsynaptic mechanisms underlying their visual responses, we recorded from X_L and X_N cells while injecting depolarizing and hyperpolarizing current pulses in combination with the presentation of visual stimuli, a strategy shown above in Figures 1 and 2. Two example on-center cells, X_L and X_N (Fig. 4*A,B*, top), showed robust responses to a bright spot con-

Figure 2. Comparison of responses to same-sign and opposite-sign contrast visual stimuli placed in the RF center. **A**, Lagged cell. Top, Same-sign contrast center stimulus evokes initial inhibition and delayed firing. Bottom, Opposite-sign contrast center stimulus evokes a hyperpolarization that effectively stops spiking activity for the duration of the stimulus. **B**, Nonlagged cell. Top, Same-sign contrast center stimulus evokes depolarization along with an increase in firing rate. Bottom, Opposite-sign contrast center stimulus evokes a response similar to the lagged cell and stops spiking activity for the duration of the stimulus.

sisting of a strong increase in the frequency of rEPSPs with an onset latency (measured from the hyperpolarized level as in Fig. 3) of 47.6 ms (Fig. 4*A*, X_L) and 43.8 ms (Fig. 4*B*, X_N). In both cells, the superimposed single trials show that the abrupt onset of visually driven rEPSPs led to strong temporal summation and sustained depolarization when the cells were held with hyperpolarizing current (current injection onset and offset indicated by upward and downward arrows, respectively). However, injection of depolarizing current revealed that the early portion of the response of the X_L cell, but not of the X_N cell, consisted of a small amplitude hyperpolarization (3 mV from -56 mV).

The effect of the early inhibition in X_L cells on the latency to firing was clear in the PSTH (Fig. 4*A,B*, bottom) calculated from the responses at the two most depolarized current levels. In this example cell, the half-rise time of the X_L cell was 100 ms after a brief and clear suppression of baseline firing and that of the X_N cell was 50 ms (for the population values see Fig. 3). In addition, the PSTH showed the characteristic lower firing rates of the X_L cells (note difference in y-axis scale in PSTH). We removed the spikes from the single trials (see Materials and Methods) and averaged the V_m responses ($n = 11$ trials at each of five current levels; Fig. 4*A,B*, bottom) to estimate the reversal potential (E_{rev}) and synaptic conductance (G_{syn} , see Materials and Methods). Underlying the brief suppression of spike output observed in the PSTH there was a hyperpolarization manifested by injection of

Figure 3. Lagged cell output is delayed relative to retinal input. **A**, PSTH (top), Average V_m and individual trials (bottom, held with hyperpolarizing current) of an X_L cell in response to a same-sign contrast stimulus placed in the RF center. LGN half-rise time was measured as the bin in which the LGN firing rate reaches a value greater than half the maximal firing rate (down arrow). rEPSP onset measured as the time point (vertical dotted line) when the averaged V_m deviates 2 SD (horizontal dotted line) from steady-state values. **B**, Example measurements for an X_N cell. **C**, Histogram of latencies to half-maximal firing rate for all cells measured as indicated in **A**. **D**, LGN half-rise time plotted against rEPSP onset time (left) and LGN half-rise time minus rEPSP onset time plotted against rEPSP onset time (right) shows that X_L cells (gray) are more delayed relative to their retinal inputs than X_N cells (black).

depolarizing current. During this early hyperpolarization the voltage–current relationship was close to linear. The value of E_{rev} (Fig. 4C, red trace) had a time course similar to the hyperpolarization and decreased rapidly to a negative peak at -63 mV just before the peak of the hyperpolarization. The value of total membrane conductance (G_{tot} ; Fig. 4C, blue trace) was 40 nS at rest and rose rapidly during the synaptic response reaching a value of 76 nS (thus, $G_{syn} = 36$ nS; see Materials and Methods) at the time of the negative peak of E_{rev} (indicated by a vertical dotted line), this change represents an increase of 90% in resting conductance. To compare across the population, we measured E_{rev} and G_{syn} values for each cell averaged between 5 and 25 ms after the onset of the synaptic response.

