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RESEARCH ARTICLE

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# Latency variability of responses to visual stimuli in cells of the cat's lateral geniculate nucleus

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**Abstract** We constructed average histograms from responses evoked by flashing stimuli and noted previously described variations in the shape of the response profile, particularly with respect to sharpness of the peak. To express this variable, we measured the half-rise latency, which is the latency from stimulus onset required to reach half the maximum response. A short half-rise latency, which is characteristic of nonlagged cells, is associated with a brisk response and sharp peak; a long half-rise latency, characteristic of lagged cells, is associated with a sluggish response and broad peak. Nonlagged cells were readily seen; we attempted to identify cells with long latencies as lagged, but we were unable to do so unambiguously due to failure to observe lagged properties other than latency. We thus refer to these latter cells as having "lagged-like" responses to indicate that we are not certain whether these are indeed lagged cells. In addition to the histograms, we analyzed the individual response trials that were summed to create each histogram, and we used spike density analysis to estimate the initial response latency to the flashing spot for each trial. We found that lagged-like responses were associated with more variability in initial response latency than were nonlagged responses. We then employed an alignment procedure to eliminate latency variation from individual trials; that is, responses during individual trials were shifted in time as needed so that each had a latency equal to the average latency of all trials. We used these "aligned" trials to create a second, "aligned" response histogram for each cell. The alignment procedure had little effect on nonlagged responses, because these were already well aligned due to consistent response latencies amongst trials. For lagged-like responses, however, the alignment made a dramatic difference. The aligned histograms looked very much like those for nonlagged responses: the responses appeared brisk, with a sharply rising peak that was fairly high in amplitude. We thus conclude that the slow build up to a relatively low peak of firing of the lagged-like response histogram is not an accurate reflection of responses on single trials. Instead, the sluggishness of lagged-like responses inferred from average response histograms results from temporal smearing due to latency variability amongst trials. We thus conclude that there is relatively little difference in briskness between nonlagged and lagged-like responses to single stimuli.

**Key words** Lagged cells · Nonlagged cells · Visual system · Thalamus · Cat

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## Introduction

Considerable attention has been focused on temporal response properties of cells in the visual system. For instance, X and Y cells in the retina of cats respond briskly to visual stimuli, and these have been called "brisk-sustained" (X) and "brisk-transient" (Y); certain types of W cell respond sluggishly, and these have been called "sluggish-sustained" and "sluggish-transient" (Cleland and Levick 1974). A similar dichotomy appears among nonlagged and lagged cells of the lateral geniculate nucleus (Mastronarde 1987a, b; Humphrey and Weller 1988a, b; Heggelund and Hartveit 1990). Compared to nonlagged responses, lagged responses exhibit a long latency to visual stimuli, and they build up to their peak response slowly and sluggishly, reaching lower peak firing levels. While lagged cells also have other distinguishing properties, such as an "inhibitory dip" or "anomalous off-discharge" (see Materials and methods for details), most attention regarding functional significance of these

two cell types has focused on their temporal properties, particularly on the delay and sluggishness of lagged responses relative to nonlagged. For instance, Saul and Humphrey (1990, 1992a, b) recently suggested that lagged and nonlagged responses represent two temporal channels to cortex, one fast and the other delayed, and that this is analogous to the two spatial X and Y channels. The idea is that these two temporal channels can be used for creation of such properties as direction selectivity in cortex (Saul and Humphrey 1990, 1992a, b).

There is an implicit assumption to all these notions about the functional significance of lagged cells, and indeed any cells with sluggish responses, that needs to be explicitly tested. Virtually all studies of these responses derive from analysis of average response histograms, particularly in response to flashed spots limited to the receptive field center (Mastronarde 1987a, b; Humphrey and Weller 1988a, b; Hartveit and Heggelund 1990, 1993; Heggelund and Hartveit 1990; Saul and Humphrey 1990; Uhlrich et al. 1990; Kwon et al. 1991; Mastronarde et al. 1991; Humphrey and Saul 1992), and the assumption is that the average response histogram accurately reflects responses to individual trials. However, certain key features deduced from these histograms may not accurately reflect responses to individual stimuli. For instance, the sluggish, slow build up of the peak response could result either from a sluggish response seen on every trial or from brisk responses to each trial but with variable latencies. The variation in latencies would create temporal smearing in the resultant histogram, giving the misleading impression of sluggish responses. We sought to test this in a population of geniculate cells that, when analyzed with average response histograms, appeared to exhibit a wide range from very brisk to quite sluggish responses. That is, in addition to generating average response histograms, we looked at individual trials of responses to determine the extent to which the histograms reflected the results of these single trials.

## Materials and methods

### Animal preparation

We performed the experiments on 6 adult cats (1.8–3.0 kg), using methods that have been described in detail elsewhere (Bloomfield et al. 1987; Bloomfield and Sherman 1988; Lo et al. 1991; Lu et al. 1992); and which are only briefly outlined here. For initial surgical preparation, we anesthetized the cats with 2–3% halothane in $N_2O/O_2$ mixed in a 1:1 ratio, and we maintained anesthesia with 0.3–1.0% halothane in a 7:3 mixture of $N_2O/O_2$ throughout the recording session. For paralysis, we administered 5.0 mg gallamine triethiodide followed by 3.6 mg/kg per hour of gallamine triethiodide plus 0.7 mg/kg per hour of *d*-tubocurarine in 5% lactated Ringer's solution. Cats were artificially respired via a tracheal cannula. We monitored rectal temperature, heart rate, and end-tidal $CO_2$ and kept them within normal physiological limits. During anesthesia and paralysis, we monitored the EEG and observed synchronous EEG activity, which is characteristic of this preparation (see Ikeda and Wright 1974; Funke and Eysel 1992).

We mounted the cat in a stereotaxic apparatus and opened the skull to allow recording from the lateral geniculate nucleus. A plastic well was built around the craniotomy, and the chamber was sealed with agar and wax to improve stability during recording. We inserted a pair of bipolar stimulating electrodes into the brain to straddle the optic chiasm and applied single pulses (0.1 ms duration, 100–500 μA, <1 Hz) across these electrodes to activate geniculate cells orthodromically from the optic tract.

The pupils were dilated, accommodation was blocked pharmacologically, and the corneas were protected with zero-power contact lenses that contained a 3 mm diameter artificial pupil. We used a fiber optic light source to plot and project retinal landmarks, including the area centralis, onto a tangent screen. Spectacle lenses focused the eyes onto the same tangent screen or onto an electronic display monitor placed in front of the cat, 28.5 cm from the nodal points of the eyes.

### Electrophysiological recording

We used fine-tipped micropipettes filled with 2–4 M KAc or 1 M NaCl to record the activity of single neurons in the A-laminae of the lateral geniculate nucleus. We pulled the pipettes to an initial impedance of 40–50 MΩ and then beveled the tip to a final impedance of 20–30 MΩ. Neuronal activity was amplified through a high-impedance amplifier. We displayed all recordings on an oscilloscope, fed them to an audio monitor, and stored them on an eight-channel FM tape recorder interfaced with a computer for off-line analysis. Action potentials were led through a window discriminator to the computer for this analysis.

