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Directional selective neurons in the awake LGN: response properties and modulation by brain state

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Hei X, Stoelzel CR, Zhuang J, Bereshpolova Y, Huff JM, Alonso JM, Swadlow HA. Directional selective neurons in the awake LGN: response properties and modulation by brain state. J Neurophysiol 112: 362–373, 2014. First published April 30, 2014; doi:10.1152/jn.00121.2014.—Directionally selective (DS) neurons are found in the retina and lateral geniculate nucleus (LGN) of rabbits and rodents, and in rabbits, LGN DS cells project to primary visual cortex. Here, we compare visual response properties of LGN DS neurons with those of layer 4 simple cells, most of which show strong direction/orientation selectivity. These populations differed dramatically, suggesting that DS cells may not contribute significantly to the synthesis of simple receptive fields: 1) whereas the first harmonic component (F1)-to-mean firing rate (F0) ratios of LGN DS cells are strongly nonlinear, those of simple cells are strongly linear; 2) whereas LGN DS cells have overlapped ON/OFF subfields, simple cells have either a single ON or OFF subfield or two spatially separate subfields; and 3) whereas the preferred directions of LGN DS cells are closely tied to the four cardinal directions, the directional preferences of simple cells are more evenly distributed. We further show that directional selectivity in LGN DS neurons is strongly enhanced by alertness, which increases responses in the preferred direction and suppresses responses in the null direction. Finally, our simulations show that these two consequences of alertness could each serve, in a vector-based population code, to hasten the computation of stimulus direction when rabbits become alert.

IN RABBITS AND RODENTS, COMPUTING the direction of visual motion begins in the retina, where directionally selective (DS) ganglion cells project to brainstem targets and the visual thalamus (Huberman et al. 2009; Simpson 1984; Stewart et al. 1971). Whereas most DS ganglion cells respond in spatially overlapping zones to both light onset and offset, some respond only to light onset (Barlow et al. 1964). Mechanisms underlying directional selectivity in retinal ganglion cells (Demb 2007) have been studied intensively in rabbits (e.g., Fried et al. 2002, 2005; Oesch et al. 2005) and more recently in mice (Huberman et al. 2009; Weng et al. 2005; Yoshida et al. 2001). Whereas brainstem projections of retinal DS neurons control aspects of eye position (Cavanaugh et al. 2012; Dhande et al. 2013; Simpson 1984; Yonehara et al. 2009), little is known about the function of the projections of retinal DS neurons to thalamocortical circuits. We have previously shown (Swadlow and Weyand 1985) that DS neurons in rabbit LGN do project to the primary visual cortex (V1), that their axons are fast conducting (similar to those of concentrically organized LGN cells), and that LGN DS neurons are much more prevalent in the representation of the upper visual field (dorsal portion of the LGN) than in the representation the visual streak (the region of high receptor and ganglion cell density representing vision along the horizon; Hughes 1971). However, the manner in which LGN DS neurons contribute to the processing of information in V1 is unknown.

Here, we target DS neurons in the LGN representation of the visual streak of awake rabbits and compare their properties with layer 4 cortical neurons recently studied in the corresponding region of primary visual cortex (V1; Zhuang et al. 2013). The representation of the visual streak is located roughly midway along the dorso-ventral axis within the rabbit LGN, whereas the upper visual field is represented dorsally (Holcombe and Guillery 1984; Hughes 1971). We examined the visual response properties of LGN DS neurons and showed that their responses are highly nonlinear, that they consist of highly overlapping ON and OFF subfields, and that their preferred directions are, like their counterparts in the retina (Oyster and Barlow 1967), restricted to the four cardinal directions. Notably, each of these visual response properties are dramatically distinct from those of V1 layer 4 simple cells studied in the same preparation using identical methods (Zhuang et al. 2013), suggesting that LGN DS neurons do not contribute strongly to the synthesis of the direction/orientation selectivity seen in V1 simple cells. Next, we show that the visual responses of LGN DS cells are strongly modulated by alertness, which increases responses in the preferred direction and suppresses responses in the null direction, making them more directional selective. Finally, we present a simple model and simulations that show how the response changes seen in LGN DS cells during alertness could result in faster computation of stimulus direction by a vector-based population code.

MATERIALS AND METHODS

Extracellular single unit recordings were made in the LGN from five awake adult female Dutch-Belted rabbits. The general surgical procedures have been reported previously (Bereshpolova et al. 2007, 2011; Stoelzel et al. 2008; Swadlow et al. 1998; Zhuang et al. 2013) and are briefly described here. All experiments were conducted under the approval of the University of Connecticut Animal Care and Use Committee in accordance with National Institutes of Health Guidelines.

Animal preparation. Initial surgery was performed while the animals were under ketamine-acepromazine anesthesia using aseptic
procedures. Stainless steel screws were installed on the dorsal surface of the skull and fused together with acrylic cement after removing skin and fascia. A stainless steel rod oriented in a rostrocaudal direction was cemented to the acrylic mass. This rod held the rabbit rigidly during electrode implantation and recording sessions. Silicone rubber was used to buffer the wound margins from the acrylic cement on the skull. After at least 10 days of recovery, neuronal activity recordings were obtained from awake rabbit through a small hole in the skull.