X_L cells ($n = 11$) had a mean E_{rev} of -64.4 ± 4.5 mV and a mean G_{syn} of 40.1 ± 22.5 nS (Fig. 4D), whereas G_{tot} at rest was 46.2 ± 15.6 nS, thus, at the peak of the early inhibition there was an increase in conductance of 88%. In comparison, X_N cells ($n =$

16) had an E_{rev} of -48.2 ± 9.0 mV (Fig. 3D), which is lower than what would be expected for a pure excitatory response (~ 0 mV) and suggests a combination of excitatory and inhibitory conductances as has been shown for sensory responses in LGN (Lindström and Wróbel, 2011), in visual (Borg-Graham et al., 1998), and in other sensory cortices (Wehr and Zador, 2003; Wilent and Contreras, 2005).

The values of E_{rev} and G_{syn} in X_L cells are strongly suggestive of an underlying GABA_A chloride-mediated IPSP (Crunelli et al., 1988; Paré et al., 1991). These results are consistent with earlier extracellular studies showing that the leading spike suppression in X_L cells can be eliminated pharmacologically by delivery of GABA_A receptor blockers at the network level (Heggelund and Hartveit, 1990). We further investigated the ionic basis of the early inhibition at a cellular level by recording from four X_L cells with KCl (3M) in the pipette rather than the control KAc solution. During impalement with 3M KCl, chloride leaks into the cell

and changes the E_{rev} of chloride-mediated responses to more depolarized values (Paré et al., 1991).

In the example X_L cell in Figure 5, a characteristic leading inhibition with an E_{rev} of -63 mV (below spike threshold of -53 mV and therefore inhibitory) effectively delayed the visually driven increase in firing rate (Fig. 5A). Fifty minutes after impalement the leading inhibitory response changed into a large depolarization leading to high firing rate (Fig. 5B) and the E_{rev} at the peak of the early inhibition had changed to -49 mV (Fig. 5C), which is above spike threshold and therefore excitatory. The latency from stimulus onset to the half-maximal spike discharge decreased continuously throughout the recording from 70 ms in the first 5 min, to 50 ms after 50 min (Fig. 5D). The absence of early inhibition eliminated the leading spike suppression and the delay to the increased firing rate, effectively transforming this X_L cell into an X_N cell. Two of three additional X_L cells recorded with 3M KCl behaved similarly; however, in a fourth cell the early inhibition never evolved into a strong depolarization. These results further substantiate the finding that responses to an excitatory contrast in the center of the RF are delayed in X_L cells by an early synaptic inhibition mediated by GABA_A receptors.

Retinal EPSPs are unique in lagged cells

Underlying the important response differences between X_L and X_N cells is the different synaptic nature of the rEPSPs. The rEPSPs of single input X_L and X_N cells are distinct in several ways. First, during spontaneous activity (monitor held at mean luminance), the rEPSPs observed in single input X_L cells are smaller in amplitude (2.9 ± 1.3 mV, $n = 7$, X_N : 8.2 ± 3.3 mV, $n = 10$, $p < 0.001$; Fig. 6A,B) and slower rising (peak slope = 4.6 ± 2.8 mV/ms, X_N : 19.5 ± 8.1 mV/ms, $p < 0.001$) compared with rEPSPs recorded from single input X_N cells. Second, whereas the rEPSPs in X_N cells appear to decay passively toward rest, rEPSPs in X_L cells decay more rapidly and terminate in a hyperpolarization (Fig. 6A,C). This late hyperpolarization was reversed by hyperpolarizing the V_m from -61 to -82 mV with current injection (Fig. 6C, bottom) strongly suggesting an active inhibition with a reversal potential below spike threshold in X_L cells. Hyperpolarization with current injection in the X_N cell from -71 to -87 mV increased the amplitude of the rEPSP but left the decay phase unchanged (Fig. 6C, top).

To quantify the difference in decay phase of the rEPSPs between the X_L and X_N cells, we averaged rEPSPs at multiple levels of current injection and calculated E_{rev} of the waveforms 8 ms after EPSP onset (Fig. 6D). The reversal potential for the population of single input X_L cells was -56.0 ± 9.1 mV ($n = 6$), much

Figure 4. Synaptic responses to a bright stimulus limited to the RF center in typical on-center X_L (A) and X_N (B) cells. Top row, Single trials of the complete visual response (represented as black rectangle) are shown for two of the five injected current levels. A, B, Bottom, Average V_m for all current levels (after spike removal) at a shorter timescale and spikes as PSTHs. C, Estimates of the reversal potential (E_{rev} , red) and total conductance (G_{tot} , blue) are shown for the X_L cell as a function of time throughout the response. The reversal potential associated with the leading hyperpolarization in the X_L response reaches -63 mV. D, X_L and X_N cells have large increases in conductance early in the synaptic response; X_L cells have more negative reversal potentials.

