Separability of stimulus parameter encoding by on-off directionally selective rabbit retinal ganglion cells

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Nowak P, Dobbins AC, Gawne TJ, Grzywacz NM, Amthor FR. Separability of stimulus parameter encoding by on-off directionally selective rabbit retinal ganglion cells. J Neurophysiol 105: 2083–2099, 2011. First published February 16, 2011; doi:10.1152/jn.00941.2010.—The ganglion cell output of the retina constitutes a bottleneck in sensory processing in that ganglion cells must encode multiple stimulus parameters in their responses. Here we investigate encoding strategies of On-Off directionally selective retinal ganglion cells (On-Off DS RGCs) in rabbits, a class of cells dedicated to representing motion. The exquisite axial discrimination of these cells to preferred vs. null direction motion is well documented: it is invariant with respect to speed, contrast, spatial configuration, spatial frequency, and motion extent. However, these cells have broad direction tuning curves and their responses also vary as a function of other parameters such as speed and contrast. In this study, we examined whether the variation in responses across multiple stimulus parameters is systematic, that is the same for all cells, and separable, such that the response to a stimulus is a product of the effects of each stimulus parameter alone. We extracellularly recorded single On-Off DS RGCs in a superfused eyecup preparation while stimulating them with moving bars. We found that spike count responses of these cells scaled as independent functions of direction, speed, and luminance. Moreover, the speed and luminance functions were common across the whole sample of cells. Based on these findings, we developed a model that accurately predicted responses of On-Off DS RGCs as products of separable functions of direction, speed, and luminance ($r = 0.98; P < 0.0001$). Such a multiplicatively separable encoding strategy may simplify the decoding of these cells’ outputs by the higher visual centers.

population coding; tuning curve; receptive field; trigger feature; visual motion

ON-OFF DIRECTIONALLY SELECTIVE retinal ganglion cells (On-Off DS RGCs) in rabbits were originally identified as having robustly asymmetrical responses to stimuli moving in opposite directions (Barlow and Hill 1963). They fire most vigorously when the stimulus is moving in one particular (preferred) direction, very little or not at all when it is moving in the opposite (null or antipreferred) direction, and produce responses between these two extremes for intermediate directions (Barlow et al. 1964). This distinctive directional selectivity lead to the direction of motion being referred to as the stimulus “trigger feature” for this class of cells (Barlow et al. 1964). The idea of a trigger feature is consistent with the findings that these cells respond in a directionally selective (DS) manner to moving stimuli independently of many stimulus parameters (dimensions), such as sign and amount of contrast, spatial configuration, spatial frequency, speed, and motion extent (Grzywacz and Amthor 2007). This robust discrimination is mediated by a number of mechanisms about which a great deal is already known (Amthor and Grzywacz 1991, 1993; Ariel and Daw 1982; Baccus et al. 2008; Barlow and Levick 1965; Caldwell et al. 1978; Grzywacz et al. 1997, 1998a,b; Kittila and Massey 1997; Taylor and Vaney 2002; Taylor et al. 2000; Poggio and Reichardt 1973; Schachter et al. 2010; Wyatt and Daw 1975).

However, the concept of directionality as a trigger feature requires further elaboration because some characteristics of On-Off DS RGCs seem to impede their signaling of the precise direction of motion. First, stimulus dimensions other than direction also affect responses of these cells. A first-order measure of response magnitude, the number of spikes elicited by a moving stimulus, depends on the contrast, speed, and spatial frequency as well. Second, these cells respond to a broad range of directions, with the response magnitude falling off monotonically from the preferred direction in a gradual manner, and, for instance, they produce responses to directions 90° from the preferred which are typically between 25% (Levick et al. 1969) to 50% (Barlow et al. 1964) of the maximum, much larger than in DS cells in the lateral geniculate nucleus (Levick et al. 1969). Therefore, despite the precise axial directional discrimination of On-Off DS RGCs, the response of a single cell is ambiguous about motion direction, as it cannot distinguish, at least by first-order response magnitude, a nonoptimal contrast stimulus moving close to the preferred direction from an optimal contrast stimulus that is farther from the preferred direction. Consequently, this raises a question about the feasibility of extracting precise information on the stimulus direction from responses of On-Off DS RGCs.

A plausible approach to de-confound stimulus parameters is population coding (Pouget et al. 2000). There is some evidence suggesting that On-Off DS RGCs may implement such a coding scheme with respect to direction. First, the preferred directions of these cells are not uniformly distributed, but are arranged in four clusters, roughly corresponding to the anterior, posterior, superior, and inferior motion in the field of view (Oyster 1968; Oyster and Barlow 1967). Second, each of the four orthogonally tuned cell subclasses tiles the retina precisely (Amthor and Oyster 1995). Consequently, every point in the field of view is projected onto the receptive fields of exactly four On-Off DS RGCs, each preferring a different cardinal direction. If all the stimulus parameters other than direction affected a quartet of cells identically, a higher visual center...
could filter out the effects of those parameters by comparing the ratios of these cells’ outputs and thus more precisely estimate the direction (van Hateren 1990). However, such an approach would require fulfillment of two, among several other, assumptions: 1) the effects of the nondirectional parameters should be separable from the effect of direction, and 2) the effects of the nondirectional parameters should be the same for all the cells in a population. To address these problems, variation in responses of On-Off DS RGCs across multiple parameters must be determined.

In this study, we pursued these problems by asking the following questions: 1) how does response vary along each stimulus dimension; 2) do the different dimensions affect cell responses independently, or do interactions between them exist; and 3) does the entire population of On-Off DS RGCs in a rabbit retina behave similarly with respect to the first two questions? Such issues have been considered previously with respect to coding strategies of visual cortical cells (Stern et al. 1993). Here we focused on three parameters of a moving stimulus: direction, speed, and luminance.

In sensory neurons, the standard method for assessment of a neuron’s response characteristics to a particular stimulus dimension is the neuronal tuning curve, which is a mapping between the stimulus parameter under study and the mean firing rate of the neuron (Dayan and Abbott 2005); thus it provides a first-order description of cell responses as a function of the parameter value. Other studies (Barlow et al. 1964) have shown that On-Off DS RGCs are broadly direction tuned with responses about half maximal to directions up to 90° from the preferred. It has also been determined that On-Off DS RGCs are broadly speed tuned and maintain directional selectivity across more than two orders of magnitude of speed, from a few tenths of a degree per second to at least 50°/s (Grzywacz and Amthor 2007). In the same study, speed tuning curves for the preferred and null directions were found to be nonmonotonic: increasing for slow speeds, exhibiting a peak around 30°/s, and decreasing for faster speeds. In another study, in which total spikes per sweep was measured rather than mean firing rate, it was found that response decreases as speed increases for the whole range of speeds studied (Wyatt and Daw 1975). The range of speeds investigated was similar in the two studies. On-Off DS RGCs have also been shown to be broadly tuned for contrast, maintaining directional selectivity over the entire range of contrasts to which they respond, even at contrasts of a few percent (Grzywacz and Amthor 2007). Contrast tuning curves for the preferred and null directions determined in that study were found to be nonmonotonic in the same rise-then-fall fashion as speed tuning curves, consistent with an earlier study in which spike count rather than mean firing rate was the response measure (Merwine et al. 1998).

To study the effects of direction, speed, and luminance on the responses of On-Off DS RGCs, we developed a model based on the tuning curves for these three parameters, but rather than the mean firing rate we used the mean spike counts. Our model was therefore constrained to first-order measure of responses, namely the mean number of spikes evoked by a particular stimulus. We found that direction, speed, and luminance had nonlinear characteristics, and we quantified these. In addition, for each cell the general shapes of direction, speed, and luminance tuning curves were independent from the other two parameters, which affected the tuning curve only by a scale factor. Moreover, the speed and luminance tuning curves appeared to be very similar across different cells. Consequently, we found that a simple multiplicatively separable model employing a Gaussian function to approximate the direction tuning curve, a power function to approximate the speed tuning curve, and an exponential rise function to approximate the luminance tuning curve closely accounted for the mean spike counts for most combinations of stimulus parameter values. Some preliminary results of this study have been previously reported in abstract form (Nowak et al. 2009).

METHODS

All experimental procedures were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (the University of Alabama at Birmingham is accredited by the American Association for Accreditation of Laboratory Animal Care). Retina preparations and electrophysiology methods were similar to those reported by Risner et al. (2010).