### Visual stimulation and classification of response properties

For the initial evaluation of neuronal responses, visual stimuli were presented on a tangent screen using a hand-held projector. We used flashed spots of light to determine ocular dominance, receptive field location, receptive field size, and on-center or off-center type. We then replaced the tangent screen with a display monitor to present sinusoidal grating and spot stimuli. These stimuli were produced with an Innisfree image generator controlled by a computer. We classified all geniculate neurons as X or Y using a standard battery of tests. This included linearity of spatial summation in response to grating stimuli, receptive field center size, response latency to electrical stimulation of optic chiasm, and response to a large, fast moving stimulus of high contrast to activate the surround (i.e., dark for an on-center cell).

The grating stimuli (40 cd/m$^2$; variable spatial frequency, temporal frequency, and contrast) were used almost exclusively to identify cells as X or Y. The new data described in this paper reflect responses to flashing dark and bright spots of light. The spot flashed bright (64 cd/m$^2$) for on-center cells and dark (16 cd/m$^2$) for off-center cells against a uniform background luminance of 40 cd/m$^2$. The stimulus for some cells was a simple square-wave, on-off flash of the spot at 1 Hz. For other cells, we used a four-part stimulus with four equal phases of background, bright spot, background, and dark spot with the luminance values for each as described above; this was used for both on and off cells and was delivered at a frequency of 0.25 Hz. We found no difference in our results from square-wave versus four-part stimulation cycles, so we pooled data across these paradigms.

We stored the spike arrival times for responses individually for each trial, and average response histograms with a bin width of 5 or 10 ms were generated from the responses to 100 stimulus cycles. We attempted to identify lagged and nonlagged responses on the basis of previously described criteria (Mastronarde 1987a, b; Humphrey and Weller 1988a, b; Uhlrich et al. 1990; Mastronarde et al. 1991). We computed the start-rise and half-rise latencies of the response histogram and searched for other features characteristic of lagged responses, such as an "inhibitory dip" to stimulus onset and an "anomalous off-discharge". These latter features are defined in terms of a centered spot that, when flashed on, is designed to excite the cell (i.e., a bright spot for an on-center cell and a dark spot for an off-center cell): the inhibitory dip is a brief period of reduced firing to stimulus onset just before the excitatory response begins; the anomalous off-discharge is a response to stimulus offset. In practice, we were generally unable to unambiguously iden-

tify lagged responses because we did not consistently observe inhibitory dips or anomalous off-discharges. We thus felt it necessary to limit our analysis to geniculate cells without a definitive nonlagged/lagged distinction (see Results and Discussion).

## Results

We recorded the extracellular responses of 61 geniculate cells (21 X and 40 Y) to a flashing dark and bright spot centered on each cell's receptive field. Unfortunately, we experienced difficulty in obtaining clear evidence of lagged cells. Originally, the major criteria for identifying these cells was response latency, and particularly half-rise latency (Mastronarde 1987a, b; Heggelund and Hartveit 1990; Uhlrich et al. 1990; Kwon et al. 1991; Humphrey and Saul 1992; Hartveit and Heggelund 1993), although the cut-off for these half-rise latencies between the faster nonlagged and slower lagged cells was variably placed between 55 and 70 ms (Mastronarde 1987b; Humhprey and Weller 1988a; Heggelund and Hartveit 1990; Saul and Humphrey 1990, 1992a; Mastronarde et al. 1991; Kwon et al. 1991). We clearly detected a number of cells with long half-rise latencies: using a conservative cut-off of 70 ms, we recorded 18 (10 X and 8 Y) cells with latencies consistent with lagged cells, and the remaining 43 (13 X and 30 Y) responded with nonlagged latencies. However, it has more recently been claimed that response latency alone is insufficient to identify lagged cells, and instead more qualitative features are required, such as the inhibitory dip to stimulus onset and an anomalous off-discharge to stimulus offset (Mastronarde 1987a, b; Humphrey and Weller 1988a, b; Mastronarde et al. 1991). With few exceptions, we failed to observe such manifestations of lagged cells, even with four-part stimuli designed to reveal them (see Materials and methods), and some inhibitory dips and anomalous off-discharges were too subtle for us to be confident of their presence.

We cannot explain our failure to clearly see inhibitory dips and anomalous off-discharges, but only two explanations seem plausible. First, it may be that our recording conditions somehow depressed these lagged properties and that those cells with long half-rise latencies, which in our case extended beyond 300 ms, were actually lagged cells. Second, we somehow failed to sample true lagged cells and instead sampled only unusual nonlagged cells or "partially lagged" cells (Mastronarde 1987a; Humphrey and Saul 1992). Perhaps, for instance, our electrodes were unsuitable for recording lagged cells. Failure to dinstinguish nonlagged and lagged cells, while disappointing, did not interfere with one major purpose of this study, which was to determine the extent to which histograms of sluggish or lagged responses accurately reflect individual response trials. Although we shall explain in the Discussion why we believe these cells with long half-rise latencies to be lagged cells, due to the uncertainty in cell classification, we have decided to adopt the following working terminology: responses with shorter half-rise latencies (<70 ms) will be refered to as "nonlagged", as there seems to be no confusion about the identity of these, while responses with longer half-rise latencies (>70 ms) will be termed as "lagged-like". Also, both will be refered to as nonlagged and lagged-like "responses" rather than "cells". This is admittedly clumsy, and we do not suggest its adoption beyond this paper, but we feel it important to remind the reader that the cells with longer half-rise latencies could not be clearly identified as being lagged. Also, as shown below, this distinction is arbitrary, being based on the single criterion of half-rise latency, which did not separate our cell sample into two distinct groups.

Figure 1 shows typical examples of nonlagged and lagged-like responses recorded extracellularly from two geniculate cells. The average response histograms to a centered flashing spot for both types of response are shown at the bottom of Fig. 1, and these reflect a common difference between nonlagged and lagged-like responses: the nonlagged response has a half-rise latency of 45 ms (Fig. 1A), while that of the lagged-like response is 95 ms (Fig. 1B). However, it must be emphasized that this apparent difference in latency and briskness of response is derived from histograms that average across many individual trials. We then addressed whether these were apparent in single trials, which seemed more relevant to the dynamic conditions under which the visual system normally functions. Responses to single trials are depicted in the raster displays at the top of Fig. 1, where it is clear that the initial response latency shows much less variability across trials for the nonlagged than for the lagged-like response.

This result raises two key questions. First, in our population of recorded cells, do lagged-like responses consistently display more variable initial response latencies to flashing spot stimuli than do nonlagged responses? Second, if so, how much of the difference between nonlagged and lagged-like responses derived from analysis of average response histograms can be explained on the basis of this latency variability? We shall try to answer both questions in the remainder of this report.

As noted in the Introduction, virtually all of the physiological data presently available for the distinction between nonlagged and lagged cells is derived from average response histograms such as those shown at the bottom of Fig. 1. However, the brain must be able to analyze ongoing, single events and does not have the opportunity to compile an average response histogram to interpret the response for each cell. In other words, do cells exhibiting lagged-like responses display "lagged" features such as the slow build up to the response on individual trials, or do lagged-like histograms (e.g., bottom of Fig. 1B) reflect individual trials with brisk responses but with considerable latency variation among trials?