more negative than the reversal of the single input X_N population (-25.3 ± 28.0 mV, $n = 5$, $p < 0.05$; Fig. 6B, right). Many single input X_N cells did not demonstrate a change in amplitude of the decay phase upon hyperpolarization, therefore it was not possible to calculate the associated E_{rev} . These results demonstrate that synaptic input from a single RGC in X_L cells actually consists of a tightly linked EPSP–IPSP complex as opposed to the simple EPSP in X_N cells.

We analyzed individual rEPSPs in X_L cells recorded with KCl pipettes to determine whether the fast hyperpolarization associated with rEPSPs was mediated by a chloride conductance. One

Figure 5. Early synaptic inhibition is mediated by chloride. Recording from an X_L cell with 3M KCl pipette solution. **A**, Three minutes after impalement, the leading inhibition to a same-sign contrast center stimulus was evident as shown by PSTH (top), the spike raster (middle), and the average V_m at different current levels (bottom). Time of visual stimulus indicated by black bar below V_m traces. **B**, Fifty minutes after impalement, the hyperpolarization had evolved into a depolarization as the reversal potential for chloride was altered by the presumed diffusion of chloride into the cell. **C**, **D**, Estimated reversal potential increased and latency to spiking decreased throughout the recording.

hour after impalement of an X_L cell with a 3M KCl-filled pipette (Fig. 6E), the hyperpolarization that followed the rEPSP early in the recording was reversed in polarity, suggesting a chloride-mediated process. In contrast, the rEPSP waveforms in X_N cells were unchanged throughout the duration of the recording with 3M KCl (data not shown). We conclude that X_L cells respond to retinal spikes with an EPSP-Cl[−]-mediated IPSP complex rather than the simple EPSP observed in X_N cells and suggest that this difference is responsible, at least in part, for the early stimulus-locked inhibition and the resulting distinct temporal behaviors of these two cell classes (Mastrorarde, 1987a; Humphrey and Weller, 1988a, b; Heggelund and Hartveit, 1990).

Summation properties of rEPSPs in lagged cells

We next asked how individual rEPSP–IPSPs seen in X_L cells produce the macroscopic leading inhibitory response to a step change in contrast. In four single input X_L cells for which this analysis was possible, the peak firing rate of rEPSPs coincided with the maximum inhibition in the V_m of the early response (Fig. 7A, left, retinal PSTH in gray, V_m in black, averaged across four cells). Furthermore, as the firing rate of the retinal input dropped to its sustained level, the X_L cell escaped from the inhibition and the membrane potential slowly depolarized leading to delayed output of the X_L cell (Fig. 7A, left). This was in contrast with the response of X_N cells, in which the increase in rate of

rEPSPs during the visual response is accompanied by depolarization (Fig. 7A, right) and accelerating firing rate.

One possible explanation for this escape from inhibition is that X_L cells have unique rates of adaptation for excitation and inhibition (Blitz and Regehr, 2005; Augustinaite and Heggelund, 2007). We explored this possibility by comparing the waveforms of averaged rEPSPs that occurred early in the response, defined as the time between the onset of the response and the peak of the hyperpolarization (Fig. 7A, Early) with the averaged rEPSPs occurring late in the response, defined as the 40 ms period following the peak of the hyperpolarization (Fig. 7A, Late). The resulting averaged early (continuous lines) and late (dotted lines) rEPSPs are shown in Figure 7B. To compare the EPSPs we measured their peak depolarization from baseline (Fig. 7C, “b–a”) and the amplitude of the hyperpolarization from the peak (Fig. 7C, “c–b”). For all four X_L cells, rEPSPs occurring early in the response exhibited stronger inhibition (c–b) than those occurring late (-2.8 ± 0.8 mV vs -0.4 ± 0.3 mV, $p < 0.001$; Fig. 7C, right; also significant measured as c–a, $p < 0.05$), but showed similar peak amplitudes (2.8 ± 1.6 mV vs 1.9 ± 0.6 mV, $p > 0.1$; Fig. 7C, b–a). As a consequence of the different dynamic behaviors of the excitatory and inhibitory phases of the rEPSP in X_L cells, the stronger inhibition early in the response causes the rapid train of rEPSPs to hyperpolarize the cell while rEPSPs later in the response are able to summate

and depolarize the cell (Fig. 7D). This hypothesis is supported by examination of single trials as in Figure 7D, in which early rEPSPs lead to sizable hyperpolarization while late rEPSPs sum temporally to generate depolarization.