**Electrophysiological recordings.** All the electrophysiological recordings were acquired by Plexon data acquisition system (Plexon, Dallas, TX). Single unit recordings from the LGN of awake rabbit were obtained using a chronically implanted seven-channel system with seven quartz-insulated platinum/tungsten (1.5–3 MΩ) electrodes organized concentrically with a spacing of ~200 μm and each independently controlled by a miniature microdrive (Swadlow et al. 2005). Microelectrodes had a maximum diameter of 40 μm and were pulled to a fine tip and sharpened. Multiunit recordings from superficial layers of superior colliculus (SC) were obtained using a three-channel system similar to the LGN system but with low impedance (≤1.5 MΩ) electrodes. Hippocampal EEG and cortical EEG were simultaneously recorded with platinum-iridium microwires for monitoring brain states (Bereshpolskaya et al. 2007, 2011; Bezdzudnaya et al. 2006; Zhuang et al. 2013).

**Receptive field and visual stimulation.** Receptive fields were plotted for the LGN cells under study and mapped by reverse correlation (Jones and Palmer 1987; Stoelzel et al. 2008) using sparse noise stimuli made of white and black squares (1°; 13.2–19.8 ms), which were pseudorandomly presented on a primary monitor (40 × 30 cm, 48 cd/m² mean luminance, and 160-Hz refresh rate). Eye movements were monitored by constant tracking of SC multiunit activity and using an infrared eye camera system (see **Monitoring eye position** for details). Receptive field properties of the cells were tested using sine-wave drifting gratings with optimal parameters (size, temporal frequency, spatial frequency, orientation/direction, and contrast). The orientation/directional tuning was measured with gratings drifting in 1 of the 8, 12, or 24 randomly interleaved directions while keeping other parameters optimal. Spatial frequency tuning was tested from 0.00825 to 1.32 cycles per degree (cpd) while keeping other parameters optimal. Each presentation lasted 3–8 s with 2-s gaps in between (mid-luminance screen was shown to the animal during the gaps). The mean presentation number per condition was 190.4 ± 7.68. For some cells, spontaneous activity was also recorded using a screen with mid-luminance.

**Cell classification.** After an LGN cell was identified, circular drifting gratings were used to determine if a cell was directionally selective, based on their selectivity to the direction of motion. A direction-selective index (DSI; see below for definition) was calculated, and only cells with DSI >0.4 were considered as DS cells.

We generally studied only DS neurons, but in some cases we compared DS neurons with concentrically organized LGN cells. Concentric cells showed strong surround inhibition with no or very poor orientation/direction selectivity. They were further classified as sustained or transient concentric cells based on their responses to stationary white or black spots, which were presented on the receptive field center of the cell for 2 s with 2-s gaps between stimulation. The spots were chosen to match the size and sign of the receptive field center.

Data from layer 4 simple cells were obtained from reanalysis of the population studied by Zhuang et al. (2013), and the methods for identifying and studying these cells can be found there.

**Search strategy for LGN DS cells.** All LGN cells studied here had receptive fields in the monocular region of visual space, 20–110° from the midline (0° being in front of the animal), at an elevation of +15 to –5° from the horizon. This retinotopic region roughly corresponds to the region of maximal receptor and ganglion cell density in rabbit retina (the visual streak; Hughes 1971), and DS neurons are relatively rare in this retinotopic region of the LGN. Thus Stoelzel et al. (2008) reported only 2/83 (2.4%) DS neurons with receptive fields in this region of visual space. Similarly, Swadlow and Weyand (1985) found only 3.4% LGN DS neurons at elevations of –5° to +15° but found 17.8% DS neurons at elevations >15° (recalculated from the original data set presented in Fig. 5 of Swadlow and Weyand 1985). Nevertheless, we chose to limit our analysis of DS neurons to the representation of visual streak to be able to compare these cells with V1 simple cells studied in this region of visual space (Zhuang et al. 2013, 2014). To achieve this, our strategy was to limit, as much as possible, recordings to DS neurons. This was accomplished by quickly abandoning non-DS neurons following brief visual testing (i.e., neurons that showed roughly equivalent responses to movement in multiple directions around the 4 cardinal directions were discarded).

**Monitoring eye position.** The rabbits generally have very stable eyes and often keep their eye position within ±0.5° for up to several minutes (Bezdudnaya et al. 2006; Collewijn 1971; Swadlow and Weyand 1985). During each recording session, the SC multiunit receptive field positions were mapped with sparse noise stimuli on a secondary LCD monitor on a secondary monitor (Acer AL1515, 23 × 20 cm, 36 cd/m² mean luminance, and 75-Hz refresh rate). The relationship between the SC and LGN receptive field center positions was set up during the mapping process when the rabbit’s eye was stable. Once an eye movement occurred during the visual stimulation, the stimulus was dynamically moved to be centered on the LGN receptive field center based on this relationship. At the same time, for most cells, the pupil position and size were monitored by an infrared camera system (ViewPoint EyeTracker System; Arrington Research), which was ~40 cm away from the rabbit eye. Data recorded ±15 s of an eye movement were discarded during offline analysis, and only data recorded during eye stable periods are reported here.

**EEG brain states.** The data were segmented into two distinct brain states, alert state and nonalert state, based on the simultaneously recorded hippocampal EEG and cortical EEG activity. The alert state was defined as hippocampal theta activity (5–7 Hz) and cortical desynchronization, while nonalert state was defined as high-voltage irregular hippocampal activity and cortical synchronization. Sometimes, novel nonvisual stimulations (e.g., random sounds and tactile stimulation) were applied to arouse the animal from nonalert to alert states. Power spectrum density graphs were generated for each cell to verify the states separation. Data reported here for alert state range from 14 to 65% of the time that the cells were studied (mean: 33 ± 2.4%) and for nonalert state from 13 to 54% (mean: 31 ± 2.1%). The remaining portions of the data sets (36%, on average) could not be classified unambiguously as either alert or nonalert and were not included in the state analyses.