### Methods of determining the initial response

One way to address the second question is to eliminate the variability in response latency from both nonlagged and lagged-like responses. This can be done by deter-

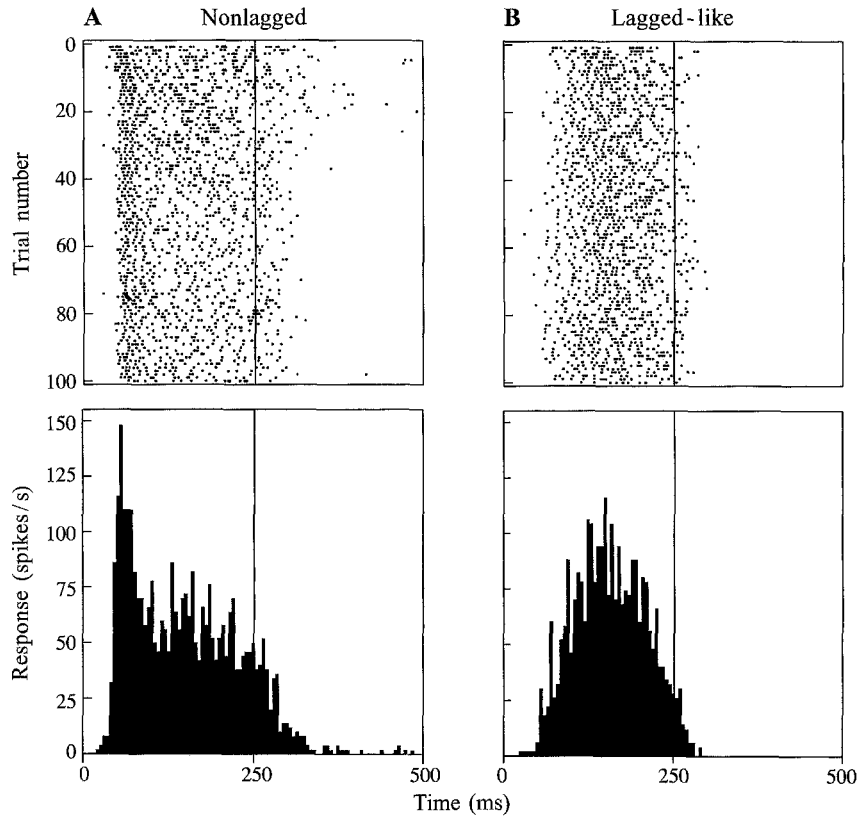


**Fig. 1A–B** Nonlagged and lagged-like responses to a centered, flashing spot of light. The spot was flashed on at time 0 and off 250 ms later (indicated by the *vertical line* through each panel). The *top panels* show responses to each of the 100 trials, each action potential in the raster displays being represented by a *dot*. The *bottom panels* represent the average response histograms. **A** Nonlagged responses; on-center cell. **B** Lagged-like responses; off-center cell

mining the initial response to the flashing spot in each trial and subsequently re-aligning the complete response on a trial by trial basis to these initial responses, creating a new average response histogram based on these aligned trials. Such a procedure minimizes timing differences amongst trials. However, since these geniculate cells display spontaneous activity, it is not an easy matter to distinguish a spontaneous spike from the initial spike in response to the visual stimulus. To solve this problem, we employed two independent means of determining the initial response: these are an analysis of spike density and analysis of interspike intervals.

*Spike density analysis*

We used a previously described method to analyze responses to single trials by creating spike density profiles (Sanderson and Kobler 1976; MacPherson and Aldridge 1979; Optican and Richmond 1987; Richmond and Optican 1987, 1990; Richmond et al. 1987, 1990; McClurkin et al. 1991a, b). Figure 2 illustrates the method as applied to a single trial, consisting of the response to the onset of a light spot for an on-center cell. We converted the actual arrival time of each action potential (Fig. 2 open circles) to a Gaussian function with a temporal spread of 15 ms at half-height. This temporal spread falls within the range previously used for spike density analyses (Sanderson and Kobler 1976; MacPherson and Aldridge 1979; Optican and Richmond 1987; Richmond and Optican 1987, 1990; Richmond et al. 1987, 1990; McClurkin et al. 1991a, b;), and the minor variations in temporal spread had little influence on our analyses. We created a spike density function by summing the individual Gaussian functions (see Fig. 2). To determine the latency of the initial response objectively, we determined the time necessary for the spike density function to reach half its maximum value (Fig. 2 dashed lines). We thus computed this initial response latency for each trial. Note that, since these are spike density functions, the determination of the actual first response may not correspond precisely to the arrival of a spike.

*Interspike interval analyis*

As a control for the spike density analysis, we chose a second, independent means of determining the initial response to each trial based on interspike intervals. We assumed that the first interspike interval to be shorter than a criterion value marked the initial response. For each cell, we used 10 different criteria, consisting of interspike intervals of 5 ms to 50 ms in 5-ms increments, to determine this initial response. We then computed the mean and standard deviation of the latency for the initial response based on each criterion, and the criterion interspike interval providing the smallest standard deviation across trials was chosen as the appropriate one for that cell.

*Data analysis*

All quantitative analyses and statistical tests were applied to both means of determining the initial response

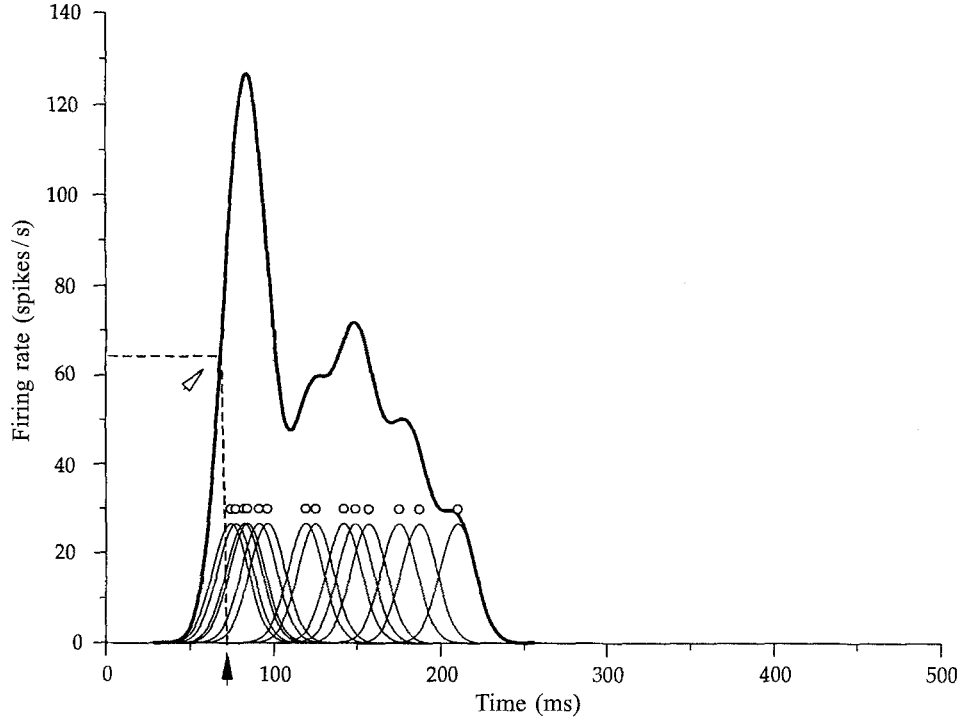


**Fig. 2** Spike density method to determine latency of first response. The example shown involves response to a single trial for an on-center cell. The centered spot of light flashed on at time 0 and off 250 ms later. Each action potential is indicated by an *open circle*. The arrival time for each of these action potentials is converted to a Gaussian function centered on that action potential and having a spread at half-height of 15 ms. These Gaussian functions are shown just beneath the action potentials. The spike density function, shown as the *thick, irregular curve*, represents the linear addition of the individual Gaussian functions. We then determined the time necessary for the spike density function to reach half its maximum value (*dashed lines* and *open arrow* on the spike density function) and used this as the estimate for the latency of the first response for that trial (*solid arrow* on the abscissa)

for each trial. With minor quantitative differences, every analysis and every statistical test reported led to the same result from both methods. This verification of the analysis by two independent methods strongly validates our approach. Since the results for both methods are nearly identical, we will report the data for only one, the spike density analysis, because this method has been used before (Sanderson and Kobler 1976; MacPherson and Aldridge 1979; Optican and Richmond 1987; Richmond and Optican 1987, 1990; Richmond et al. 1987, 1990; McClurkin et al. 1991a, b).