Retina Preparation

Small weight New Zealand albino rabbits (1.6-4.2 kg) of both sexes were dark adapted and anesthetized initially by intraperitoneal injections of urethane (2 g/kg; Sigma-Aldrich, St. Louis, MO), followed by administration of Nembutal (Ovation Pharmaceuticals, Lake Forest, IL) through the marginal ear vein until no change in heart rate and no reflexive movement resulting from a pinch to the paw was observed. Under dim red light, the eye was enucleated and the animal was euthanized by an intravenous injection of 1 ml Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI). The eye was hemisected in ice-cold oxygenated bicarbonate-buffered (95% O2 and 5% CO2) Ames medium (Sigma-Aldrich, St. Louis, MO), and the lens and vitreous were removed. The remaining eyecup was bisected along the line crossing the optic disc and orthogonal to the fiber band. One of the halves was mounted on a domed chamber where it was superfused with heated (33–35°C) oxygenated bicarbonate-buffered Ames medium (~3.5 ml/min). Ganglion cell somas were visualized using Azure B (Sigma-Aldrich) solution, a few drops of which were added to the superfusate flowing over the retina (Amthor et al. 2002).

Cell Selection and Categorization

It is known that On-Off DS RGCs in rabbits occur in four broadly tuned directions (Oyster and Barlow 1967), and there is evidence that different directions have different distributions of cholinergic receptor subtypes (Strang et al. 2007). It is also known that the receptive fields of these cells are larger at higher eccentricities than centrally (Levick 1967). In this study, On-Off DS RGCs were recorded from both inferior and superior retina at various eccentricities but mostly just below the visual streak, and the location in the retina and the preferred direction of each recorded cell were noted. However, our sample size was too small to investigate whether any properties related to directional selectivity varied systematically as a function of location or particular preferred direction. Thus our analyses of On-Off DS RGCs were lumped together with respect to particular preferred direction and eccentricity.

Visual Stimuli and Receptive Field Mapping

Stimuli were displayed on a standard 15-inch color CRT monitor (model SyncMaster 15GLi, Samsung, Ridgefield Park, NJ) with 640 × 480 resolution and 100-Hz refresh rate. The monitor’s nonlinear relationship between its luminance and applied grayscale value was measured using a photometer (model LS-110; Minolta). The displayed image was reflected by a mirror that projected it through the
epiillumination pathway obtained by removal of the fluorescence lamp housing from a microscope (model Optiphot-2; Nikon), and having passed a ×2 eye piece followed by a ×4 objective, it was focused on the retina. The aperture of the optical system was 13 mm, and the overall demagnification factor was ~9. The display of the stimuli on the monitor was driven by a custom-developed application DataAcquirer II/AIN (Amthor Laboratory, University of Alabama at Birmingham) running on a personal computer under Microsoft Windows XP Professional operating system (Microsoft, Redmond, WA). During manual receptive field mapping, the stimulus consisted of one small white rectangle flashed or jittered repeatedly on a dark background at different locations. For subsequent data collection, a bar moving perpendicular to its long side was used, with the length of this side being equal to the diameter of the receptive field center. Depending on the experimental protocol, the bar was swept in a number of directions with all the other parameters held constant, or its speed or luminance was varied, either separately or in combination. Bar speed was measured in millimeters per second on the retina, but it was also converted to degrees per second using the retinal magnification factor of 0.173 mm/° calculated for a rabbit schematic eye by Hughes (1972). In experiments with constant speed, the speed was 2.84 mm/s (16.42/°), whereas in experiments with variable speeds, five speeds varying between 0.71 mm/s (4.10/°) and 11.36 mm/s (65.66/°) were used. Light intensity was reported in terms of luminance, but since luminance is a measure specific to the human observer and not to the retina preparation under the optical system used, the corresponding retina irradiance was also determined. For this purpose, the relationship between the applied grayscale value and the retina irradiance was measured using a light detector (model SED 033/FW; International Light, Newburyport, MA) connected to a radiometer (model IL 1700; International Light). The readings of the radiometer were subsequently corrected to account for the fact that the stimulating light did not cover the whole area of the integrating sphere. In experiments with constant luminance level, the bar luminance was 57.58 cd/m² (irradiance: 9.73 μW/cm²) and the background was dark (luminance: 0 cd/m² and irradiance: 0.57 μW/cm²). In experiments with variable luminance levels, five luminance levels varying between the value slightly above the cell threshold determined earlier (the lowest value across all cells was 1.03 cd/m², irradiance: 0.73 μW/cm²) and 57.58 cd/m² were used for the bar, while the background was also kept dark. In experiments involving positive and negative contrasts, three positive and three negative contrasts were used, and the background was mid-gray with the luminance of 28.62 cd/m² (irradiance: 5.09 μW/cm²). The contrasts were calculated as Weber contrasts from the retinal luminance levels, five luminance levels varying between the value slightly above the cell threshold determined earlier (the lowest value across all cells was 1.03 cd/m², irradiance: 0.73 μW/cm²) and 57.58 cd/m² were used for the bar, while the background was also kept dark. In experiments involving positive and negative contrasts, three positive and three negative contrasts were used, and the background was mid-gray with the luminance of 28.62 cd/m² (irradiance: 5.09 μW/cm²). The contrasts were calculated as Weber contrasts from the formula \[ \frac{L_b - L_o}{L_b} \frac{L_b}{L_o} \text{ where } L_b \text{ denotes the stimulus luminance and } L_o \text{ denotes the background luminance, measured in candelas per meters squared.} \]

Data Recording and Analysis

Extracellular recordings from single cells were obtained using a single carbon-fiber tipped, copper wire in-glass electrode plated with silver chloride (Amthor et al. 2003; Armstrong-James and Millar 1979). The signal was amplified (A-M Systems, Sequim, WA), filtered with a custom-made learning filter to remove 60-Hz noise, thresholded with a custom-made Schmitt trigger, and digitized at a 1-kHz sampling rate using PCI-DAS1002 acquisition board (Measurement Computing, Norton, MA). Action potentials were verified to stem from a single cell by observation on an oscilloscope (Hitachi) and by listening to the audio output. On-line spike extraction was performed by the DataAcquirer II/AIN application and the resulting spike times were stored to the nearest millisecond on the hard disk for further off-line analyses. Each recording comprised 35 trials in each of which unique conditions (particular combinations of direction, speed, and luminance level) were arranged in a shuffled random order. The collected data were subsequently analyzed off-line using routines written in MATLAB 7.7 (MathWorks, Natick, MA).

Assessment of Directional Selectivity

Directional selectivity of each cell was quantified off-line using the direction selectivity index proposed by Taylor and Vaney (2002):

\[ D = \frac{\sum d_i}{\sum r_i} , \]

where \( D \) represents the direction selectivity index, the summations are performed over all the tested directions, and each \( d_i \) is a vector having the same direction as the corresponding stimulus and the length \( r_i \) equal to the mean number of spikes elicited by that stimulus. This index ranges from 0, when the mean spike counts are equal in all tested directions, to 1, when a nonzero spike count is obtained only for one direction. Consequently, larger index values indicate stronger directional selectivity.

Fitting of Tuning Curves

Direction, speed, and luminance tuning curves were fitted off-line with various functions using the MATLAB Optimization Toolbox (Version 4.1). The fitting procedure involved a nonlinear least-squares method based on trust-region reflective Newton algorithm (Optimization Toolbox User’s Guide 2008). To assess the robustness of the fits, 95% confidence intervals were computed using MATLAB Statistics Toolbox (Version 7.0). The goodness of fit of different functions was compared by means of adjusted coefficient of determination (adjusted \( R^2 \)), which was preferred over coefficient of determination (\( R^2 \)) because of unequal number of the fitted parameters in those functions. Details of the fitting are given in the APPENDIX.