In summary, a Cl[−]-dependent repolarization of rEPSPs in X_L cells, but not in X_N cells, is responsible for the early inhibitory response to same-sign contrast stimuli of X_L cells. Furthermore, the smaller EPSPs in X_L cells lead to slower summation rates and lower firing rates in X_L cells compared with X_N cells.

Discussion

Our results demonstrate for the first time *in vivo* the presence of fast, chloride-dependent inhibition following single rEPSPs at short latency (~ 1 ms) during visual responses in the cat LGN. This form of visually driven feedforward inhibition is only present in a subclass of X-cells, the lagged cells (X_L), and is responsible for their two defining visual response properties: (1) the delay of spike output in response to a same-sign contrast center stimulus and (2) the lower and more sustained firing rate in their visual responses compared with nonlagged X-cells (X_N). The rapid temporal summation of the IPSPs at response onset generates a powerful early inhibition that suppresses spike output and delays the visual response. However, the IPSP rapidly adapts thus allowing rEPSPs to summate and depolarize the cell leading to the delayed spike output. The lower firing rates in X_L cells are due to the

smaller and faster decaying rEPSPs as they are shaped by the presence of Cl^- -dependent inhibition. Indeed, our intracellular recordings with KCl-filled pipettes reversed the IPSP associated with the rEPSP and modified the response properties of X_L cells leading to (1) the loss of the early spike suppression, (2) the elimination of the delay to an increase in firing rate by which X_L cells are defined, and (3) increase in firing rate during visual response. KCl recordings from X_N cells were indistinguishable from control recordings with KAc. Similar effects on response properties of X_L cells were observed in extracellular recordings after local application of GABA_A antagonists (Heggelund and Hartveit, 1990).

We attribute this fast form of inhibition to the action of the triadic synapses that characterize X_L cells (Humphrey and Weller, 1988b) and provide the first intracellular evidence *in vivo* supporting a functional role of this unique synaptic structure in thalamus.

Lagged cells

Lagged cells were discovered by Mastronarde (1987a) in the cat thalamus using simultaneous extracellular recordings of RGCs and their postsynaptic LGN neurons. Mastronarde (1987a) distinguished X_N and X_L cell classes based in part on a parametric analysis that included a bimodal distribution of visual latencies. However, he excluded cells that he called “partially lagged.” A subsequent study showed that the visual latency distribution is essentially continuous (Wolfe and Palmer, 1998) but refers to cells at the extremes of the time course distribution as lagged and nonlagged due to their drastically different response properties. Our intracellular data also show a continuous and wide distribution of visual latencies. However, relative to their retinal inputs, all X_L cells had latencies >20 ms whereas all X_N cells had latencies <20 ms (Fig. 3C).

This delay in firing in X_L cells is due to the strong initial synaptic inhibition, which defines our population of lagged cells.

In Mastronarde's (1987a) study, cross-correlation of retinal and geniculate lagged cells exhibited a large dip following the monosynaptic peak. Furthermore, they were poorly driven by their retinal excitatory afferents despite the fact that these lagged cells were single input cells as were their nonlagged counterparts. Our results show that these two response properties are likely due to feedforward inhibition recruited by each rEPSP, and the smaller rEPSP amplitudes in X_L cells.