## Latency of the initial response

We used the spike density distributions to determine the latency of the initial spike in response to the flashing spot stimulus for each trial. From this, we computed for each of the 61 cells in this study the mean and standard deviation of this latency across the 100 stimulation trials. We also computed from the average response histograms the latency each cell needed to reach half its maximum firing rate in response to the stimulus (the "half-rise" latency; see Mastronarde 1987b; Humphrey and Weller 1988a; Heggelund and Hartveit 1990; Saul and Humphrey 1990, 1992a; Kwon et al. 1991; Mastronarde et al. 1991). Figure 3 summarizes these data, showing how both the mean latency and standard deviation for the initial response correlates with the half-rise latency. The vertical shaded regions in the panels of Fig. 3 represent half-rise latencies of 55–70 ms because, as noted above, various authors have used values in this range to distinguish nonlagged from lagged cells (Mastronarde 1987b; Humphrey and Weller 1988a; Heggelund and Hartveit 1990; Saul and Humphrey 1990, 1992a; Kwon et al. 1991; Mastronarde et al. 1991). We used a latency of 70 ms as the border between lagged-like and nonlagged responses.

It may seem odd that the half-rise latency can often occur before the initial response (Fig. 3). This can happen because of how half-rise latency is defined (Mastronarde 1987a, b): it is the latency after stimulus onset required for the cell to reach half its peak firing rate. Thus it begins with stimulus onset and not with the beginning of the response. As an example, a cell with an initial response latency to a spot onset of, say, 50 ms and that reaches peak firing by 70 ms after spot onset has a half-rise latency of 35 ms, which occurs before the initial response.

As Fig. 3A illustrates, half-rise latency correlates well with the mean initial response latency ($r$=+0.86; $P$<0.001), which is no surprise. However, Fig. 3B shows a significant correlation between half-rise latency and the standard deviation of the initial response latency ($r$=+0.76; $P$<0.001). In other words, the more lagged-like the response, the more variable the timing of the initial response. This confirms the impression conveyed by Fig. 1. Furthermore, cells with lagged-like responses show a greater standard deviation in this initial response latency than do cells with nonlagged responses ($P$<0.001 on a Mann-Whitney $U$-test).

## Alignment of average response histograms

It follows from the above discussion that a potentially important contributor to the differences in average response histograms between lagged-like and nonlagged

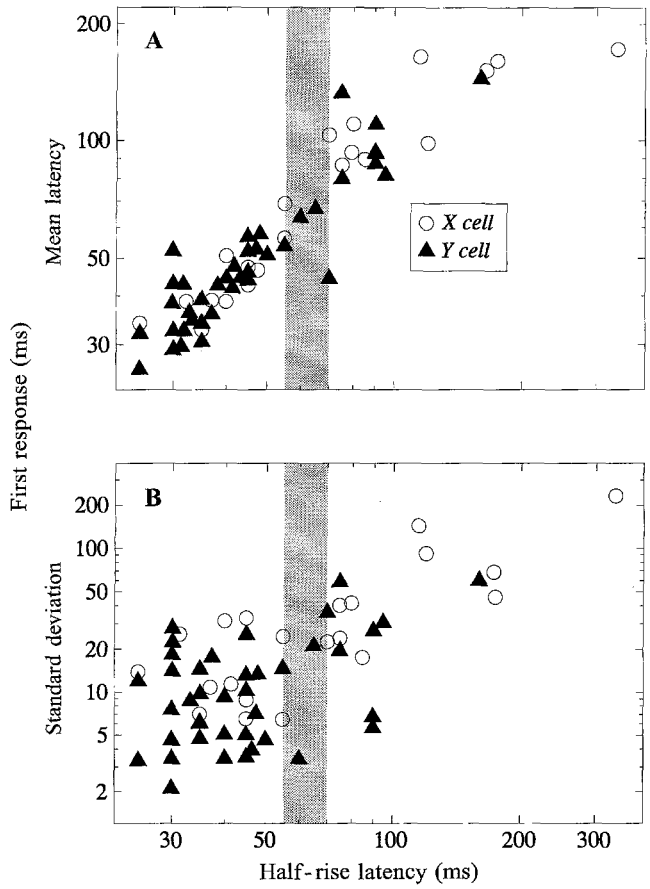


**Fig. 3A–B** Mean and standard deviation of the latency of the first response to a flashing spot plotted against half-rise latency. Data for X and Y cells are separately plotted. The half-rise latency is derived from each average response histogram and represents the time after spot onset needed for the cell to achieve half of its peak response. The *vertical shaded region* in each panel indicates half-rise latencies of 55–70 ms, because various authors have used values in this range to distinguish nonlagged (<55–70 ms) from lagged cells (<55–70 ms), those within this range being somewhat ambiguous as to their identify (see text for details). The latency of the first response to each trial was determined by the method illustrated in Fig. 2. **A** Mean latency of first response versus half rise latency. **B** Standard deviation of the first response latency versus half-rise latency

responses is the greater variability in the lagged-like response latencies amongst individual trials. To test this, we eliminated this source of variability in all responses by temporally shifting the response of each individual trial for a cell so that all trials had the same response latency. For a given cell, we chose the mean initial response latency as the alignment point for the initial response in each trial. After all the individual trials were temporally aligned so that the initial response occurred with the same latency, we generated a new average response histogram. Thus we generated for each cell a non-aligned histogram based on the actual data and an aligned histogram as described.

Figures 4 and 5 show typical examples of this alignment procedure for a nonlagged and lagged-like response. Note that the alignment procedure leads to rather small differences for the average response histograms of cells with nonlagged responses (Fig. 4). This is because individual trials are already well aligned temporally. However, alignment does have a pronounced effect on average response histograms for lagged-like responses (Fig. 5). After alignment, the response appears much brisker, reaching a higher peak of firing faster. Indeed, the aligned histogram of the response represented by Fig. 5 looks much more nonlagged than lagged-like.

## Parameters of aligned histograms

To determine more quantitatively the effect of the alignment procedure on average response histograms, we computed several parameters before and after alignment.

### *Slope*

From the average response histograms, we determined the slope of the response to the flashing spot in the following manner. We determined the first bins in which the response exceeded 10% and 90% of the bin with the largest response, and we divided the response difference between these bins by their time difference. Figure 6 summarizes this analysis. As in Fig. 3, Fig. 6 plots these slope values as a function of half-rise latency, and the shaded vertical stripes represent the half-rise latency range of 55–70 ms. Figure 6A shows this relationship before alignment. Here, there is a significant negative correlation between half-rise latency and slope ($r$=–0.46; $P$<0.001), and thus slopes of nonlagged responses are greater than those of lagged-like responses ($P$<0.001 on a Mann-Whitney $U$-test). This is expected, since lagged-like responses as gleaned from average response histograms build much more slowly than do nonlagged (Mastronarde 1987b; Humphrey and Weller 1988a; Saul and Humphrey 1990; Mastronarde et al. 1991). Even after alignment (Fig. 6B), the correlation between half-rise latency and slope is significant ($r$=–0.34; $P$<0.01), and nonlagged slopes are greater than lagged-like ($P$<0.001 on a Mann-Whitney $U$-test).