Similarity Index

Depending on the experimental protocol, various direction, speed, luminance, or contrast tuning curves were obtained for a single cell at different stimulus conditions. Within each tuning category, the similarity of the shapes of those tuning curves was quantified off-line using a similarity index. This index was similar to that introduced by Rapela et al. (2010) except for two differences: it was based on the median rather than the mean as the measure of central tendency and on the Kendall’s tau rank correlation coefficient rather than the Pearson product-moment correlation coefficient as the measure of correlation. This modification made it robust against outliers, possibly related to the weakest stimuli. The index was computed in three steps: first, the tuning curves were normalized with respect to their peaks, then the median normalized tuning curve for the cell was determined, and finally the Kendall’s tau rank correlation coefficient between the data constituting that median curve and the data corresponding to all the normalized tuning curves was computed yielding the index value. As shown by Rapela et al. (2010), this type of index gives the upper bound of how well a curve can be predicted from the others. Since it is a correlation index, its value ranges from 0, when the curves are statistically unrelated (which would happen, for example, if they were generated by noise), to 1, when the curves are identical, meaning perfect similarity, or, in the terminology adopted by Rapela et al. (2010), a perfect ability to predict a curve from the others. This index, owing to its statistical nature, also provides a corresponding \( P \) value that represents the probability of obtaining a particular value solely by chance, when in fact there is no association among the curves.
Direction Tuning Curves

General characteristics of direction tuning curves. On-Off DS RGCs respond asymmetrically to a stimulus moving in different directions, producing the strongest response when the stimulus is moving in the preferred direction, the weakest response when it is moving in the null direction, and intermediate responses when it is moving in intermediate directions (Barlow et al. 1964). Therefore, their direction tuning curves are unimodal with the peak at the preferred direction and fall gradually in both directions from that preferred direction toward the null direction, at which they reach the minimum. Here, we investigated direction tuning curves of all 26 On-Off DS RGCs with more precision than reported previously in the literature, using 32 directions separated by ~11.25° rather than the typical 8 or 16 directions. We swept a bright bar (of luminance 57.58 cd/m²) on a dark background at a constant speed (2.84 mm/s as measured on the retina, equivalent to 16.42°/s) across the receptive field. For each cell, we first assessed its directional selectivity using the direction selectivity index proposed by Taylor and Vaney (2002). We found the indexes to vary between 0.25 and 0.68, with the mean of 0.49 and the SD of 0.11. The latter two values appear similar to those reported by Taylor and Vaney (2002) (mean = 0.57; SD = 0.08) and Zeck and Masland (2007) (mean = 0.58; SD = 0.10), although our mean value is slightly lower.

We next examined the shapes of the direction tuning curves. A polar plot of a representative direction tuning curve obtained from a typical cell is shown in Fig. 1A. Our results confirmed previous findings that direction tuning curves fall off in both hemi-planes from the preferred direction in a generally monotonic and symmetrical manner (Barlow et al. 1964; Oyster 1968). This is demonstrated in Fig. 1B, which replots the data from Fig. 1A in Cartesian coordinates with additional small dots representing spike counts in individual trials. Even though the individual trials exhibited some considerable variability, in a number of cells such as this one, there was effectively no overlap between the responses near the preferred and near the null directions.

We also examined the widths of the direction tuning curves. For each cell, we fitted the mean spike counts with a Gaussian function (1 of the 3 fitted functions used during subsequent functional approximation of direction tuning curves, as described later) and obtained an estimate of the direction tuning width as the half width at half maximum. Figure 1C shows a histogram of these estimates for all the 26 cells. Our results confirmed that On-Off DS RGCs are broadly direction tuned (Barlow et al. 1964; Levick et al. 1969). Specifically, we found that although there was some variability in direction tuning widths as they ranged between ~38° (resulting in more elliptical-like tuning curves) and 106° (resulting in more cardioid-like tuning curves), the tuning was generally broad, with the median tuning width of 75.5°. We noted that the shape of the histogram shown in Fig. 1C strongly indicated that the direction tuning widths could stem from a normal distribution. To verify this hypothesis, we compared the actual distribution of the direction tuning widths with a theoretical distribution being an appropriately scaled normal distribution with the same parameters as those calculated from the data (mean = 76.6°; SD = 17.91°). As demonstrated in Fig. 1C, a curve representing the theoretical distribution, when overlaid upon the histo-

Fig. 1. Direction tuning curves and their widths. Direction tuning curves were determined from rabbit On-Off directionally selective retinal ganglion cells (On-Off DS RGCs) at 32 directions separated by ~11.25° by averaging spike counts from 35 trials per direction. Stimulus was a bar of luminance of 57.58 cd/m² swept at the speed of 2.84 mm/s as measured on the retina: A: polar plot of a representative direction tuning curve determined from a typical On-Off DS RGC. Tuning width computed as the half width at half maximum of a Gaussian fit was 75.5°. B: Cartesian plot of the same direction tuning curve (black solid line) superimposed on spike counts from individual trials (gray dots). Positions of the dots are slightly jittered for better visibility. Bars on the curve show SE. For reference, mean number of spikes for no-stimulus (blank) condition is also shown (black broken line at the bottom). C: histogram of the direction tuning widths for each of the 26 cells. Each width was computed as the half width at half maximum of a Gaussian fit to the cell mean spike counts. Bin width is 10°, and the highest bin occurs between 70~80°. Overlying line represents the theoretical scaled normal distribution with the same mean and SD as those calculated from the data. D: quantile-quantile plot comparing the distribution of the direction tuning widths for the 26 cells to the theoretical scaled normal distribution with the same mean and SD. Line representing the identity relationship (dotted black) is shown as a reference. E: plot of the total of the mean spike counts being a sum over all directions vs. the direction tuning width for each of the 26 cells. F: plot of the mean spike counts for no stimulus (blank) condition vs. the direction tuning widths for the 26 cells. In E and F, the linear regression line (solid gray) is shown for reference and the linear correlation coefficient (r) is quantified.
direction tuning width is related to the overall excitability of the cell, possibly reflecting the cell health condition. For this purpose, for each of the 26 cells we compared its direction tuning width with the corresponding total of the mean spike counts that was the sum over the 32 tested directions. Likewise, we compared the direction tuning width with the cell spontaneous activity represented by the mean spike counts for no-stimulus (blank) condition. In both cases we did not find any correlation. Figure 1E shows a plot of the total mean spike counts for each cell vs. the direction tuning width. Apparently, the points are randomly scattered, indicating no association, which is confirmed by a very small Pearson product-moment correlation coefficient ($r = 0.12; P = 0.56$). A similar plot of the mean spike counts for no-stimulus (blank) condition vs. the direction tuning width is shown in Fig. 1F. The points are also randomly scattered, yielding equally small Pearson product-moment correlation coefficient ($r = -0.07; P = 0.72$).

**Functional approximation of direction tuning curves.** We investigated whether the direction tuning curve can be approximated by some simple functions through fitting the relationship between the stimulus direction and the mean spike counts. We chose three functions with a small number of free parameters: mirror-symmetric Archimedes’ spiral (Eq. 2 in Appendix), cosine (Eq. 3), and Gaussian (Eq. 4). The mirror-symmetric Archimedes’ spiral was a concatenation of two standard Archimedes’ spirals, each confined to one hemi-plane from the preferred direction, as shown in Fig. 2A. This function was selected because it makes two assumptions, which might simplify decoding: 1) the response magnitude is symmetric about the preferred-null direction axis, and 2) in each hemi-plane the response magnitude declines linearly away from the preferred direction. The other two nonlinear functions were selected as they are widely used for fitting neuronal tuning curves (Dayan and Abbott 2005). The cosine function is not as simple as the mirror-symmetric Archimedes’ spiral, as it is quadratic in the neighborhood of the peak and linear on the flanks, but it has the advantage of being smooth at the peak. Moreover, a quartet of cosine functions allows representation of the positive projections of direction onto four axes. The Gaussian function, like cosine, is smooth at the peak, and also offers an additional degree of freedom governing its width. In the case of this function, we implemented extra terms to ensure mirror symmetry in both hemi-planes from the preferred direction (see Appendix). A typical fit of the mirror-symmetric Archimedes’ spiral, using the data points producing the same direction tuning curve as depicted in Fig. 1, A and B, is shown in Fig. 2B, while Fig. 2, C and D, shows the corresponding fits of the cosine and Gaussian functions, respectively. We found that generally the data points along the slopes were fitted closely by all of the three functions, but in the regions immediate to the preferred and null directions, in which the direction tuning curves were rather flat, the mirror-symmetric Archimedes’ spiral could not follow the data points precisely, as opposed to the two nonlinear functions. This failure of the mirror-symmetric Archimedes’ spiral was due to its piecewise linear nature resulting in its pointed shape in these regions. Most commonly, the closest fit was offered by the Gaussian function owing to the additional parameter governing its width. This additional flexibility presented an advantage over the other two functions for two reasons. First, the tuning widths expressed in terms of the half width at half maximum of both

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**Fig. 2. Functional approximation of direction tuning curves.** Direction tuning curves determined at 32 directions were fitted with 3 functions: A: polar plot of an example mirror-symmetric Archimedes’ spiral function (with arbitrary parameter values). This function combines two standard Archimedes’ spiral functions, one of which (black thin line) is shown over 180° (a single hemi-plane) and symmetrically merged around the preferred direction, they form a mirror-symmetric Archimedes’ spiral function, which has a cardioid shape. B, C, and D: fits of the 3 functions: mirror-symmetric Archimedes’ spiral (B), cosine (C), and Gaussian (D) (black lines) to the mean spike counts (gray dots) producing the direction tuning curve of a typical cell (the same as in Fig. 1, A and B). Bars on each line show 95% confidence intervals of the fitted function. E: box plot showing the spread of a measure of goodness of fit (adjusted $R^2$) for the 3 functions for the 26 cells. Central broken line in each box represents the median, the solid edges represent the first (bottom) and third (top) quartiles, and the whiskers extend to the most extreme values not considered outliers, whereas potential outliers are individually plotted as crosses.