**Data analysis.** Spike waveforms were identified online and verified offline by Plexon cluster analysis software. All data were then analyzed by Plexon NeuroExplorer (Nex Technologies) and MATLAB (The MathWorks).

Data of the first two cycles of each presentation were removed to discard transient responses to stimulus onset. Then, with the use of Fourier analysis, the mean firing rate (F0) and first harmonic component (F1) of peristimulus time histograms (PSTH) graphs were calculated for further analysis. Unlike concentric cells in the LGN, which respond to a drifting grating with strong modulations of the temporal frequency tested (F1 modulation), the DS cells responded mainly with an increase in the mean firing rate (F0). F1 modulations were hardly seen in LGN DS cells (see below for one exception). Therefore, except for measurements of spatial frequency tuning, all other measurements are reported as F0 responses only (total firing rate, spontaneous rate was not subtracted).

Burst is defined as a cluster of at least 2 spikes with an interspike interval ≤4 ms and with the first spike of the burst having a preceding interspike interval of ≥100 ms (Bezdudnaya et al. 2006; Lu et al. 1992).

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Statistical significance was determined by independent sample t-test, unless otherwise stated. Means ± SE are represented for figures with bar graphs (*P < 0.05; **P < 0.01; ***P < 0.001).

Receptive field structure. The receptive field structure was measured by reverse correlation (Jones and Palmer 1987; Stoezl et al. 2008) with sparse noise stimuli, and ON/OFF receptive field matrices were generated with a 20-ms time window around the peak response. The matrices were smoothed with a Gaussian filter, and a 30% threshold was applied. Contour plot lines were fitted using bicubic interpolation, and each line represents a 10% decrement. To measure the overlap between ON and OFF responses, the local similarity index (LSI) was calculated as follows:

\[
LSI = \sum (RF_{ON} \cdot RF_{OFF}) / \sqrt{\sum (RF_{ON} \cdot RF_{ON}) \cdot \sum (RF_{OFF} \cdot RF_{OFF})}
\]

RF_{ON} and RF_{OFF} are the ON and OFF receptive field matrices after applying the filter and threshold. The values of LSI range from 0 to 1. LSI equals 1 when ON and OFF receptive fields totally overlap with each other and equals 0 when they are entirely separated.

Directional tuning. Directional tuning for each cell was measured as the average of F0 responses measured with gratings pseudorandomly drifting in 8, 12, or 24 directions. For each cell, the preferred direction, DSI, and orientation-selective index (OSI) were calculated. In addition, we calculated direction selectivity based on circular variance (CVd) and orientation selectivity based on circular variance (CVo) (Piscopo et al. 2013). The preferred direction was computed as the vector sum of the responses in all the directions. The DSI, OSI, CVd, and CVo were calculated as follows:

\[
\begin{align*}
DSI &= (R_{\text{pref}} - R_{\text{null}}) / (R_{\text{pref}} + R_{\text{null}}) \\
OSI &= (R_{\text{pref}} - R_{\text{orth}}) / (R_{\text{pref}} + R_{\text{orth}}) \\
CVd &= \left( \sum R_j e^{\theta_j} / \sum R_j \right) \\
CVo &= \left( \sum R_j e^{\theta_j^2} / \sum R_j \right)
\end{align*}
\]

where \( R_{\text{pref}} \) is the F0 response in the measured preferred direction, which was defined as the stimulus direction closest to the vector sum of responses across all directions. \( R_{\text{null}} \) is the F0 response in the stimulus direction 180° opposite of the preferred direction; \( R_{\text{orth}} \) is the averaged F0 response in the stimulus directions 90° away from the preferred direction; \( R_j \) represents all the directions tested; and \( R_j \) and \( \theta_j \) are the F0 responses and angles (in radians) in \( j \)th direction. DSI, OSI, CVd, and CVo range from 0 to 1; all of them approach 1 in neurons with strong direction or orientation selectivity and approach 0 in neurons with poor direction or orientation selectivity. DSI and OSI only take the responses from two or three points of the tuning curve into account, while CVd and CVo measure the selectivity from all the points of the tuning curve and are more robust. Only cells with DSI > 0.4 were considered as DS cells.

The tuning curve of each cell was fitted by the von Mises distribution, modified from Elstrott et al. (2008):

\[
R = R_0 + R_{\text{max}} e^{\cos(x - \mu)} / e^k
\]

where \( R \) is the F0 response in any given direction \( x \); \( R_{\text{max}} \) is the maximum F0 response; \( \mu \) is the preferred direction in radians; and \( k \) is the concentration parameter for tuning width. The half-tuning width at half height (HWHH) of the fitted tuning curve was measured as follows:

\[
\text{HWHH} = \theta = \text{acos} \left[ \ln(0.5 \cdot e^k + 0.5 \cdot e^{-k}) / k \right]
\]

This HWHH parameter shows the sharpness of the orientation/directional tuning.

Spatial frequency and response linearity. Spatial frequency tuning responses were measured with the spatial frequency of the grating ranging from 0.00825 to 1.32 cd/p (for some cells: 0.05 to 1.32 cd/p). For measuring the response linearity, the spatial frequency tuning of both F0 and F1 responses was measured and analyzed. The tuning curves were fitted by Gaussian model:

\[
y = R_0 + R_{\text{pref}} \cdot e^{\left(-\left(x - SF_{\text{peak}}\right)^2/2\sigma_y^2\right)}
\]

where \( y \) is the F0 or F1 response to each spatial frequency (x) tested; \( R_0 \) is the baseline activity; \( SF_{\text{peak}} \) is the spatial frequency that elicits the maximum response (\( R_{\text{pref}} \)); and \( \sigma_y \) is the standard deviation of the spatial tuning curve (the width of the Gaussian function). Here, the sum of \( R_0 \) and \( R_{\text{pref}} \) was considered as the peak F0 or peak F1 response.