However, alignment does seem to increase the slope of the average response histogram of lagged-like responses more than that of nonlagged responses. This is illustrated in Fig. 6C, which plots the relationship between the half-rise latency and slope ratio for each cell; the slope ratio is simply the slope of the aligned histogram divided by that of the non-aligned histogram, and the higher the ratio, the greater effect of alignment. The relationship in Fig. 6C has a significant positive correlation ($r$=+0.78; $P$<0.001), and these ratios are greater for lagged-like than nonlagged responses ($P$<0.001 on a Mann-Whitney $U$-test). Thus even though lagged-like responses still have lower slopes than nonlagged after alignment, alignment does increase these slopes much more for the lagged-like responses.

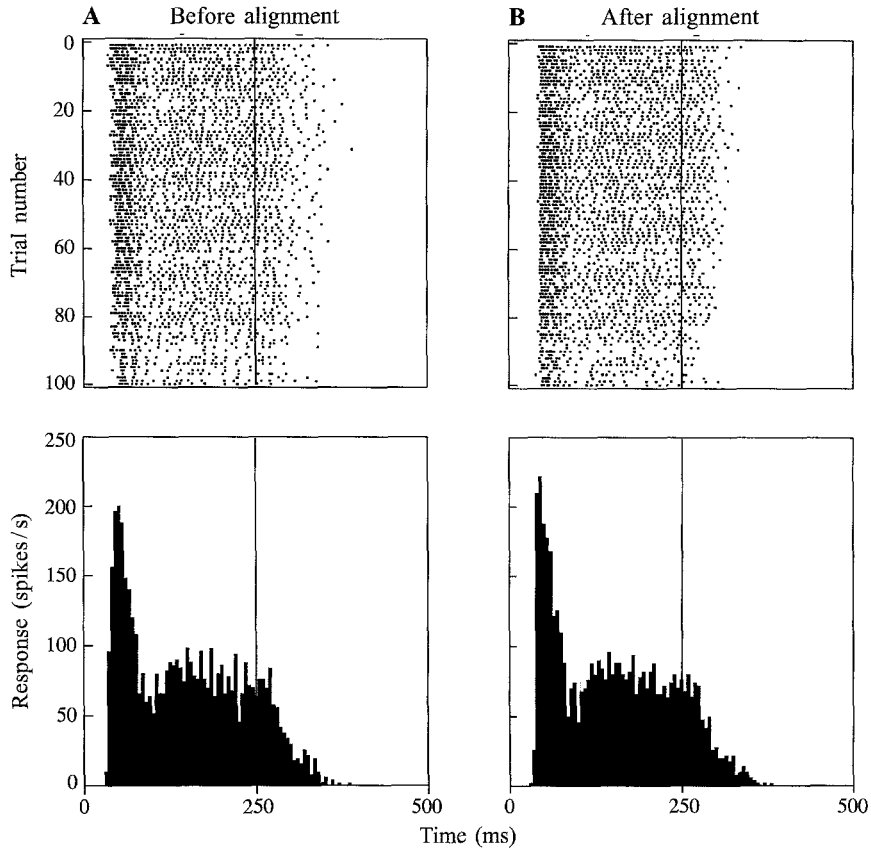


**Fig. 4A–B** Effect of alignment procedure on average response histogram for nonlagged response. The example shows the response of an on-center cell to a centered light spot flashed on at time 0 and off 250 ms later, with the rasters above showing responses on individual trials and the average response histograms shown below; conventions as in Fig. 1. **A** Average response histogram before alignment. **B** Average response histogram after alignment. Alignment was performed by determining the mean latency of the first response to each trial and then time shifting each trial by the amount needed to set the first response of that trial to the mean for all trials. Note that this alignment procedure has remarkably little effect on the average response histogram, because even before alignment, there is little variation among trials in the latency of the first response

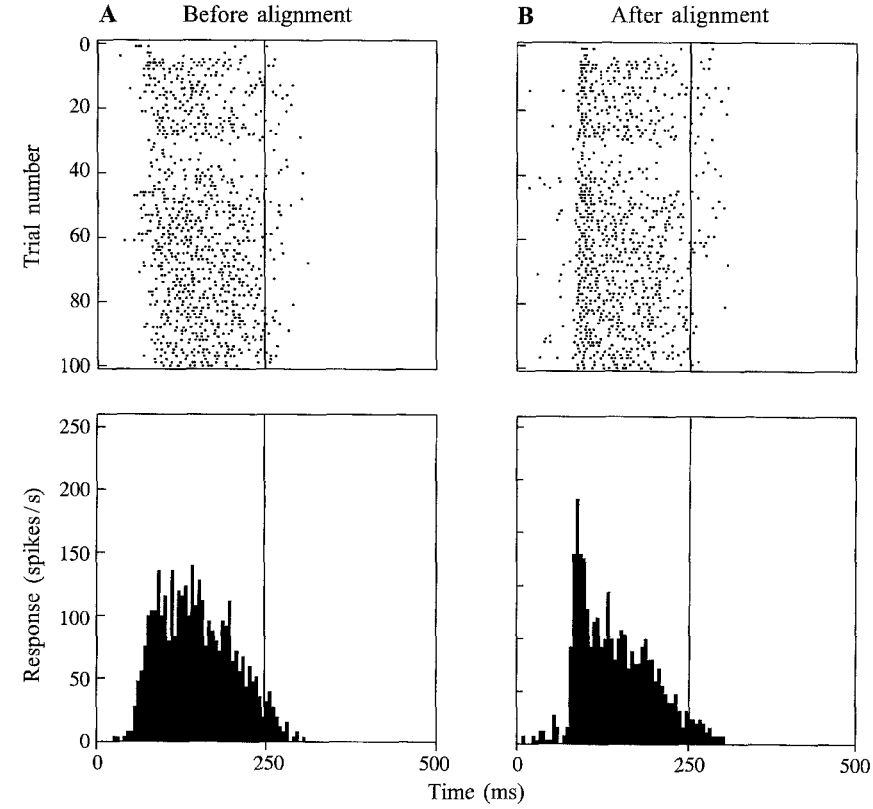


**Fig. 5A–B** Effect of alignment procedure on average response histogram for lagged-like response. This is also an on-center cell, and conventions are as in Fig. 1. **A** Average response histogram before alignment. **B** Average response histogram after alignment. Note that, unlike the case for the nonlagged response in Fig. 4, this alignment procedure has a dramatic effect on the average response histogram (see text for details)