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the mirror-symmetric Archimedes’ spiral and the cosine functions were fixed at 90°, whereas the respective median direction tuning width for all the 26 cells proved to be 75.5° (as described earlier), which means that both of the functions were on average too broad. Second, the width parameter enabled the Gaussian function to adjust even more precisely depending on the individual tuning widths, which could be slightly broader or narrower than the average. Our observations were confirmed when we assessed the goodness of fit of the 3 functions for all the 26 cells by means of an adjusted coefficient of determination (adjusted $R^2$), the spread of which is shown in Fig. 2E in the form of a box plot. As expected, the Gaussian function provided the best fit, although the goodness of fit of all the three functions was very high and comparable, with the median values of 0.92, 0.94, and 0.97, for the mirror-symmetric Archimedes’
spiral, cosine, and Gaussian functions, respectively. Moreover, the interquartile range of the coefficients (depicted as the box height in Fig. 2E) was substantially smaller for the Gaussian function compared with the other two. Even though this function required one extra parameter to be fitted, the additional degree of freedom seemed justified since the adjusted $R^2$, which penalizes extra parameters, still had the highest value and the smallest variation for the Gaussian function.

**Invariance of direction tuning curves to other stimulus parameters.** We next investigated how different stimulus parameters affect the shape of the direction tuning curve. First, we examined the effect of speed. Here, we varied speed and direction systematically, sweeping a bright bar (of luminance 57.58 cd/m²) on a dark background in 16 directions separated by $\sim 22.5^\circ$ at five speeds across the receptive field. Other stimulus parameters except direction and speed were held constant. The data came from 10 cells from 10 different retinas. The results were consistent for nine cells, whereas one cell was found to be anomalous (as its responses were sluggish and not equally DS at all speeds), and thus it was omitted from the analyses. Figure 3A shows a Cartesian plot of the direction tuning curves obtained for the various speeds from the cell that yielded the least noisy data. Generally, as the speed increased, the mean number of spikes decreased, and this outcome was independent of direction (except for the null direction, for which the number of spikes was almost equal at all the speeds). Figure 3B shows the same direction tuning curves normalized with respect to the peak mean spike count for each speed. The normalization resulted in all the curves reducing to the same generic curve, which suggested that the effect of speed on the direction tuning curve can be represented by a multiplicative scale factor. For some cells, this robust normalization failed somewhat only at the fastest speed, which elicited poor responses for most directions away from the preferred, producing the respective normalized direction tuning curve slightly shifted or having a slightly different width compared with the other normalized curves. To assess the extent to which the direction tuning curves for different speeds normalized to one generic curve in a given cell, we calculated a similarity index (see METHODS). Figure 3C shows a histogram of these similarity indexes quantified for all the nine analyzed cells. We found the similarity to be high, with the minimum correlation equal to 0.71, the maximum correlation equal to 0.90, and the median correlation equal to 0.85. All the corresponding $P$ values were below 0.0001.

We also examined the effect of luminance on the direction tuning curve. Here, luminance and direction were varied systematically, using a bright bar at five luminance levels against a dark background, and sweeping it in 16 directions separated by $\sim 22.5^\circ$ at a constant speed (2.84 mm/s as measured on the retina, equivalent to 16.42°/s) across the receptive field. Other stimulus parameters except direction and luminance were held constant. The data came from nine cells from nine different retinas, but the results were consistent for eight cells. One cell was found to yield rather anomalous results, and thus it was omitted from the analyses (the same cell that was anomalous when speed was varied). Figure 3D shows a Cartesian plot of the direction tuning curves obtained for the various luminance levels from one of the cells that produced the least noisy data. Generally, as the luminance increased, the mean number of spikes also increased, and this outcome was independent of direction (again except for the null direction, for which the number of spikes was almost equal at all the luminance levels). It is worth pointing out that the $\sim 40$-fold variation of luminance in the plot shown in Fig. 3D is reduced to an $\sim 2$-fold variation in mean spike count, which constitutes a 20-fold compression. In Fig. 3E, the same direction tuning curves were normalized with respect to the peak mean spike count for each luminance level. As in the case of speed variation, the normalization mapped all the curves onto the same generic curve, which indicated that the effect of luminance on the direction tuning curve can also be represented by a multiplicative scale factor. This mapping, for some cells, failed somewhat at the lowest luminance level, with the result that the respective normalized direction tuning curve was slightly shifted or changed in width compared with the other normalized curves. As before, we applied the similarity index to each cell to assess the extent to which the direction tuning curves for different luminance levels normalized to one generic curve. Figure 3F shows a histogram of the similarity indexes quantified for all the eight analyzed cells. Again, we found the similarity to be high, with the minimum correlation being 0.75, the maximum correlation being 0.91, and the median correlation being 0.88, and with all the corresponding $P$ values being below 0.0001.

We also examined the effect of covariation of speed and luminance on the direction tuning curve. Here, we used eight directions separated by $\sim 45^\circ$ and all combinations of three speeds and three luminance levels for sweeping a bright bar across the receptive field. The data came from three cells from three different retinas. A Cartesian plot of the direction tuning curves obtained for all the nine combinations of speeds and luminance levels from the cell that yielded the least noisy data is shown in Fig. 3G. Generally, in accord with our previous findings, the mean number of spikes increased as the speed decreased or as the luminance increased; thus the strongest parameter combination was that of the slowest speed and the highest luminance level, whereas the weakest combination comprised the fastest speed and the lowest luminance level. However, at least for those parameter values that we tested, we found that speed contributed a more profound effect on cell response than luminance. As before, we normalized the direction tuning curves with respect to each one’s peak mean spike count. Figure 3H shows the normalized versions of the curves depicted in Fig. 3G. Following normalization, all of the curves, except for that obtained for the weakest parameter combination, were mapped onto the same generic curve. The weakest combination comprised a special case as it yielded cell activity not different than the spontaneous activity, which resulted in the shape of the corresponding direction tuning curve being extremely dependent on random noise. Despite this exception, the results indicated that the combined effect of both speed and luminance on the direction tuning curve can still be represented by a multiplicative scale factor. Using the similarity index, we assessed for each cell the extent to which the direction tuning curves for different combinations of speeds and luminance levels normalized to one generic curve. Figure 3I shows a histogram of similarity indexes quantified for all the three cells. The similarity was still high, with the minimum correlation of 0.69, the maximum correlation of 0.79, and the median correlation of 0.72, and with all the corresponding $P < 0.0001$. Although these index values were slightly smaller than those in the previous experiments, this reduction was mostly caused by the weakest parameter combination, not yielding any signifi-
When this combination was omitted from computations, the correlations were on average 0.09 higher, with the minimum correlation increased to 0.78, the maximum correlation increased to 0.86, and the median correlation increased to 0.82.

**Speed Tuning Curves**

General characteristics and invariance of speed tuning curves. The mean firing rates of On-Off DS RGCs for the preferred and null directions have been shown to depend on the stimulus speed in a nonmonotonic fashion: they increase for slow speeds, exhibit a peak around 30°/s, and decrease for faster speeds (Grzywacz and Amthor 2007). However, it has also been shown that, in the preferred direction, the spike count (integral of the spike rate) decreases monotonically with speed (Wyatt and Daw 1975). This is possibly because at lower speeds a longer time on receptive field is not offset by a lower firing rate. Here, we investigated speed tuning curves more thoroughly than reported in the literature, employing more directions than just the preferred and null ones.