The functional role served by lagged cells is likely the generation of direction selectivity in cortex. It has been proposed that the convergence of LGN cells whose RFs are offset in space and time (X_N and X_L cells) produces RFs of thalamorecipient L4 cortical cells that are oriented in space-time (Saul and

Figure 6. Differences in X_L and X_N cell rEPSPs. **A**, Five sample V_m traces showing spontaneous rEPSPs for X_N (top) and X_L (bottom) LGN cells. Traces displaced vertically. **B**, Distribution of rEPSP amplitudes for both cell types. Means indicated by downward arrows. SDs indicated by horizontal lines. **C**, Superimposed average of rEPSPs made at resting V_m (black trace) and during hyperpolarizing current injection (gray trace) for X_N (top) and X_L (bottom) cells, showing a clear reversal of the late hyperpolarization in the example X_L cell. **D**, Reversal potentials measured 8 ms after start of the rEPSP have lower values in X_L cells. Means indicated by circles. SDs indicated by vertical lines. In four cells, reversal potentials could not be estimated and are represented at the top of the plot (NA). **E**, Averaged spontaneous rEPSPs in an X_L cell just after impalement (left) and 1 h after impalement (right) with 3M KCl in the pipette. Black and gray traces as in **C**.

Humphrey, 1990) and direction selective. As predicted by Adelson and Bergen (1985) and demonstrated by McLean and Palmer (1989), direction-selective L4 simple cells do indeed exhibit space-time oriented RFs and the preferred direction and velocity can be largely accounted for by a linear mechanism (Reid et al., 1987).

Inhibition in thalamus and triadic synapses

Inhibitory input to LGN relay cells originates from two types of GABAergic neurons, the neurons of the perigeniculate nucleus (PGN) and local circuit interneurons interspersed within the LGN. Inputs from PGN are located on more distal dendrites than those from interneurons and their role in visual processing is still unknown.

Thalamic interneurons form GABAergic release sites from axonal terminals (F1 terminals) and from enlarged presynaptic spe-

Figure 7. EPSP–IPSP waveform evolves during the response to flashed visual stimuli. **A**, Average LGN V_m (black trace) and normalized PSTH (light gray) of RGC firing following a flashed same-sign contrast stimulus. Plots are averages of four X_L (left) and four X_N (right) cells. Maximal RGC firing rate occurs during hyperpolarization of the X_L cell V_m . In X_N cells, peak RGC firing rate closely matches peak depolarization. **B**, Averaged rEPSPs taken from early (solid line) and late (dashed line) in the macroscopic responses of four X_L cells. **C**, IPSP amplitude is measured as the difference of the peak and trough of the waveform, and is reduced late in the response when the V_m begins to depolarize, as shown in the bar graph at right. Change in IPSP amplitude is significant while the change in the EPSP amplitude is not. **D**, Single trials that show that the rapid train of rEPSPs initially hyperpolarize the cell and later depolarize the cell.

cializations formed at the end of long, thin dendritic processes (F2 terminals). Many F2 terminals are part of triadic arrangements in which they are postsynaptic to RGC axons and presynaptic to thalamic relay neuron dendrites. These triadic synaptic structures are a feature characteristic of most thalamic nuclei (Famiglietti and Peters, 1972; Hamos et al., 1985). The triadic postsynaptic sites to F2 terminals are particularly large in X_L cells and form clusters that have been described as grape-like appendages, perhaps betraying their functional importance (Humphrey and Weller, 1988b). Triadic synapses are ensheathed by glial processes into structures called glomeruli.

The simplest interpretation of our results is that the rEPSP–IPSP complex seen in X_L cells is mediated by the triadic synaptic structures within the glomeruli. Although other schemes are compatible with our data, the use of the feedforward inhibitory synapse would inherently allow for the omnipresence of the inhibitory component of the rEPSP–IPSP complex seen only in X_L cells. This idea is supported by the anatomical studies of Humphrey and Weller (1988b) who showed the X_L cell pathway to be dominated by the glomerular circuits.

The presence of small and short latency GABA_A-mediated IPSPs in thalamic relay cells probably of glomerular origin was first shown in anterior thalamic nuclei in cat *in vivo* (Paré et al., 1991), which are devoid of reticular input. In the LGN, the presence of GABAergic inhibition time locked (~ 1 ms delay) to single electrically evoked rEPSPs was demonstrated in cat *in vivo* (Bloomfield and Sherman, 1988) and in mouse LGN slices *in vitro* (Blitz and Regehr, 2005). In response to brief electrical stimulation of the optic tract, 34% of mouse LGN relay neurons showed inhibition reliably following, and with the same activation threshold, as single inputs from the retina, while the remaining LGN neurons showed inhibition with varying latencies and a

variable threshold. The time-locked inhibition was a GABA_A, Cl[−]-mediated current most likely mediated by the triadic synapse and it is virtually identical to the inhibition we show here in X_L cells *in vivo*. Finally, a recent study in cat LGN by Lindström and Wróbel (2011) demonstrated that X-cells respond to same-sign contrast center stimuli with strong feed-forward IPSPs and suggested that this is likely a result of triadic circuitry, although they did not find differences between lagged and nonlagged cells.