To measure the response linearity of the cell, the F1/F0 ratio was calculated. Since the spatial frequencies that elicited the strongest F0 and F1 responses were not always the same, we implemented a previously described method (Chen et al. 2009; De Valois et al. 1982) to calculate it. First, we selected the three spatial frequencies that generated the largest combined F1 and F0 responses. Then, we obtained the F1/F0 ratio by averaging the F1 and F0 responses for these three spatial frequencies. Therefore, the F1/F0 ratio is represented by F1 average/F0 average ratio.

LGN DS simulation. To further understand the effect of alertness on LGN DS cells, a simple model of vector summation was constructed that uses LGN DS responses to predict stimulus direction in the alert and nonalert states. Then, we asked what changes in LGN DS response contribute to minimizing the prediction error. To build the model, the tuning curve of each LGN DS cell was normalized in both alert and nonalert states by the maximum mean firing rate in the alert state. Then, the normalized tuning curves were aligned by their preferred directions to obtain population averages for the four cardinal directions of movement: anterior, superior, posterior, and inferior. These population averages were used as the tuning of the four simulated LGN DS cells that we used in the model.

The mean firing rates of the four simulated DS cells to a given stimulus direction were extracted from their tuning curves. Then, the four mean firing rates were used to generate four spike trains by Poisson process and the four spike trains were integrated within a certain time window and fed to a vector sum detector. The resulting angle from the vector sum, the predicted stimulus direction, was compared with the stimulus direction to calculate the prediction error. The differences between the stimulus direction and predicted stimulus direction were plotted for the alert, nonalert, and nonalert scaled (the nonalert state tuning curve was scaled to the maximum firing rate of the alert state) states. The integration time ranged from 0 to 0.5 s with 10-ms increments. A thousand simulation iterations were performed for each cell in each condition.

RESULTS

Nineteen DS cells were studied in five awake Dutch-Belted rabbits. One additional DS cell was found but was held for only a short period of time, and the data from this cell are not included here. In these same microelectrode penetrations, we studied 264 other neurons that did not meet the criterion (see MATERIALS AND METHODS) for being DS. In addition, we tested other non-DS neurons in these penetrations that were quickly abandoned after brief testing (see Search strategy for LGN DS cells) and adequate records for such non-DS cells were not always kept. Therefore, we cannot here provide a precise value for the proportion of all encountered LGN neurons that were DS, except to say that it was <20/284.

Drifting gratings were presented over their receptive field center, and were optimally matched in size, temporal frequency, spatial frequency, and luminance contrast and then the orientation/direction was randomly changed to test 8, 12, or 24 directions. The orientation/directional tuning of one example DS cell is shown in Fig. 1. Black dots represent the F0 responses in the grating directions tested. Solid curve and
The distributions of LSI for DS cells and concentric cells are significantly bimodal (Hartigan’s test, $P < 0.001$) with the LSI for all DS cells being $>0.35$ (mean = $0.75 \pm 0.04$, Fig. 2B, black) and LSI for concentric cells $<0.02$ (mean = $0.0009 \pm 0.0009$, most of them have an LSI of 0, Fig. 2B, grey). Simple cells have pure ON or pure OFF or segregated ON/OFF receptive fields (Zhuang et al. 2013), so it is not surprising to see a significant difference of LSI between LGN DS and layer 4 simple cells (mean: DS vs. simple: $0.75 \pm 0.04$ vs. $0.03 \pm 0.007$, $P < 0.001$, Fig. 2B). The distribution of LSI for LGN DS and layer 4 simple cells is also significantly bimodal (Hartigan’s test, $P < 0.01$).

DS cells show strong direction selectivity by responding maximally in the preferred direction, and weakly in the null direction (180° opposite of the preferred direction; mean: preferred vs. null: $39.58 \pm 4.3$ vs. $4.07 \pm 1.1$ spikes/s, paired $t$-test, $P < 0.001$, Fig. 2C, population averages are shown in inset).

To quantify the direction selectivity of the cells, both DSI and CVD were computed. DSIIs were calculated based on the preferred and null direction responses (see MATERIALS AND METHODS) and were close to 1 in cells with strong direction selectivity and close to 0 in cells with poor direction selectivity. As noted in MATERIALS AND METHODS, only LGN cells with DSIIs of $>0.4$ were classified as DS cells, and the distributions of DSIIs for all LGN DS (black) and simple (white) cells are shown in Fig. 2D. Note that most of the simple cells are very direction selective (47 out of 56 cells have DSI $>0.4$). We also calculated the CVD, which is based on the entire orientation/ directional tuning curve (see MATERIALS AND METHODS) and ranges from 0 to 1, with larger values representing shaper directional tuning. The distribution of CVD of LGN DS cells ranged from 0.2 to 0.8 (mean: $0.56 \pm 0.04$, Fig. 2D, inset, black) suggesting that some DS cells are relatively more sharply tuned than others. The CVDs for simple cells (Fig. 2D, white) are similar to that of LGN DS cells (mean: simple: $0.49 \pm 0.03$, $P = 0.144$). OSI and CVo were also computed to quantify the orientation selectivity of the cells. The value of OSI and CVo, as for DSI and CVD, ranged from 0 to 1, with values close to 0 representing poor orientation selectivity and those close to 1 representing strong orientation selectivity. Cortical simple cells had better orientation selectivity than LGN DS cells, as measured by CVo (Fig. 2E, OSI: mean: LGN DS vs. simple: $0.67 \pm 0.05$ vs. $0.8 \pm 0.05$, $P = 0.072$; CVo: mean: LGN DS vs. simple: $0.24 \pm 0.03$ vs. $0.47 \pm 0.03$, $P < 0.05$).