![Fig. 3. Invariance of direction tuning curves.](image)

A: direction tuning curves of the least noisy cell for five speeds. Curves (solid lines) were determined at 16 directions separated by 22.5° for a bar of the luminance of 57.58 cd/m². B: same direction tuning curves after normalization with reference to the peak of each curve. Similarity index is 0.90. C: histogram of similarity indexes quantified for normalized direction tuning curves determined for different speeds for each of the 9 analyzed cells. Bin width is 0.01 unit, and the highest bin occurs between 0.84–0.85. D: direction tuning curves of one of the least noisy cells for five luminance levels. Curves (solid lines) were determined at 16 directions separated by 22.5° for a bar swept at the speed of 2.84 mm/s. E: same direction tuning curves after normalization with reference to the peak of each curve. Similarity index is 0.90. F: histogram of similarity indexes quantified for normalized direction tuning curves determined for different luminance levels for each of the 8 analyzed cells. Bin width is 0.01 unit and the highest bin occurs between 0.89 and 0.90. G: Direction tuning curves of the least noisy cell for the 9 combinations of 3 speeds and 3 luminance levels. Curves (solid, broken, and dotted lines) were determined for a bar at 8 directions separated by 45°. H: same direction tuning curves after normalization with reference to the peak of each curve. Similarity index is 0.79. I: histogram of similarity indexes quantified for normalized direction tuning curves determined for different combinations of speeds, luminance levels, and for each of the 3 cells. Bin width is 0.01 unit and the similarity indexes are 0.69, 0.72 and 0.79, respectively. In A, D, and G, mean number of spikes for no-stimulus (blank) condition is shown as a reference (broken line with dots), while bars on each line show SE. In B, E, and H, bars on the lines show normalized SE, but for a better visibility they are shown only for a single direction and are slightly jittered along the abscissa.
Fig. 4. Speed tuning curves and their invariance. Speed tuning curves were determined for five speeds (measured in mm/s on the retina) from the same data that were used for the investigation of invariance of direction tuning curves. A: speed tuning curves for 8 almost equidistant directions (reported as an angle in degrees) for the least noisy cell. For reference, mean number of spikes for no-stimulus (blank) condition is also shown (broken line with dots at the bottom). B: same speed tuning curves after normalization with reference to the peak of each curve. Similarity index is 0.86. C: histogram of similarity indexes quantified for normalized speed tuning curves determined for different directions for each of the nine analyzed cells. Bin width is 0.01 unit, and the highest bin occurs between 0.73 and 0.74. D: normalized speed tuning curves for the preferred directions of the 9 analyzed cells. For each curve, normalization was carried out with reference to its peak. In A, bars on the lines show SE, while in B and D, they show normalized SE.
on receptive field, these curve fits imply that, over the limited range of the speeds tested, the spike rate increases with speed.

**Luminance Tuning Curves**

General characteristics and invariance of luminance tuning curves. The responses of On-Off DS RGCs for the preferred and null directions have been shown to depend nonmonotonically on the stimulus contrast: they increase at low contrasts and, after reaching a peak, decrease at high contrasts, both when the mean firing rate (Grzywacz and Amthor 2007) and the mean spike count (Merwine et al. 1998) are considered. Here, we investigated a closely related cell characteristic, namely luminance tuning curves, employing more directions than just the preferred and null ones. We used the same data from the eight cells that were analyzed during the investigation of direction tuning curves at five different luminance levels (as described earlier). This time we plotted the mean spike count vs. luminance for eight almost equidistant directions, including the preferred and the null directions. Figure 6A shows a plot of such luminance tuning curves from the cell that yielded the least noisy data. Generally, as the luminance increased, the mean number of spikes also increased, but in a highly nonlinear fashion: at the low luminance levels it exhibited a rapid rise followed by a plateau indicating saturation at the high luminance levels. Contrary to the results reported by Merwine et al. (1998) for contrast, we did not observe a fall in response at high luminance levels, possibly because the luminance levels that we tested were not high enough to elicit this effect. As in the case of speed, we found that the luminance tuning curves were similar in shape independent of direction, although they differed in maximum and minimum values. Figure 6B shows the same luminance tuning curves normalized with respect to the peak mean spike count for each direction. As with speed, the normalization reduced all the curves to the same generic curve, suggesting that the effect of direction can be represented by a multiplicative scale factor. In the same manner as before, we applied the similarity index to each cell to assess the extent to which the luminance tuning curves

Fig. 6. Luminance tuning curves and their invariance. Luminance tuning curves were determined for five luminance levels (measured in cd/m²) from the same data that were used for the investigation of invariance of direction tuning curves. A: luminance tuning curves for 8 almost equidistant directions (reported as an angle in degrees) for the least noisy cell. For reference, mean number of spikes for the no-stimulus (blank) condition is also shown (broken line with dots at the bottom). B: same luminance tuning curves after normalization with reference to the peak of each curve. Similarity index is 0.80. C: histogram of similarity indexes quantified for normalized luminance tuning curves determined for different directions for each of the 8 analyzed cells. Bin width is 0.01 unit and there is no dominant value. D: normalized luminance tuning curves for the preferred directions of the 8 analyzed cells. For each curve, normalization was carried out with reference to its peak. In A, bars on the lines show SE, while in B and D, they show normalized SE.
for different directions normalized to one generic curve, utilizing for its computation the luminance tuning curves for all the 16 directions available from the data. Figure 6C shows a histogram of the similarity indexes quantified for all the eight analyzed cells. We found that the similarity was generally high, with the median correlation of 0.53 and the maximum correlation of 0.80, except for one outlier that was equal to 0.04 (the second smallest value was 0.42). All the corresponding $P$ values apart from that associated with the outlier ($P = 0.63$) were below 0.0001. The outlier stemmed from the fact that for this particular cell the luminance tuning curves were very noisy and often not monotonic (surprisingly, the similarity index for the corresponding direction tuning curves was as high as 0.75).

We examined whether the characteristics of luminance tuning curves are individual to each cell or instead common across various cells, and to this end we determined the luminance tuning curves for the preferred direction for each of the eight analyzed cells. As in the case of speed, we found them to vary considerably in magnitude due to different excitability in those cells, and likewise, we normalized them with respect to each one’s peak mean spike count. The normalized curves are shown in Fig. 6D, but there is one significant difference from the corresponding plot of speed tuning curves. The speeds at which the responses were measured were exactly the same for all the cells, whereas the luminance levels, apart from the highest one, mostly differed among the cells due to different luminance thresholds and the resultant different partitioning of the available range. Despite this dissimilarity, it appears that all of the normalized luminance tuning curves substantially overlap for all the luminance levels except for the lowest ones, which were close to individual cell thresholds.

**Functional approximation of luminance tuning curves.** We investigated whether the luminance tuning curve can be approximated in a functional form by attempting to fit the normalized luminance tuning curves obtained for the preferred directions from all the eight analyzed cells with three functions that generally resembled their shape: exponential rise (Eq. 7 in **APPENDIX**), rectangular hyperbola (i.e., Michaelis-Menten equation; Eq. 8), and logarithmic (Eq. 9). Figure 7A shows the fit of the exponential rise, whereas the fit of the rectangular hyperbola and the fit of the logarithmic function are shown in Fig. 7, B and C, respectively. The exponential rise yielded the following values of the fitted parameters: gain = 0.78 [95% CI = (0.68, 0.88)], offset = 0.19 [95% CI = (0.09, 0.30)], and rise constant = 0.11 [95% CI = (0.08, 0.15)]. The rectangular hyperbola produced the following parameters: gain = 0.99 [95% CI = (0.84, 1.13)], offset = 0.09 [95% CI = (–0.07, 0.26)], and rate constant = 5.46 [95% CI = (2.43, 8.48)]. The logarithmic function delivered the following parameters: gain = 0.21 [95% CI = (0.18, 0.24)] and offset = 0.18 [95% CI = (0.09, 0.27)]. For all these functions we assessed the goodness of fit in the form of the adjusted $R^2$, which was 0.88 for both the exponential rise and the rectangular hyperbola, and 0.85 for the logarithmic function, respectively. We found that the fits of all the three functions were good, although at the low luminance levels cells had different thresholds resulting in some scatter of the initial points of the individual luminance tuning curves. We also found that the logarithmic function overestimated the luminance tuning curves at the high luminance levels, because, being an increasing function, it was not able to fit their characteristic saturation.

