Spike train signatures of retinal ganglion cell types

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Abstract
The mammalian retina deconstructs the visual world using parallel neural channels, embodied in the morphological and physiological types of ganglion cells. We sought distinguishing features of each cell type in the temporal pattern of their spikes. As a first step, conventional physiological properties were used to cluster cells in eight types by a statistical analysis. We then adapted a method of P. Reinagel et al. (1999: J. Neurophysiol., 81, 2558–2569) to define epochs within the spike train of each cell. The spike trains of many cells were found to contain robust patterns that are defined by the (averaged) timing of successive interspike intervals in brief activity epochs. The patterns were robust across four different types of visual stimulus. Although the patterns are conserved in different visual environments, they do not prevent the cell from signaling the strength of its response to a particular stimulus, which is expressed in the number of spikes contained in each coding epoch. Clustering based on the spike train patterns alone showed that the spike train patterns correspond, in most but not all cases, to cell types pre-defined by traditional criteria. That the congruence is less than perfect suggests that the typing of rabbit ganglion cells may need further refinement. Analysis of the spike train patterns may be useful in this regard and for distinguishing the many unidentified ganglion cell types that exist in other mammalian retinas.

Introduction
An early step in vision is the decomposition of the visual input by the retina into separate neural channels, each tuned to a particular aspect of the visual scene. On anatomical grounds, the number of channels in mammalian retinas generally seems to be around 12. The attempt to differentiate the sensory coding of these ganglion cells has a long history (Masland, 2001; Wasse, 2004). In the classic characterizations the cells were classified by a qualitative, teleologically based, description of the responses (Kuffler, 1953; Lettvin et al., 1959; Hubel & Wiesel, 1960; Barlow & Levick, 1965; Levick, 1967; Levick & Thibos, 1983). The first persuasive attempt to replace these informal criteria used grating stimuli, which for some of the simpler cell types provide a powerful and concise way to characterize them (Enroth-Cugell & Sharpey, 1973; Hochstein & Sharpey, 1976). Those cells possess a simple receptive field structure (Kuffler, 1953), and their response to laboratory stimuli can be predicted using a linear model followed by a static non-linearity and Poisson-like firing statistics (Berry et al., 1997). However, these methods are generally less successful when confronted with cells that have complex, non-linear properties such as direction-selective (DS) cells, local edge detectors or uniformity detectors (Levick, 1967; Carandini et al., 2005). This is in large part because of the problem of stimulus sets; it has not been easy to find stimuli that are analytically tractable, such as a white-noise stimulus, and at the same time representative of the statistical complexity of the natural visual world (Carandini et al., 2005; Felsen & Dan, 2005; Rust & Movshon, 2005).

One of the first attempts to overcome stimulus limitations classified ganglion cells in the rabbit (DeVries & Baylor, 1997) using the temporal structure of the spike trains (the autocorrelation function) in response to dim light. It was suggested that autocorrelation functions are characteristic of retinal ganglion cell types but the spike trains were only evaluated qualitatively. This approach was expanded in a recent study in the salamander retina by Segev et al. (2006). Based on the spike train autocorrelation function alone, three broad cell classes were identified by a quantitative cluster algorithm; the number of clusters increased to six, taking into account the spatial integration properties of the ganglion cells. Here, we sought a stimulus-independent way to identify microstructures in the spike trains of rabbit retinal ganglion cells. We began by identifying cells according to a set of traditional criteria; the cells were subjected to a quantitative statistical cluster analysis on the basis of traditionally used response properties (Barlow & Levick, 1965; Levick, 1967). We then analysed spike trains in response to different stimulus sets, including spontaneous activity in dim light, artificial white noise or more naturalistic stimuli. We found that a stimulus-invariant firing pattern exists in the spike train of each of these types of retinal ganglion cells, mainly defined by the timing of successive interspike intervals (ISIs) in brief activity epochs.

The spike train patterns taken by themselves could be used to separate the cells into types, and most of the new clusters corresponded to types identified by conventional criteria in the pre-classification. However, an overlapping fraction suggests additional types, not described by conventional criteria. This study did not attempt an exhaustive analysis, which would require a finer pre-classification and a much larger sample of cells. It does represent a demonstration of principle, suggesting that an exhaustive analysis of the ganglion cell population would be both possible and physiologically meaningful.
Materials and methods

Experiments were performed on whole-mount retinas in accordance with the animal use committee of the Massachusetts General Hospital. Procedures have been described previously (Yang & Masland, 1994; Koizumi et al., 2004). Briefly, rabbits and mice were anesthetized by intramuscular injection of ketamine (30–100 mg/kg) and xylazine (5–10 mg/kg), and killed by an overdose of pentobarbital (200 mg/kg). The enucleated eye was hemisected and the retina peeled off the pigment epithelium. The retina was superfused with oxygenated Ames medium at 35 °C and mounted, ganglion cell side down, on a multielectrode array (Multichannel Systems, Reutlingen, Germany). The recorded rabbit ganglion cells were located in the mid-periphery, ~10 mm inferior to the myelinated fiber bundles.