The preferred direction of each cell was calculated by vector sum and the distribution is shown in Fig. 2F. It is very obvious that the distribution of LGN DS cell preferred directions (black) forms four groups (anterior, posterior, superior, and inferior), resembling the distribution of ON_OFF DS ganglion cells in the rabbit retina (Oyster and Barlow 1967). In contrast, the preferred directions of cortical simple cells (red) are more homogeneously distributed. LGN DS cells are much more likely to prefer movement in the directions within 15° of cardinal axes than are simple cells (mean: DS vs. simple: 17 out of 19 vs. 27 out of 54, $\chi^2$ test, $P = 0.002$, Fig. 2F). This suggests that, compared with simple cells, LGN DS cells better code movements in the four directions, anterior, posterior, inferior, and superior.
Normalized and averaged population tuning curves are shown for each of the four cardinal groups (green: anterior population, \(n=7\); black: superior population, \(n=2\); cyan: posterior population, \(n=5\); magenta: inferior population, \(n=5\); error bar: SE, Fig. 2G). The LGN DS cells are relatively broadly tuned with four prominent peaks in the four cardinal directions (tuning curves from Fig. 2G are superimposed in Fig. 2H).

**Linearity of spatial summation.** Spatial frequency tunings of 10 LGN DS cells and 20 concentric cells were tested, and each frequency was examined for both the F0 and F1 responses. A orientation/directional tuning curve for one example DS cell is shown in Fig. 3A, and the corresponding spatial frequency tuning curve in Fig. 3B, respectively. Dots are the responses measured under different spatial frequencies, and solid and dotted lines represent the best fit of a von Mises function.
dashed curves are the fitted curves of F0 and F1 responses by the Gaussian model (see MATERIALS AND METHODS). Nine of 10 LGN DS cells had higher F0 responses than F1 responses at all of the spatial frequencies tested (e.g., Fig. 3B); however, one LGN DS cell had higher F0 responses at high spatial frequencies and higher F1 responses at low spatial frequencies (data not shown). In the population of LGN DS cells studied, all but one cell (9 out of 10) had higher peak F0 response than peak F1 response (the exception is indicated by the grey arrow, Fig. 3C). Population averages are shown in Fig. 3C, inset. The preferred spatial frequency of LGN DS cells ranges from 0.34 to 0.98 cpd with a mean of 0.65 cpd (data not shown).

F1/F0 ratio is a common way to classify simple and complex cells in the cortex with simple cells having a F1/F0 ratio > 1 and complex cells a F1/F0 ratio < 1 (Movshon et al. 1978). To better compare the linearity of LGN DS, LGN concentric and V1 simple cells, we also calculated the F1/F0 ratios to drifting grating stimulation. We chose the three spatial frequencies that elicited the maximum combined F0 and F1 responses. Then, we calculated the F1/F0 ratio by averaging the F1 and F0 responses for these three spatial frequencies. The F1/F0 ratios for all but one DS cells were < 0.4 (mean for all the DS cells with a F1/F0 ratio < 1: 0.24 ± 0.029). By contrast, 18 out of 20 LGN concentric cells and 40 out of 44 layer 4 simple cells had a F1/F0 ratio > 1 (mean for all LGN concentric cells: 1.41 ± 0.27; mean for all V1 simple cells: 1.46 ± 0.048). LGN DS vs. simple: P < 0.01, LGN DS vs. LGN concentric: P < 0.05, Fig. 3D). The LGN DS cell that has a F1/F0 ratio > 1 is indicated by the gray arrow (Fig. 3D). Therefore, LGN DS are much more nonlinear compared with LGN concentric cells and layer 4 simple cells.

Response modulations by brain state. Awake rabbits shift between alert and nonalert states both spontaneously and in response to diverse sensory stimulation. Notably, this shift in brain state is associated with profound changes in LGN and V1 responses. We have previously shown that LGN concentric cells have higher spontaneous firing rate, lower burst rate, and higher response gain in the alert state than in the nonalert state (Bereshpolova et al. 2011; Bezudhnyay et al. 2006; Cano et al. 2006). Like LGN concentric cells, here we show that DS cells also have significantly higher spontaneous firing rates and lower burst rate in the alert state (mean spontaneous firing rate: alert vs. nonalert: 15.36 ± 1 vs. 9.95 ± 0.95 spikes/s, paired t-test, P < 0.001, Fig. 4A); mean burst rate: alert vs. nonalert: 0.17 ± 0.04 vs. 2.42 ± 0.31 bursts/s, paired t-test, P < 0.001, Fig. 4B). However, unlike concentric LGN cells, alertness increased the visual responses of LGN DS cells selectively around their preferred direction of movement and had an opposite suppressive effect around the null direction (Fig. 5). As concentric LGN cells, LGN DS cells generated stronger responses during the alert (Fig. 5A) than nonalert (Fig. 5B) states and their spontaneous rates were also higher during the alert state (Fig. 5C, dotted lines). However, the response to the nonpreferred direction was weaker in the alert state (Fig. 5C). As a population (Fig. 6), alertness enhanced the visual responses of DS cells in the preferred direction and suppressed them in the null direction (mean response in the preferred direction: alert vs. nonalert: 51.73 ± 5.24 vs. 37.04 ± 6.21 spikes/s, paired t-test, P < 0.01, Fig. 6A; mean response in the
measured (OSI and CVo) and the tuning HWHH did not increase or suppress by alertness, the orientation selectivity strength of the LGN DS visual responses could be strongly nonalert: 0.93 increased significantly in the alert state (mean for DSI: alert vs. nonalert: 0.24 ± 0.04 vs. 0.24 ± 0.05, paired t-test, \( P = 0.881 \), Fig. 6F; mean for HWHH: alert vs. nonalert: 56.45 ± 3.97 vs. 52.52 ± 4.49, paired t-test, \( P = 0.3 \), Fig. 6G).