**Response Model**

**Model development.** One important consequence of our finding that the effects of speed and luminance on direction tuning curve could be reduced to multiplicative scale factors, as could the effect of direction on the speed and luminance tuning curves, is that the response measured as the mean spike count is affected by these three stimulus parameters independently, and thus their effects could be multiplicatively separated. Therefore, we attempted to develop a model predicting the mean spike count in On-Off DS RGCs that would be based on a multiplication of three independent functions (Eq. 1 in **APPENDIX**): the first one accounting for the effect of direction (direction factor), the second one accounting for the effect of speed (speed factor), and the third one accounting for the effect of luminance (luminance factor). Drawing from our results on fitting of the tuning curves (as described earlier), we chose the Gaussian function to represent the direction factor, the power function to represent the speed factor, and the exponential rise function to represent the luminance factor. In the light of different cell excitability as well as the known differences in the preferred directions, we assumed in the model that the direction tuning curve should be fitted on an individual cell basis. On the other hand, our findings indicating that the speed...
and luminance tuning curves could be common to all On-Off DS RGCs allowed us to employ in the model the population fits of the speed and luminance tuning curves. In fact, these fits were obtained for the normalized speed and luminance tuning curves, but this was actually desired from the model perspective, as the model assumed that the direction tuning curve would be fitted using that combination of speed and luminance level which yielded the strongest responses.

Model tests. To test our model, we used the same data from the three cells that were analyzed during the investigation of direction tuning curves at the nine combinations of three different speeds and three different luminance levels (as described earlier). It should be noted that none of these cells were utilized for fitting either the normalized speed tuning curves (shown in Fig. 5) or the normalized luminance tuning curves (shown in Fig. 7). For each of the three cells, we performed the following procedure. First, we fitted the Gaussian function to the direction tuning curve obtained for the slowest speed and the highest luminance level, as this combination produced the strongest responses. Second, for each combination of speed and luminance level, we computed model predictions of the mean spike counts for all eight directions tested, using the fits shown in Figs. 5A and 7A. Finally, we compared those predictions with the actual mean spike counts. Figure 8 shows such a comparison for one of the cells (the same cell as in Fig. 3, G and H). The results are divided into separate plots for each combination of speed and luminance levels, each of which contains eight points corresponding to the eight directions. We found that the model was accurate for almost all stimuli. The only deviation from accuracy occurred for the weakest stimuli. Specifically, for all three cells the model overestimated the mean spike counts for the combination of the fastest speed and the lowest luminance level, doing the same for two cells at the combination of the fastest speed and the intermediate luminance level. As mentioned earlier, the combination of the fastest speed and the lowest luminance level comprised a special case, as it yielded cell activity not different than the spontaneous activity. As shown in Fig. 8, except for this one combination at which the stimulus failed to drive the cell, the correlation coefficients were in the range 0.96 – 0.99 (0.99 for 5 of the 8 combinations), indicating that the model captured the data very well.

The summarized results of the model tests on all three cells are shown in Fig. 9. Figure 9A shows a comparison between the predicted spike counts vs. the mean spike counts for all the combinations of speeds and luminance levels separately for each cell. To quantify the accuracy of the predictions, for each cell we computed a linear correlation between the predictions and the actual mean spike counts individually for every combination of speeds and luminance levels. The spreads of the Pearson product-moment correlation coefficients are shown in Fig. 9B. We found these coefficients to be generally close to 1 (mean for the 3 cells of the median r = 0.98; P < 0.0001). For each cell, we also performed a linear regression analysis, again considering each combination of speeds and luminance levels individually. Figure 9C shows the spreads of the slopes of the linear regression, which we found to be generally close to 1 (mean median slope = 1.08). Figure 9D, in turn, shows the spreads of the intercepts of the regression, which were usually close to 0 (mean median intercept = 0.15).

Figures 8 and 9 demonstrate that a multiplicatively separable model in which the speed and luminance factors are based on population fits while the direction factor is fitted on an individual cell basis provides a very good account of the first-order measure of On-Off DS RGC responses over a substantial range of speeds and luminance levels. Consequently, directionality is invariant up to a scale factor determined by the product of speed and luminance factors.

Luminance vs. contrast. Although we developed our model employing luminance rather than contrast, we also examined whether the effect of direction is separable from the effect of contrast. Here, we varied contrast and direction systematically, using a mid-gray background (of luminance 28.62 cd/m²) and a bar that was either lighter or darker than the background. Three negative and three positive contrasts were employed for the bar, which was swept in 16 directions separated by 22.5° at a constant speed (2.84 mm/s as measured on the retina, equivalent to 16.42°/s) across the receptive field. Other stimulus parameters except direction and contrast were held constant. The data came from nine cells from eight different retinas. Figure 10A shows a Cartesian plot of the direction tuning curves obtained for the various contrasts from the cell that produced the least noisy data. Generally, as the absolute contrast increased, the mean number of spikes also increased, and this outcome was independent of direction (except for the
null direction, for which the number of spikes was almost equal at all the contrasts). This result is similar to that yielded by luminance variation (see Fig. 3D). Figure 10B shows the same direction tuning curves normalized with respect to the peak mean spike count for each contrast. As a result of this normalization, all the curves reduced to the same generic curve, which indicated that the effect of contrast on the direction tuning curve can be represented by a multiplicative scale factor just as the effect of luminance can (see Fig. 3E).

For each cell, we also assessed the extent to which the direction tuning curves for different contrasts normalized to one generic curve by applying the similarity index. Figure 10C shows a histogram of the similarity indexes quantified for all the nine cells. We found that the similarity was high, with the minimum correlation of 0.54, the maximum correlation of 0.84, and the median correlation of 0.80, and with all the corresponding $P$ values below 0.0001, although we noticed that these index values were slightly smaller than those obtained for luminance variation (see Fig. 3F). Especially, the minimum correlation was comparatively low, but this outcome was explained by the fact that for this particular cell the weakest positive and the weakest negative contrasts yielded activity not different than the spontaneous activity, which resulted in the shapes of the corresponding direction tuning curves being extremely dependent on random noise. When these two contrasts were omitted from computations for this cell, the correlation increased to 0.82.

DISCUSSION

In this study, we quantitatively investigated how three parameters of a moving stimulus, namely its direction, speed, and luminance, affect the mean spike count responses of On-Off DS RGCs in rabbits. We thus addressed in these cells a portion of the encoding problem involving the interactions among the three parameters. This investigation was motivated by the question of whether, given the exquisite preferred-null axial selectivity for direction exhibited by On-Off DS RGCs on the one hand but the general ambiguity of their responses about the
On-Off DS RGCs. Moreover, we confirmed previous findings that direction tuning curves appear to be common across the whole population of these cells and that the effects of these three parameters are multiplicatively separable; and that the effect of the nondirectional parameters is multiplicatively separable from the effect of direction. A population code subject to the above assumptions presents an additional advantage; namely, it permits isotropic estimates of direction in the presence of noise, that is estimates without systematic errors (unbiased) and with similar random errors (equally accurate) in any direction (van Hateren 1990).

**Direction Decoding Problem and Population Coding**

Van Hateren (1990) indicated that when responses of visual movement detectors also depend on nondirectional stimulus parameters, such as contrast and speed, the output of a single detector does not permit the precise determination of the stimulus direction because the effects of the nondirectional parameters confound the information on direction. However, a population code in which at least two detectors are used can resolve the ambiguity as direction can be uniquely represented by the ratio of their responses. This is possible when at least two detectors in the population meet the following criteria: 1) each detector has a different preferred direction, and these preferred directions are sufficiently separated from each other (otherwise the ratio of responses may change very little over a range of directions); 2) the detectors have overlapping direction tuning curves, meaning that these curves must be sufficiently broad; 3) the direction tuning curves vary for different directions (because constant functions will not yield a change in their ratio for different directions); 4) responses of the detectors depend in the same manner on the nondirectional stimulus parameters; and 5) the effect of the nondirectional parameters is multiplicatively separable from the effect of direction. A population code subject to the above assumptions presents an additional advantage; namely, it permits isotropic estimates of direction in the presence of noise, that is estimates without systematic errors (unbiased) and with similar random errors (equally accurate) in any direction (van Hateren 1990).

**Broad Direction Tuning**

In this study, we confirmed previous findings that direction tuning curves of On-Off DS RGCs are broad and appear to be smooth, monotonic, and symmetrical functions of direction away from the preferred direction (Barlow et al. 1964; Levick et al. 1969). Although we found some spread in direction tuning width, the distribution appeared to be normal, meaning that there were no systematic differences across different cells. Moreover, the tuning widths seemed not to be related to the cell health condition because tuning width did not correlate with overall excitability.