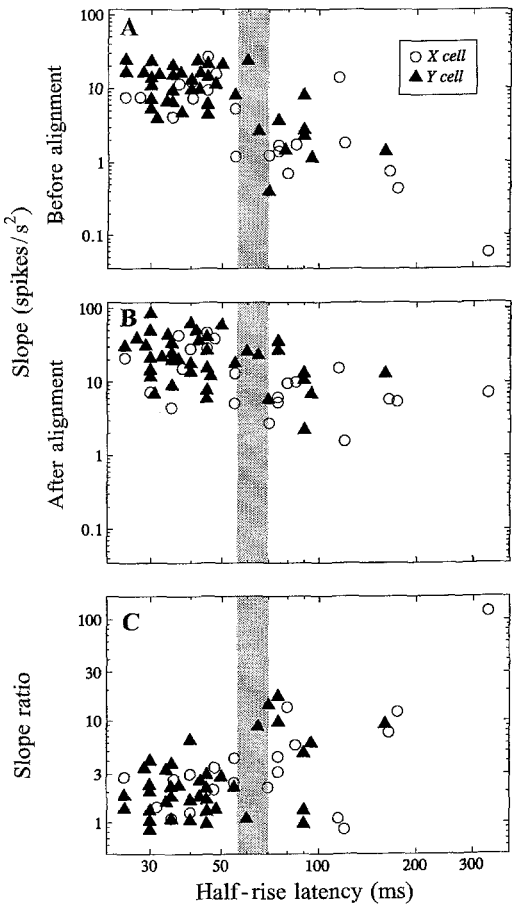


**Fig. 6A–C** Effect of alignment on the slope of the response to a flashing spot of light versus half-rise latency. Data are shown separately for X and Y cells, and conventions for half rise latency are the same as for Fig. 3. Slope was determined from average response histograms before and after alignment by determining the 10% and 90% values of the peak response and dividing the response difference between these values by the time interval between them. **A** Slope versus half-rise latency before alignment. **B** Slope versus half-rise latency after alignment. **C** Slope ratio versus half-rise latency. The slope ratio is the slope of the aligned histogram for each cell divided by that of the non-aligned histogram (see text for details)

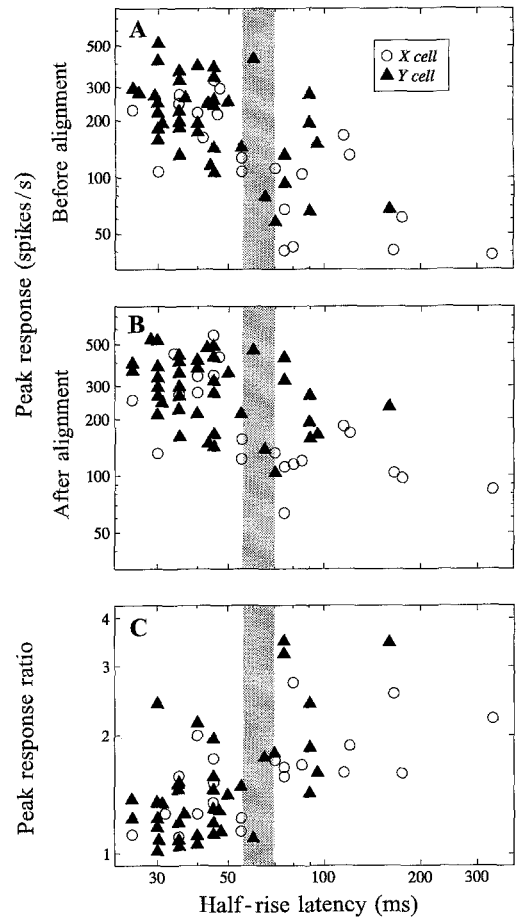


**Fig. 7A–C** Effect of alignment on the peak response to a flashing spot of light versus half-rise latency; conventions as in Fig. 3 and 6. Peak response was estimated by subtracting the response attained at 10% of the peak value from that attained at 90% of this value. **A** Peak response versus half-rise latency before alignment. **B** Peak response versus half-rise latency after alignment. **C** Peak response ratio versus half-rise latency. The peak response ratio is the peak response of the aligned histogram for each cell divided by that of the non-aligned histogram (see text for details)

*Response*

We determined the peak response in a fashion similar to our estimate for the slope, taking the difference between 10% and 90% of the largest response as our estimate of peak response. Figure 7A, B shows that, as is the case with slope, half-rise latency correlates negatively with peak response both before ($r$=–0.49; $P$<0.001) and after alignment ($r$=–0.45; $P$<0.001), and nonlagged peak responses are greater than those of lagged-like responses both before and after alignment ($P$<0.001 on Mann-Whitney $U$-tests for both comparisons). However, as with slope, we considered the effect of alignment on the peak response ratio (Fig. 7C), by dividing the peak response of the aligned histogram by that of the non-aligned. These ratios are positively correlated with half-rise latency ($r$=+0.39; $P$<0.01), and are greater for lagged-like responses than for nonlagged ($P$<0.01). In other words, even though peak response levels remain lower for cells with lagged-like responses after alignment, the alignment nonetheless increases these levels more for lagged-like than nonlagged responses.

*Rise time*

Figure 8 summarizes the effects of alignment on the rise time of the initial response to the flashing spot as calculated from the average response histograms. The rise time is, in effect, the peak response divided by the slope (see above). In other words, it is the time between the first bins to exceed 10% and 90% of the largest response. Figure 8A shows that, before alignment, there is a positive correlation between half-rise latency and rise time ($r$=+0.81; $P$<0.001), and that the rise time is greater for lagged-like than for nonlagged responses ($P$<0.001 on a

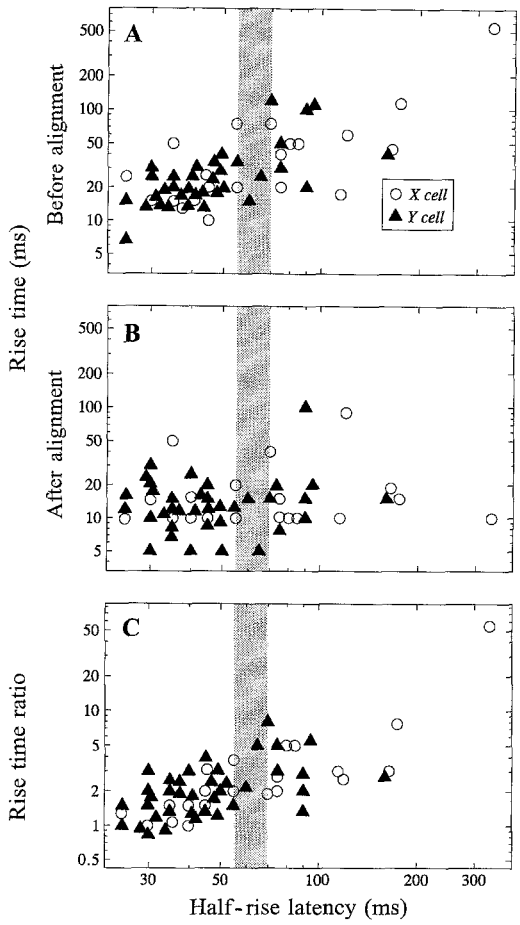


**Fig. 8A–C** Effect of alignment on the rise time of the response to a flashing spot of light versus half-rise latency; conventions as in Fig. 3 and 6. Rise time was estimated as the time interval needed for the response to rise from 10% of the peak value to 90% of this value. **A** Rise time versus half-rise latency before alignment. **B** Rise time versus half-rise latency after alignment. **C** Rise time ratio versus half-rise latency. The rise time ratio is the rise time of the aligned histogram for each cell divided by that of the non-aligned histogram (see text for details)

Mann-Whitney *U*-test). Of particular interest is the effect of alignment on this parameter (Fig. 8B), because after alignment, there is no correlation between half-rise latency and rise time ($r$=+0.12; $P$>0.1), and rise times of lagged-like and nonlagged responses are indistinguishable ($P$>0.1 on a Mann-Whitney *U*-test). Figure 8C shows the effect of alignment on the rise time ratio, which is the value of this parameter in the aligned histogram divided by that in the non-aligned histogram. As expected from Fig. 8A, B, this ratio is positively correlated with half-rise latency ($r$=+0.78; $P$<0.001), and is greater for lagged-like than for nonlagged responses ($P$<0.001 on a Mann-Whitney *U*-test).