In both states, visual responses in the null direction were strongly suppressed two to eight times below the spontaneous firing rate (Fig. 6H, mean in the alert state: null vs. spontaneous: 1.85 ± 0.67 vs. 15.18 ± 1.15 spikes/s, \( P < 0.001 \); mean in the nonalert state: null vs. spontaneous: 4.51 ± 0.6 vs. 9.32 ± 0.85 spikes/s, \( P < 0.01 \)). Moreover, this response suppression in the null direction was approximately two times higher in the alert than nonalert states (mean: alert vs. nonalert: 87.98 ± 3.99 vs. 45.45 ± 10.12%, \( P < 0.001 \)).

**LGN DS simulation.** The LGN DS cells code movements in the four cardinal directions with relatively broad and partially overlapped directional tuning curves (Fig. 2H). The main two effects of the alert state on LGN DS cells were the enhancement of visual responses in their preferred direction and the suppression of their responses in the null direction. To investigate the relative contribution of these two effects in the speed at which stimulus direction could be detected, we developed a simple model, which is illustrated in Fig. 7A. The firing rates of four simulated DS cells to a particular stimulus direction (green arrow at left) were extracted from average tuning curves using a Poisson process. Then, the spike rates were integrated over different time windows and fed to a vector sum detector that returned the predicted stimulus direction.

Figure 7B shows the population tuning curves in the alert (solid lines) and nonalert (dashed lines) states used to predicted three different directions 90° (Fig. 7C1), 112.5° (Fig. 7C2), and 135° (Fig. 7C3), indicated by the gray arrows above the curves. The relationships between the integration time and the mean predicted error (differences between stimulus direction and predicted direction) were obtained from the average tuning curves for the alert state (Fig. 7C, red), nonalert state (Fig. 7C, blue), and a nonalert state scaled to match the maximum response of the alert state (Fig. 7C, black). The contribution of response enhancement (Gain in Fig. 7C3) was estimated by comparing the nonalert and nonalert-scaled conditions (notice that the only difference between them is the increased response gain in the nonalert-scaled state). The contribution of response

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**Fig. 4.** Brain state, spontaneous activity, and bursting of LGN DS cells. Spontaneous firing rate (A) is significantly higher, while burst rate (B) is significantly lower in the alert than in the nonalert state. A2 and B2: population averages (alert in black and nonalert in white). ***\( P < 0.001 \).

null direction: alert vs. nonalert: 1.7 ± 0.62 vs. 4.24 ± 0.6 spikes/s, paired \( t \)-test, \( P < 0.01 \), Fig. 6B). As a consequence, the direction selectivity measured as both DSI and CVd also increased significantly in the alert state (mean for DSI: alert vs. nonalert: 0.93 ± 0.02 vs. 0.71 ± 0.07, paired \( t \)-test, \( P < 0.01 \), Fig. 6C; mean for CVd: alert vs. nonalert: 0.64 ± 0.05 vs. 0.52 ± 0.06, paired \( t \)-test, \( P < 0.05 \), Fig. 6D). Importantly, while the strength of the LGN DS visual responses could be strongly enhanced or suppressed by alertness, the orientation selectivity measured (OSI and CVo) and the tuning HWHH did not change with state (mean for OSI: alert vs. nonalert: 0.66 ± 0.08 vs. 0.64 ± 0.09, paired \( t \)-test, \( P = 0.71 \), Fig. 6E; mean for CVo: alert vs. nonalert: 0.24 ± 0.04 vs. 0.24 ± 0.05, paired \( t \)-test, \( P = 0.881 \), Fig. 6F; mean for HWHH: alert vs. nonalert: 56.45 ± 3.97 vs. 52.52 ± 4.49, paired \( t \)-test, \( P = 0.3 \), Fig. 6G).