Given that the preferred directions of On-Off DS RGCs cluster in four groups, roughly corresponding to the anterior, posterior, superior, and inferior motion in the field of view (Oyster 1968; Oyster and Barlow 1967), curves with these characteristics appear to meet three of the criteria set forth by van Hateren (1990). First, the four preferred directions are distinct and separated by ~90°. With the direction tuning width taken into account, this separation is sufficient to avoid constant response ratios. Second, the distribution of the preferred directions and the direction tuning width imply that direction tuning curves belonging to different groups are sufficiently broad to overlap with one another. Finally, the direction tuning curves vary with direction in a well-behaved manner as determined by their shape. Consequently, broad direction tuning of On-Off DS RGCs does not seem an impediment from the perspective of direction coding but, on the contrary, appears as an advantage facilitating population coding of direction in these cells.

**Fig. 10. Invariance of direction tuning curves with respect to sign of contrast.** Direction tuning curves were determined for 3 positive and 3 negative contrasts (calculated as Weber contrasts) at 16 directions separated by ~22.5° for a bar swept at the speed of 2.84 mm/s against a mid-gray background of luminance 28.62 cd/m². A: direction tuning curves (solid lines) of the least noisy cell. Mean number of spikes for no-stimulus (blank) condition is shown as a reference (broken line with dots). Bars on each line show SE. B: same direction tuning curves after normalization with reference to the peak of each curve. Bars on the lines show normalized SE, but for a better visibility they are shown only for a single direction and are slightly jittered along the abscissa. Similarity index is 0.84. C: histogram of similarity indexes quantified for normalized direction tuning curves determined for different contrasts for each of the 9 cells. Bin width is 0.01 unit, and the highest bin occurs between 0.82–0.83.
Universal Nondirectional Tuning Curves, Separability, and Hypothesis of “Equivalent Contrast” Normalization

Our finding that all the On-Off DS RGCs that we studied shared the same speed and luminance tuning curves accords with another criterion set forth by van Hateren (1990). Since our sample consisted of cells located at various eccentricities in both inferior and superior retina, we may assume that these curves are indeed universal and not just common to cells in a specific region. However, from the perspective of direction decoding, universal tuning of all the nondirectional stimulus dimensions is not strictly required. Instead, what the criterion implies is that the tuning of every nondirectional parameter should be common within each local population, which, in turn, would allow some variability among different populations, for example, at different retinal locations. This relaxation could affect such stimulus dimensions that may be, for instance, closely related to the receptive field size. This cell characteristic is actually known to vary regionally: the receptive fields are larger in On-Off DS RGCs at higher eccentricities than those located centrally (Levick 1967); within a given region, however, their sizes appear to be similar. Therefore, although we examined the tuning curves for those two parameters only, we believe that a similar outcome is likely to apply to other nondirectional stimulus dimensions, either in a truly universal or locally restricted manner.

The last criterion set forth by van Hateren (1990) is addressed by another finding of ours, namely that the effects of stimulus direction, speed, and luminance were multiplicatively separable. Again, we examined only these three parameters of a moving stimulus out of the many possible, but we believe that the separability of the effect of direction from the effects of all the other nondirectional parameters not considered in our study also holds true. As before, however, it is important to emphasize that from the perspective of direction decoding it is not necessary for each nondirectional parameter to be completely separable from all the other parameters. What is essential is that the combined effects of all the nondirectional parameters be separable from the sole effect of direction (see later discussion on selectivity of temporal vs. spatial frequencies).

The above findings together with the idea, supported by some previous evidence (Amthor and Oyster 1995; Oyster 1968; Oyster and Barlow 1967), that every point in the field of view is projected onto the receptive fields of exactly four On-Off DS RGCs, each preferring a different cardinal direction, lead us to the following hypothesis of “equivalent contrast” normalization. It states that for a quartet of cells with receptive fields at a given locus all the nondirectional stimulus parameters, such as contrast, speed, or size, can be lumped together into one “equivalent contrast” super-parameter, accounting for their combined effects. Consequently, this “equivalent contrast” super-parameter could be subsequently isolated from the only parameter that is strongly different among such a local population of On-Off DS RGCs, namely their direction preference, which, in turn, would permit extraction of the stimulus direction. This extraction, as proposed by van Hateren (1990), could be achieved by comparing ratios of responses from the four cells in the population or by other straightforward computations.

Luminance vs. Contrast

Although we developed our multiplicatively separable model based on luminance tuning curves rather than contrast tuning curves, we expect that similar results would be obtained when using the latter, for the following reasons. First, our data from the experiments involving positive and negative contrasts show that direction tuning curves normalize to the same generic curve irrespective of the amount or sign of contrast (see Fig. 10), which indicates that the effect of direction is separable from the effect of contrast in the same manner as it is separable from the effect of luminance. Second, On-Off DS RGCs are generally equally responsive and equally DS for light and dark stimuli (Barlow and Levick 1965; Taylor and Vaney 2002). Third, these cells exhibit similar dendritic ramifications in the inner and outer plexiform layers (Amthor et al. 1984, 1989).

Unlike the mean spike count speed response, which varied over a wide range of speeds, the mean spike count luminance response varied over only a narrow range of luminance values. Consequently, as luminance saturated above some moderate value, it did not contribute to the response magnitude. Luminance thus behaves as a compressive function, and as a result it permits more of the channel capacity to be devoted to motion-related stimulus dimensions. This finding is similar to what is seen in cortical area MT for contrast (Sclar et al. 1990). Indeed, several similarities between the responses of On-Off DS RGCs and MT cells in primates have been observed (Grzywacz and Amthor 2007), and there is also some evidence in primates for a direct projection from lateral geniculate nucleus to area MT, bypassing V1 (Sincich et al. 2004).

We note that, contrary to the results reported by Merwine et al. (1998) for contrast, we did not observe a fall in response magnitude at high luminance levels. This, however, may be explained by the possibility that the luminance levels that we tested were not high enough to elicit this effect.

Other Stimulus Dimensions and Types

If On-Off DS RGCs indeed utilize “equivalent contrast” normalization for coding stimulus direction, it should perform universally across various stimulus dimensions and types. Here we examined only one type of stimulus, namely a bar, and only two nondirectional stimulus parameters, namely speed and luminance. Therefore, the question whether similar results could be obtained for other stimulus parameters, such as size or shape, as well as other kinds of stimuli, such as gratings, dot, or texture patterns, remains open.

There is some evidence indicating that the answer would be positive. For instance, Grzywacz and Amthor (2007) have shown that directional selectivity of On-Off DS RGCs appears quantitatively similar for both spots and sinewave gratings. However, some other evidence suggests that it may not be so straightforward. Hammond and Smith (1983), for example, have shown in complex cells of cat striate cortex that direction tuning curves may be subject to change depending on the exact stimulus configuration. They have shown that the preferred direction for a bar and for a texture in the same cell may be different, the direction tuning curves for these two stimuli may have different bandwidths, and the shape of the direction tuning curve for texture alone is affected by speed. Some findings suggest that similar phenomena may occur in On-Off...
DS RGCs. Grzywacz and Amthor (2007) have observed in a proportion of On-Off DS RGCs a splitting of the preferred direction into two “horns” off the original preferred-null axis for sinusoidal gratings at high spatial frequencies. They proposed an explanation that at spatial frequencies higher than optimum a grating oriented off-axis has a projection on the original preferred-null axis at a lower equivalent spatial frequency, closer to the optimum, and thus evokes a stronger response than the same grating oriented on-axis. A similar splitting of direction tuning curves for textures at high speeds has been observed by Hammond and Smith (1983) in their study on cat complex cells, again only in a proportion of cells, but an analogous explanation, although considered, was rejected in that study. Grzywacz and Amthor (2007) have also found the bandwidths of speed tuning of On-Off DS RGCs for a grating and for a spot to be slightly different, the latter being somewhat broader. Moreover, they reported that responses of On-Off DS RGCs to drifting gratings of varying spatial and temporal frequencies are roughly separable and not oriented along an isospeed contour in spatial frequency vs. temporal frequency contour plots. Those plots indicate that these cells are tuned to a particular spatial frequency irrespective of temporal frequency (see also He and Levick 2000), and hence they are not strictly speed tuned. Consequently, selectivity for speed is not an invariant characteristic of the responses of these cells since the optimum speed changes as a function of stimulus composition. From this perspective, On-Off DS RGCs are similar to other DS cells in the higher visual centers, such as V1, which also show spatiotemporal separability rather than speed invariance (Holub and Morton-Gibson 1981; Ikeda and Wright 1975; Tolhurst and Movshon 1975). Nevertheless, although the optimum speed varies with stimulus composition, this does not preclude the use of “equivalent contrast” normalization to extract direction from a local population of On-Off DS RGCs all experiencing the same complex stimulus.