## Discussion

Our data suggest that the use of average response histograms to evaluate nonlagged and lagged-like responses can be misleading. Such histograms suggest that, compared to nonlagged responses, lagged-like responses are much more sluggish because they require much more time to reach peak levels, and these peak levels are low. This mistakenly assumes that the average response histogram accurately reflects responses to single trials. We have shown that a main difference between nonlagged and lagged-like responses is that the latter display considerably more latency variability among individual trials than do the former. When this variability is eliminated, many of the differences between nonlagged and lagged-like responses disappear in the average response histogram. This analysis also shows that, in individual trials, lagged-like responses are nearly as brisk as nonlagged responses. Responses in individual trials may well prove to be a more relevant index of visual system function than do average response histograms.

### Cells with lagged-like responses versus lagged cells

We have tried to make clear that our cells with lagged-like responses are not necessarily the same as lagged cells. While lagged-like responses include long half-rise latencies, like those of lagged cells, they lack other characteristics of lagged cells, such as inhibitory dips and anomalous off-discharges. Several authors have emphasized the need to use a battery of tests including these features, and not just the half-rise latency, to identify lagged cells (Mastronarde 1987a, b; Humphrey and Weller 1988a; Mastronarde et al. 1991; Humphrey and Saul 1992). There does, however, seem to be some confusion here, since other authors chiefly depended on response latencies to identify these cells (Heggelund and Hartveit 1990; Uhlrich et al. 1990; Kwon et al. 1991; Hartveit and Heggelund 1993), and not all cells defined as lagged expressed inhibitory dips or anomalous off-discharges (Mastronarde 1987a, b; Humphrey and Weller 1988a; Mastronarde et al. 1991; Humphrey and Saul 1992). Even those authors espousing the employment of a battery of tests provide data indicating that half-rise latency alone can distinguish many lagged cells. For instance, Humphrey and Saul (1992) state: "all nonlagged...cells had half-rise latencies < 60 ms; all lagged cells had latencies > 65 ms". Table 1 of Mastronarde (1987a) shows that the longest half-rise latency of his nonlagged cells was 58 ms, and the shortest of lagged cells was 56 ms.

We thus believe that most or all of our cells with lagged-like responses are indeed lagged cells. However, we emphasize the proviso that we could not generally verify this with evidence of inhibitory dips or anomalous off-discharges (see also Kwon et al. 1991). Perhaps we experienced an odd sampling problem due to our electrodes or animal preparation (e.g., anesthesia protocol) and failed to record lagged cells. Thus our cells with lagged-like responses may be "partially lagged" (Mastronarde 1987a; Humphrey and Saul 1992) or some other unusual type. This does seem improbable, because con-

cluding that our cells with long half-rise latencies are not lagged would require that we have observed a totally new cell type: no such nonlagged or partially lagged cells have yet been described (Mastronarde 1987a, b; Humphrey and Weller 1988a; Mastronarde et al. 1991; Humphrey and Saul 1992). In any case, we think it very likely that lagged cells, like our cells with lagged-like responses, are not as sluggish as might be suggested from their average response histograms, that this apparent sluggishness is largely a result of temporal blurring due to variability in response latency among trials, and that on single trials lagged cells respond much more like nonlagged cells.

## Nonlagged and lagged-like responses as different temporal channels

Several authors have argued that nonlagged and lagged cells help to analyze temporal features in the visual scene by creating two parallel temporal channels (Mastronarde 1987a, b; Saul and Humphrey 1990, 1992a, b). Saul and Humphrey (1990) have shown that, in the temporal domain, nonlagged cells are tuned to high temporal frequencies at a short latency, and lagged cells are tuned to low temporal frequencies at a long latency. A more specific recent suggestion is that visual cortex makes use of these two distinct temporal channels to create direction selectivity in neuronal receptive fields (Saul and Humphrey 1990, 1992b). This hypothesis is analogous to the notion that X and Y cells signify two spatial channels, X for high spatial frequencies and Y for low.

From a teleological perspective, one would not expect a cell type responsible for temporal processing to display as much temporal variability in responses to visual stimuli as do cells showing lagged-like responses. In other words, the variable latencies seen in examples of lagged-like responses such as that shown in Fig. 1B seem poorly suited to encoding temporal patterns in the visual scene. Also, part of the argument for two temporal channels originally derived from descriptions of nonlagged responses being transient and lagged-like responses being sustained. However, this is largely due to analyses of average response histograms. On single trials, lagged-like responses are nearly as brisk and transient as are nonlagged responses, but the latency variation of lagged-like responses leads to their temporal smearing during averaging, and this makes them appear more sluggish and sustained than they truly are. Also, the shortest response latencies of the more variable lagged-like responses were typically as short as were those of nonlagged. Therefore, such is the variability in latency that lagged-like responses often appear as *early* and as *briskly* on single trials as do nonlagged responses. If many of our cells with lagged-like responses are lagged cells, our data do not support the notion of different temporal channels.

Unfortunately, while our analysis of lagged-like responses seems at odds with the notion of different temporal channels for these cells types, we cannot offer an alternative hypothesis. It is hard to imagine what useful purpose can be served by responses that are relatively unreliable in the temporal domain. Perhaps, as has been suggested previously (Uhlrich et al. 1990; McCormick 1991), lagged-like responses are the result of other cellular properties of geniculate relay cells and do not represent a functional cell type or sensory channel. Before considering the functional significance of these responses, it is necessary to understand their origin.

## Possible origin of lagged-like responses

An intriguing possibility for the origin of some features of lagged-like responses, but not all, and that is consistent with our data is the existence of a voltage-dependent $K^+$ conductance known as the A-current or $I_A$. This conductance is found in many neurons throughout the brain (Rogawski 1985; Storm 1988; Hille 1992), but more specifically, it has been identified for geniculate relay cells (McCormick 1991). $I_A$ is inactivated at depolarized membrane potentials, but depolarization from more hyperpolarized levels can activate it. Thus a retinogeniculate EPSP can activate $I_A$ in a hyperpolarized relay cell. Because $I_A$ involves an increased outward flow of $K^+$, it serves to hyperpolarize the cell, thereby opposing the effort of the EPSP to depolarize. The result is a slowing down and diminution of the depolarization induced by the EPSP. This could explain both the increased latency and reduced peak levels seen in lagged-like responses. Furthermore, $I_A$ has a robust voltage sensitivity such that fluctuations of a few millivolts of membrane potential can significantly affect the amplitude and timing of the evoked $I_A$. During intracellular recording in vivo, such fluctuations of membrane potential commonly occur (unpublished observations), and it follows that membrane potential will vary somewhat between repeated trials of visual stimulation. Therefore, EPSPs evoked by a visual stimulus on different trials would elicit different amplitudes of $I_A$. This, in turn, would translate to different response latencies to the visual stimulus on different trials, because a larger $I_A$ would require more time for an EPSP to reach firing threshold.