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**Fig. 5.** Brain state and orientation/directional tuning. The effects of brain state on 1 DS cell are shown. A and B: tuning curves in alert (A) and nonalert (B) state with PSTHs shown for 12 different directions. Spontaneous rates are shown in dashed circles (alert in red, nonalert in blue). C: superimposed fitted tuning curves in alert (red) and nonalert (blue) state. Spontaneous firing rates are also shown in dashed lines.
Fig. 6. Population data of directional tuning properties of LGN DS cells during alert and nonalert state. Preferred direction responses (A1) are significantly higher, and null direction responses (B1) are significantly lower in the alert state. DSI (C1) and CVd (D1) are higher in the alert state than in the nonalert state. Orientation-selective indices (OSI; E1), CVo (F1), and half-tuning width at half height (HWHH; G1) do not change with states. H1: response suppression (reduction) in the null direction from the spontaneous firing rate is greater in the alert state than in the nonalert state. The cell with a negative %suppression had an increase in the null direction response beyond spontaneous rate in the nonalert state. Population averages are shown in the bar graphs in A2–H2 (alert in black and nonalert in white). *P < 0.05 and **P < 0.01.
Fig. 7. Simulation showing faster computation of stimulus direction in the alert state than in the nonalert state. A: diagram of LGN DS model using 4 DS cells with preferred movements in the cardinal axes. Firing rates of the DS cells when presented with a particular stimulus direction were used to generate spike trains by Poisson process. The resulting firing rates provided inputs to a vector sum detector, using firing rates calculated in different integration times, to get the predicted direction. B: population tuning curves in the alert (solid lines) and nonalert (dashed lines) state for 4 cells, each preferring 1 of the 4 cardinal directions. The tuning curve of the inferior direction (black) in the nonalert state was scaled to have the same maximum firing rate of that in the alert state, as shown in the dotted line. C1–C3: relationships between integration time and mean predicted errors for 3 stimulus directions (SD: 90°, 112.5°, and 135°) in the alert (red), nonalert state (blue), and nonalert scaled state (black). Black arrows on the left show the 20° threshold we used. Arrows in C3 indicate the prolonged integration time due to suppression and gain, respectively. Insets: population averages of integration time in alert, nonalert, and nonalert scaled states. ***P < 0.001.
suppression was estimated by comparing the alert and nonalert-scaled conditions (suppression in Fig. 7C3). Interestingly, although the response enhancement seems more pronounced than the response suppression in our results (e.g., Fig. 5C), the contributions of both response enhancement and suppression were relatively similar. It should be noted that, for all three stimulus directions tested, the integration time to get a 20° mean predicted error was shorter in the alert state than in the nonalert state (mean time for stimulus direction = 90° in the alert vs. nonalert scaled state: 0.099 ± 0.0017 vs. 0.1435 ± 0.0024 s, paired t-test, P < 0.001; nonalert vs. nonalert scaled state: 0.1955 ± 0.0027 vs. 0.1435 ± 0.0024 s, paired t-test, P < 0.001, Fig. 7C1; mean time for stimulus direction = 112.5° in the alert vs. nonalert scaled state: 0.1235 ± 0.0017 vs. 0.1765 ± 0.0022 s, paired t-test, P < 0.001; nonalert vs. nonalert scaled state: 0.2365 ± 0.003 vs. 0.1765 ± 0.0022 s, paired t-test, P < 0.001, Fig. 7C2; mean time for stimulus direction = 135° in the alert vs. nonalert scaled state: 0.1845 ± 0.002 vs. 0.2615 ± 0.0034 s, paired t-test, P < 0.001; nonalert vs. nonalert scaled state: 0.3575 ± 0.0046 vs. 0.2615 ± 0.0034 s, paired t-test, P < 0.001, Fig. 7C3). Also, the detection time was longer when the stimulus direction was least aligned with the cardinal axes for both alert and nonalert state (paired t-test, P < 0.001). Therefore, the results suggest that both the response increase in the preferred direction and response suppression in the null direction are important to enhance the signal to noise ratio and increase the speed of detection of a stimulus direction during the alert state. The simulation also predicts that stimuli moving closer to the direction of the cardinal axes will be detected faster by populations of LGN DS cells.

**DISCUSSION**

DS cells were first reported in rabbit retina >50 yr ago (Barlow and Hill 1963) and have been also characterized in squirrels (Michael 1966) and mice (Weng et al. 2005; Yoshida et al. 2001). Despite the relative large proportion of retinal DS ganglion cells (18.6–41% in rabbits and mice; Barlow and Hill 1963; Barlow et al. 1964; Sun et al. 2002, 2006; Weng et al. 2005), LGN DS cells are more rare (Levick et al. 1969; Marshall et al. 2012; Montero and Brugge 1969; Piscopo et al. 2013; Swadlow and Weyand 1985), with all but one of these studies reporting <7% of LGN cells to be DS. Furthermore, DS neurons are not uniformly distributed within the LGN. Indeed, in rabbit LGN (Stoelzel et al. 2008; Swadlow and Weyand 1985), DS neurons are much more prevalent in the representation of the upper visual field than in the representation of the visual streak (see MATERIALS AND METHODS). Similarly, evidence for nonuniform distribution of DS neurons has also been reported in mouse LGN (Marshall et al. 2012; Piscopo et al. 2013), which also has a visual streak-like increase in retinal ganglion cell density along the representation of the horizon (Drager and Olsen 1981). Here, we only studied DS neurons in the LGN representation of the visual streak to compare these cells with V1 simple cells studied, using the same methods, in this region of visual space (Zhuang et al. 2013, 2014).

**Axonal projections of LGN DS cells.** The presence of DS cells in the LGN raises questions concerning their cortical targets and the potential role in shaping the well-tuned direction/orientation selectivity seen in rabbit/rodent V1 (Piscopo et al. 2013; Scholl et al. 2013). Previous work (Swadlow and Weyand 1985) showed that LGN DS neurons do project to V1 and that their axons have conduction velocities similar to those of concentric LGN neurons, but the terminal layer of these axons could not be determined with the antidromic methods that were employed. Although our preliminary studies (Hei et al. 2013) indicate that at least some LGN DS neurons provide a strong synaptic impact in layer 4, recent work in mouse (Cruz-Martin et al. 2014) indicates that LGN DS neurons located in the shell region of the LGN selectively target the superficial layers of V1. Future work will resolve this issue.