Another concern regarding a universal performance of the “equivalent contrast” normalization is related to the fact that a single bar that we used as a stimulus is not very representative for natural scenes since natural sensory inputs usually do not consist of isolated simple patterns (Felsen and Dan 2005). Therefore, it would be compelling to confirm the “equivalent contrast” normalization using movies from rabbit’s natural environment. Likewise, we did not consider here an issue concerning complex effects of adaptation and relative motion between local area and background (Chiao and Masland 2003; Olivczky et al. 2003, 2007), which also should be addressed.

Consequently, the stimulus regimes under which “equivalent contrast” normalization might be valid are still to be thoroughly investigated.

Spike Count as a Measure

In this study, we used mean spike count as the measure of cell responses, but there are other characteristics of responses of On-Off DS RGCs that could conceivably figure in the refinement of coding for direction. This is because different response measures can yield somewhat different tuning profiles, and thus the same response can be simultaneously characterized in more than one dimension, which, in turn, may facilitate coding precision. For instance, Wyatt and Daw (1975) have demonstrated such a change in tuning profile in On-Off DS RGCs for speed examined at the preferred direction by showing that as the total number of spikes decreases monotonically when the stimulus speed increases, at the same time the peak firing rate increases for low speeds, reaches a maximum ~30/s, and then decreases. They concluded that although it was not clear which response measure is better for characterizing cell activity, the peak firing rate is the one that subsequent neurons can analyze in realistically short time intervals. Amthor et al. (2005) have described another example of coding strategy exhibited by On-Off DS RGCs, namely correlated firing. They have found that synchronous spikes among neighboring On-Off DS RGCs carry additional information about moving stimuli extending across receptive fields of these cells, indicating that the cells are responding together to a contour of a common object. Correlated firing for contours in this and other classes of retinal ganglion cells has been further elaborated by Chatterjee et al. (2007). Such coding could conceivably be robust in the cases where our model was weakest, that is for fast speed or low contrast stimuli near threshold. Lastly, there is evidence that neither total spikes nor firing rate convey all the information about a stimulus that is encoded by ganglion cells (Jacobs et al. 2009).

The limitation of using spike counts is clearly problematic for assessment of speed tuning, for several reasons. First, counting the total spikes produced by the passage of a discrete stimulus effectively means different integration times for different speeds. This problem gets further complicated by the fact that for the same speeds large textured objects yield responses that last longer than those elicited by small objects, meaning that speed becomes confounded with object size in a spike count. Second, reliable inference by the brain on such rapidly changing stimulus parameters as speed requires use of an “instantaneous” firing rate rather than a mean firing rate, as otherwise decoding of those parameters could become corrupted by changes in the visual scene over long periods.

Model Limitations

Although our multiplicatively separable model generally predicted mean spike counts with a remarkable accuracy, it systematically failed for the weakest stimuli (comprising the combinations of the fastest speed and the lowest or the intermediate luminance level), for which it yielded predictions larger than the corresponding data. As a possible solution, we expect that a simple extension of the model employing a low-response threshold-like nonlinearity could improve its performance for such stimuli. This nonlinear stage would follow the current multiplicatively separable stage, the output of which for the weakest stimuli might often be below the threshold. Consequently, the small predictions obtained for these stimuli from the multiplicatively separable stage would be transformed into even smaller overall predictions, which, in turn, would make the model closer to the data.

Another limitation of our model stems from the fact that it was developed based on neuronal tuning curves, which are derived from responses to the same stimulus averaged over multiple presentations. In the real world, however, the brain does not need repetitions of stimuli to process them but instead operates on their single occurrences. Therefore, if separability of stimulus parameters is to be utilized for direction decoding, it should also be applicable on a single trial basis. This issue is
not addressed here, neither is a related problem of noise present in response magnitude over different trials.

Conclusions

Notwithstanding the above considerations, the findings reported here substantiate two important conclusions. First, the precise preferred-null axial discrimination but broad direction tuning otherwise makes sense if On-Off DS RGCs convey information on stimulus direction by means of a population code. Moreover, such an arrangement might offer an additional advantage in terms of minimizing the number of axons in the optic nerve necessary to transmit this information to the higher visual centers in the brain. Direction representation in these centers could then be transformed from fewer broadly tuned On-Off DS RGCs to a larger number of more narrowly tuned DS cells in some combinatorial coding scheme (Osborne et al. 2008). Second, separability of parameter encoding by On-Off DS RGCs would allow the higher visual centers to normalize the effects of the nondirectional stimulus parameters through comparing ratios of the responses from the cells in a population or by other straightforward computations. Assuming that such a population comprises a quartet of orthogonally tuned On-Off DS RGCs with overlapping receptive fields, this could give rise to discrimination of many more than the four directions preferred by those cells. This idea is analogical to color perception, where hundreds of colors can be discriminated from just three photoreceptor classes.

Finally, we conclude that these findings add a strong support to the concept that On-Off DS RGCs in rabbits use a population code for signaling direction, which, in turn, would simplify direction decoding by the higher visual centers in the brain.

APPENDIX

General Form of the Model

We investigated a multiplicatively separable model of responses of On-Off DS RGCs in the following form:

\[ r = f(d) \cdot g(s) \cdot h(l), \]

where \( r \) denotes cell response expressed as mean number of spikes, \( f \) denotes function representing the effect of direction \( d \), \( g \) stands for function representing the effect of speed \( s \), and \( h \) stands for function representing the effect of luminance \( l \).

Direction Fitting

For derivation of the function representing the effect of direction, we fitted direction tuning curves with three functions: mirror-symmetric Archimedes’ spiral, cosine, and Gaussian. All these functions depended on a single variable \( d \) denoting stimulus direction [measured in degrees, \( d \in (0,360) \)]. The mirror-symmetric Archimedes’ spiral function employed three parameters: \( a \) (gain), \( b \) (offset), and \( d^* \) (preferred direction), and took the following form:

\[ f_d(d) = a \cdot \frac{180 - |d - d^*|}{2c^2} + b. \]  

The cosine function employed the same three parameters: \( a \) (gain), \( b \) (offset), and \( d^* \) (preferred direction), and took the following form:

\[ f_c(d) = a \cdot \cos \left( \frac{\pi}{180} \cdot (d - d^*) \right) + b. \]

The Gaussian function employed four parameters: \( a \) (gain), \( b \) (offset), \( c \) (tuning width; expressed in the form of SD), and \( d^* \) (preferred direction), and took the following form:

\[ f_g(d) = a \cdot \exp \left( - \frac{180 - |180 - |d - d^*||^2}{2c^2} \right) + b. \]  

Additional expressions involving absolute values and the number 180 in the formula of the Gaussian function were used to ensure mirror symmetry of this function in both hemi-planes from the preferred direction.

Speed Fitting

For derivation of the function representing the effect of speed, we fitted speed tuning curves with two functions: power and exponential decay. Both functions depended on a single variable \( s \) denoting stimulus speed (measured in mm/s on the retina). The power function employed three parameters: \( a \) (gain), \( b \) (offset), and \( c \) (exponent), and took the following form:

\[ g_p(s) = a \cdot s^c + b. \]

The exponential decay function also employed three parameters: \( a \) (gain), \( b \) (offset), and \( c \) (decay constant), and took the following form:

\[ g_e(s) = a \cdot \exp(-c \cdot s) + b. \]

Luminance Fitting

For derivation of the function representing the effect of luminance, we fitted luminance tuning curves with three functions: exponential rise, rectangular hyperbola, and logarithmic. All these functions depended on a single variable \( l \) denoting stimulus luminance (measured in cd/m²). The exponential rise function employed three parameters: \( a \) (gain), \( b \) (offset), and \( c \) (rise constant), and took the following form:

\[ h_r(l) = a \cdot (1 - \exp(-c \cdot l)) + b. \]

The rectangular hyperbola also employed three parameters: \( a \) (gain), \( b \) (offset), and \( c \) (rate constant), and took the following form:

\[ h_r(l) = a \cdot \frac{l}{c + l} + b. \]

The logarithmic function employed two parameters: \( a \) (gain) and \( b \) (offset), and took the following form:

\[ h_l(l) = a \cdot \ln(l + 1) + b. \]

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DISCLOSURES

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