Adaptation of inhibition

In contrast to the small variability in rEPSP amplitude, the amplitude of the associated IPSP is highly variable suggesting complex underlying dynamics. Changes in IPSP amplitude are particularly important at the onset of the visual response. The fast and strong IPSP summation, which generates the characteristic early inhibition of X_L cells, and which coincides with the highest firing frequency of the retinal input, is followed by rapid adaptation that allows X_L cells to escape inhibition and generate a sustained and delayed discharge. While different mechanisms could underlie this adaptation, one possibility is that synaptic depression of the

triadic-mediated IPSP builds during the phasic portion of the RGC discharge. In fact, Blitz and Regehr (2005) showed such adaptation in mouse LGN *in vitro*; however, it was extremely fast since only the first rEPSP was followed by an IPSP. In our data, the IPSP does not diminish entirely after the first rEPSP, but over the first ~ 20 ms of the response, possibly a difference in the dynamics of adaptation in cat LGN. Another possibility for the lability of inhibition is a change in the E_{rev} of chloride ions due to postsynaptic chloride accumulation. In hippocampal pyramidal cells, repetitive activation of GABA_A IPSCs led to changes in the reversal potential of chloride without any changes in conductance and were thus not attributable to GABA_A desensitization (Huguenard and Alger, 1986). This shift occurred in seconds rather than a few milliseconds as in our data; however, recordings in the hippocampus were done in the soma of large dissociated pyramidal cells. In contrast, the postsynaptic elements of X_L cells are only a few micrometers across.

An alternate explanation for the larger amplitude and phasic nature of the early inhibition in response to a visual stimulus is the engagement of axodendritic feedforward inhibition elicited by interneuronal spiking (Paré et al., 1991; Blitz and Regehr, 2005; Acuna-Goycolea et al., 2008). Interneuronal spiking would generate an F1-mediated IPSP and back propagate into the dendrites activating *all* F2 synapses (Acuna-Goycolea et al., 2008; Casale and McCormick, 2011). This scenario may account for the discrepancy between the E_{rev} of the IPSPs associated with the visually evoked macroscopic response (-64 mV) and the E_{rev} of rEPSPs occurring outside of visual responses (-56 mV), in which perhaps only a subset of F2 terminals and no F1 terminals are activated. Indeed, LGN interneurons possess intrinsic properties that boost excitability and dramatically change propagation of excitation within the somatodendritic tree by engaging sodium

and calcium dendritic conductances and making it likely that complex patterns of interneuronal activation are engaged by visual input (Acuna-Goycolea et al., 2008).

An additional contributor to the build-up of excitation after the early inhibition and the sustained and prolonged discharge of X_L cells during the visual response may be the activation of NMDA receptors, which have been shown to mediate a moderate to a large portion of X_L cell excitatory responses (Heggelund and Hartveit, 1990; Kwon et al., 1991; Augustinaite and Heggelund, 2007).

Other roles of triadic synapses

In addition to causing a delay in lagged cells on the order of tens of milliseconds, it has been proposed that the main role of triadic synapses is to modulate the gain of retinogeniculate transmission on the timescale of seconds (Sherman, 2004). For example, activation of metabotropic glutamate receptors in F2 terminals postsynaptic to retinal inputs generate long lasting (>100 ms) depolarization of the GABAergic terminals increasing visually driven inhibition and consequently decreasing the gain of the retinogeniculate synapse (Cox and Sherman, 2000). In addition, the amplitude of triadic inhibition is modulated by acetylcholine, as activation of muscarinic M2 type receptors in the F2 terminal reduces GABA release (Cox and Sherman, 2000). Indeed, electrical stimulation of the parabrachial nucleus, which supplies cholinergic input to the glomeruli, leads to a 300–500% increase of the responses to optic chiasm stimulation (Wolfe and Palmer, 1998).

Here we have shown that lagged cells have unique synaptic events, which cause their delayed visual response. Triadic synapses are likely responsible for the distinct temporal dynamics in lagged cells by supplying a feedforward inhibition tightly locked to retinal excitation.

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