**Which cells in V1 receive input from LGN DS neurons?** Orientation preference in cats and primates is columnar and is believed to originate, in part, from selective LGN inputs with ON or OFF receptive field centers precisely aligned with the cortical receptive field subfields (Alonso et al. 2001; Hubel and Wiesel 1962; Reid and Alonso 1995; Tanaka 1983; but see Mata and Ringach 2005). By contrast, orientation selectivity in rodents and rabbits is not columnar (Bonin et al. 2011; Bousfield 1977; Drager 1975; Girman et al. 1999; Metin et al. 1988; Okhi et al. 2005; Van Hooser et al. 2005) and the mechanism(s) generating sharp orientation/direction tuning may differ (e.g., Scholl et al. 2013). It is tempting to think that the DS input could contribute significantly to the orientation/directional properties of simple cells. Our results and recent findings in the mouse (Piscopo et al. 2013) suggest otherwise (also see Lien and Scanziani 2013). Thus we found that 1) whereas LGN DS neurons have spatially overlapping ON and OFF subfields, those of simple cells are spatially segregated; 2) whereas the F1/F0 ratios of simple cells indicate a linear spatial summation, those of LGN DS cells are highly nonlinear; and 3) whereas LGN DS cells have preferred directions lying on the four cardinal directions, the simple cells have more broadly distributed preferred directions. Together, these differences suggest that LGN DS neurons do not “drive” (Sherman and Guillery 1998) V1 simple cells and convey their receptive field properties upon them.

By contrast, fast-spiking inhibitory interneurons [suspected inhibitory interneurons, (SINs); Swadlow 1988; Swadlow and Weyand 1987; Zhuang et al. 2013] are likely targets of LGN DS neurons. These cells, like the LGN DS cells, have spatial receptive fields with overlapping ON/OFF zones and very low (nonlinear) F1/F0 ratios. Notably, V1 SINs lack the directional selectivity seen in LGN DS cells. However, SINs are known to receive a convergent, promiscuous input from topographically aligned thalamic neurons that display a variety of properties. Thus, individual V1 SINs, which have overlapping ON/OFF subfields, may receive input from both ON center and OFF center LGN neurons (Zhuang et al. 2013). Moreover, SINs in layer 4 barrel cortex show little directional preference for whisker movements but receive highly convergent input from ventrobasal thalamic neurons with a diversity of directional preferences (Swadlow and Gusev 2002). Similar results are seen in rat barrel system (Bruno and Simons 2002). Targeting of layer 4 SINs by LGN DS neurons would suggest a role for LGN DS neurons in driving fast and strong feed-forward inhibition that could sharpen sensory tuning of recipient simple cells around the four cardinal directions of motion. Of course, it is also possible that LGN DS terminals in layer 4 target the descending dendrites of layer 2/3 complex cells or ascending apical dendrites of layer 5 complex cells. Indeed, many corti-
cotectal neurons of rabbit layer 5 have complex, DS receptive fields similar to those of LGN DS neurons (Swadlow 1988; Swadlow and Weyand 1987). Cross-correlation studies of retinotopically aligned LGN DS neurons and cortical populations (e.g., Alonso et al. 1996, 2001; Swadlow and Gusev 2002) would be well suited to test these hypotheses.

**Effects of brain state on LGN DS neurons.** In awake rabbits, frequent shifts between alert and nonalert brain states are associated with significant changes in spontaneous activity, burst firing (Guido and Weyand 1995; Sherman and Guillery 1996; Weyand et al. 2001), and visually driven responses of LGN concentric neurons (Bezdudnaya et al. 2006; Cano et al. 2006), and some such changes are conveyed to V1 layer 4 simple cells (Bereshpolova et al. 2011; Zhuang et al. 2014). Our results in LGN DS neurons are consistent with the results for LGN concentric cells, in showing higher spontaneous firing rates, lower burst rates, and stronger responses to visual stimulation in the preferred direction when alert. Our results also show that alertness increases response suppression to stimuli moving in the null direction (Levick et al. 1969). Thus the response enhancement (gain) in the preferred direction and the response suppression in the null direction both contribute to an increase of the signal-to-noise ratio when alert, even though there is no change in sharpness of tuning and direction preference (the HWHH, Fig. 6G). The mechanism of the enhanced null-direction suppression when alert could involve feed-forward and/or feed-back inhibition mediated by brainstem or cortical (Briggs and Usrey 2008) inputs. However, since there is little evidence for feed-forward inhibition within rabbit LGN (Lo 1981), the mechanism probably involves enhanced feedback inhibition, via the thalamic reticular nucleus, and there is evidence for such arousal induced enhancement of feed-back inhibition in LGN projection cells (Swadlow and Weyand 1985). Alternatively, LGN DS neurons could be selectively suppressed by neuromodulatory inputs associated with arousal (e.g., McCormick 1992).

**Functional role of LGN DS cells.** Our results show that LGN DS cells provide a dedicated thalamocortical channel to transfer motion signals about the four cardinal directions to the primary visual cortex. The relatively broad and overlapping directional tuning characteristics of LGN DS channels suggest that they could form the core elements of a vector-based population code for extracting directional information. We do not know the function of this thalamocortical directional information, but our simulations show how alertness could allow more rapid extraction of this information. In this regard, it is worth noting that rabbits are frequent targets of birds of prey, that they naturally evade these predators (Pongrácz and Altwäcker 2000), and that DS neurons are most prevalent in the LGN representation of the upper visual field (Swadlow and Weyand 1985). These results and observations suggest that LGN DS cells could be useful in determining the angle of approach of areal predators, and our model and simulations indicate that alertness would hasten this computation. This would allow more rapid decisions about behavioral output (e.g., both go/no-go decisions and the direction of escape responses).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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