The suggestion is that, when a hyperpolarized relay cell expresses $I_A$, it responds in a lagged-like fashion; when it is depolarized, $I_A$ is inactivated, and it responds in a nonlagged fashion. While direct evidence for this hypothesis is lacking, it is interesting to consider, because it can account for many of the observations concerning nonlagged and lagged-like responses. As mentioned in the above paragraph, $I_A$ can account for the variable response latency and lower amplitude of the lagged-like responses. Without $I_A$ and even with minor fluctuations in membrane potential, the retinogeniculate EPSP has no variable hyperpolarization against which it operates. The EPSP thus develops with a consistent latency and more fully depolarizes the cell, leading to a higher firing rate evoked at a relatively fixed latency. To the extent that many of our cells with lagged-like re-

sponses are lagged cells, this notion would implicate $I_A$ as an important feature of responses of lagged cells. Nonetheless, certain features characteristic of lagged cells, such as their anomalous off discharges, the long conduction time of their axons projecting to cortex, and certain morphological features (Mastronarde 1987a; Humphrey and Weller 1988a, b) are not simply explained by $I_A$, indicating that, whatever role this membrane current plays in lagged responses, it cannot explain all the features that distinguish lagged cells.

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## References


Bloomfield SA, Sherman SM (1988) Postsynaptic potentials recorded in neurons of the cat's lateral geniculate nucleus following electrical stimulation of the optic chiasm. J Neurophysiol 60:1924–1945

Bloomfield SA, Hamos JE, Sherman SM (1987) Passive cable properties and morphological correlates of neurones in the lateral geniculate nucleus of the cat. J Physiol (Lond) 383:653–692

Cleland BG, Levick WR (1974) Brisk and sluggish concentrically organized ganglion cells in the cat's retina. J Physiol (Lond) 240:421–456

Funke K, Eysel UT (1992) EEG-dependent modulation of response dynamics of cat dLGN relay cells and the contribution of corticogeniculate feedback. Brain Res 573:217–227

Hartveit E, Heggelund P (1990) Neurotransmitter receptors mediating excitatory input to cells in the cat lateral geniculate nucleus. II. Nonlagged cells. J Neurophysiol 63:1361–1372

Hartveit E, Heggelund P (1993) Brain-stem influence on visual response of lagged and nonlagged cells in the cat lateral geniculate nucleus. Vis Neurosci 10:325–339

Heggelund P, Hartveit E (1990) Neurotransmitter receptors mediating excitatory input to cells in the cat lateral geniculate nucleus. I. Lagged cells. J Neurophysiol 63:1347–1360

Hille B (1992) Ionic channels of excitable membranes. Sinauer Associates, Sunderland, MA, USA

Humphrey AL, Saul AB (1992) Action of brain stem reticular afferents on lagged and nonlagged cells in the cat lateral geniculate nucleus. J Neurophysiol 68:673–691

Humphrey AL, Weller RE (1988a) Functionally distinct groups of X-cells in the lateral geniculate nucleus of the cat. J Comp Neurol 268:429–447

Humphrey AL, Weller RE (1988b) Structural correlates of functionally distinct X-cells in the lateral geniculate nucleus of the cat. J Comp Neurol 268:448–468

Ikeda H, Wright MJ (1974) Sensitivity of neurones in the visual cortex (area 17) under different levels of anesthesia. Exp Brain Res 20:471–484

Kwon YH, Esguerra M, Sur M (1991) NMDA and non-NMDA receptors mediate visual responses of neurons in the cat's lateral geniculate nucleus. J Neurophysiol 66:414–428

Lo F-S, Lu S-M, Sherman SM (1991) Intracellular and extracellular in vivo recording of different response modes for relay cells of the cat's lateral geniculate nucleus. Exp Brain Res 83:317–328

Lu S-M, Guido W, Sherman SM (1992) Effects of membrane voltage on receptive field properties of lateral geniculate neurons in the cat: contributions of the low threshold $Ca^{2+}$ conductance. J Neurophysiol 68:2185–2198

MacPherson JM, Aldridge JW (1979) A quantitative method of computer analysis of spike train data collected from behaving animals. Brain Res 175:183–187

Mastronarde DN (1987a) Two classes of single-input X-cells in cat lateral geniculate nucleus. II. Retinal inputs and the generation of receptive-field properties. J Neurophysiol 57:381–413

Mastronarde DN (1987b) Two classes of single-input X-cells in cat lateral geniculate nucleus. I. Receptive field properties and classification of cells. J Neurophysiol 57:357–380

Mastronarde DN, Humphrey AL, Saul AB (1991) Lagged Y cells in the cat lateral geniculate nucleus. Vis Neurosci 7:191–200

McClurkin JW, Gawne TJ, Optican LM, Richmond BJ (1991a) Lateral geniculate neurons in behaving primates. II. Encoding of visual information in the temporal shape of the response. J Neurophysiol 66:794–808

McClurkin JW, Gawne TJ, Richmond BJ, Optican LM, Robinson DL (1991b) Lateral geniculate neurons in behaving primates I. Responses to two-dimensional stimuli. J Neurophysiol 66:777–793

McCormick DA (1991) Functional properties of a slowly inactivating potassium current in guinea pig dorsal lateral geniculate relay neurons. J Neurophysiol 66:1176–1189

Optican LM, Richmond BJ (1987) Temporal encoding of two-dimensional patterns by single units in primate inferior temporal cortex. III. Information theoretic analysis. J Neurophysiol 57:162–178

Richmond BJ, Optican LM (1987) Temporal encoding of two-dimensional patterns by single units in primate inferior temporal cortex. II. Quantification of response waveform. J Neurophysiol 57:147–161

Richmond BJ, Optican LM (1990) Temporal encoding of two-dimensional patterns by single units in primate primary visual cortex. II. Information transmission. J Neurophysiol 64:370–380

Richmond BJ, Optican LM, Podell M, Spitzer H (1987) Temporal encoding of two-dimensional patterns by single units in primate inferior temporal cortex. I. Response characteristics. J Neurophysiol 57:132–146

Richmond BJ, Optican LM, Spitzer H (1990) Temporal encoding of two-dimensional patterns by single units in primate primary visual cortex. I. Stimulus-response relations. J Neurophysiol 64:351–369

Rogawski MA (1985) The A-current: how ubiquitous a feature of excitable cells is it? Trends Neurosci 8:214–219

Sanderson AC, Kobler B (1976) Sequential interval histogram analysis of nonstationary neuronal spike trains. Biol Cybern 22:61–71

Saul AB, Humphrey AL (1990) Spatial and temporal response properties of lagged and nonlagged cells in cat lateral geniculate nucleus. J Neurophysiol 64:206–224

Saul AB, Humphrey AL (1992a) Evidence of input from lagged cells in the lateral geniculate nucleus to simple cells in cortical area 17 of the cat. J Neurophysiol 68:1190–1208

Saul AB, Humphrey AL (1992b) Temporal-frequency tuning of direction selectivity in cat visual cortex. Vis Neurosci 8:365–372

Storm JF (1988) Temporal integration by a slowly inactivating K current in hippocampal neurons. Nature 336:379–381

Uhlrich DJ, Tamamaki N, Sherman SM (1990) Brainstem control of response modes in neurons of the cat's lateral geniculate nucleus. Proc Natl Acad Sci USA 87:2560–2563