The experimental setup and recording technique have been described in a previous report (Zeck et al., 2005). Briefly, the activity of ganglion cells in a small patch of the retina (about 200 × 200 μm²) was recorded via electrodes spaced 30 μm apart. Each electrode was connected to its own differential pre-amplifier (1200× gain, bandpass filter 10–3000 Hz). Extracellular waveforms were recorded (DSP signal processors, Cyberkinetics, Foxborough, MA, USA) when their amplitude exceeded a threshold set at 2.5 SD above mean noise level. The waveforms of 1.5 ms duration were stored at 33 l/s time-resolution. Typically each electrode recorded waveforms from more than one cell and each cell was recorded on several adjacent electrodes. A supervised neural network algorithm (Spike Sorter IV, Cyberkinetics) separated the waveforms and performed a K-means analysis was performed using user-written routines in MATLAB.

Light stimuli

Visual stimuli were generated using visionworks software (Vision Research Graphics, Durham, NH, USA) and projected with a calibrated cathode ray tube monitor (Dell P780). The stimulus was reflected via a substage mirror and focused through a 20× objective (LCF Plan FI NA 0.4, Olympus Optical, Japan) onto the multielectrode array. The light entered the retina through the array substrate, which had transparent indium tin-oxide leads, from the ganglion cell side.

Pseudo-random flickering checkerboard

The receptive field was mapped using a 16 × 16 pseudo-random flickering checkerboard stimulus; the luminance of each square was independently modulated by an m-sequence (Reid et al., 1997). The light intensities at the position of the retina ranged from 0 to 2 cd/m². The update rate of the checkerboard frames was 50 Hz and the width of each square was either 55 or 75 μm (the total size of the stimulating field was therefore 880 or 1200 μm²). The size of the receptive field of each cell was calculated by reverse correlating stimulus and spike response, and considering checkers whose intensity at the temporal maximum of the mean effective stimulus exceeded by a factor of 3 the SD of the squares in the background (DeVries & Baylor, 1997).

Area–response protocol

Once we knew the location of the cell’s receptive field we flashed a series of spots of different sizes centered onto these cells (Carcieri et al., 2003; Zeck et al., 2005). The small sampling area (~2 patches of ~0.04 mm² per recording) allowed spots to be presented centered at two locations only. For each location we presented two protocols, either spots with higher intensity than background or spots with lower intensity than background. The spots were presented for 2 s interleaved with a homogeneous background for 8 s. We counted the spikes in the whole presentation time and normalized to the maximum average rate individually. The first protocol excited ON and ON–OFF cells, whereas the second protocol excited OFF and ON–OFF cells.

Full-field flicker stimulus

A third stimulus was a 30 s white-noise full-field flicker stimulus (temporal flat power spectrum in the 1–30 Hz range). The stimulus was repeated between 20 and 30 times.

Moving grating

Direction selectivity was tested by using a square-wave spatial grating moved in eight equally separated directions. For each direction the grating (spatial frequency 1 cycle/mm) was presented for 6 s at a temporal frequency of 1 Hz followed by a stimulus-free interval of the same length. The total stimulus length ranged from 600 to 1200 s. The spatial extent of the moving grating was generally ~2500 μm on the retina. For one protocol (Fig. 7) it was confined to a diameter of 500 μm, approximating the diameter of the receptive fields of the direction-selective (DS) cells.

Movie clip

In addition to laboratory stimuli, we also used a movie clip (van Hateren & Ruderman, 1998). This movie was taken from a car moving through Groningen on a quiet, wintry Sunday morning. Major features of the movies are vertical orientations (trees, buildings, etc.) moving at different speeds. A total of 31 cells comprising all cell types were stimulated with a 192 s clip repeated five times.

Cell classification and clustering using the Gaussian mixture model approach

A quantitative statistical cluster analysis was performed based on the characterization of each cell by the following four indices: direction selectivity index, bias, response duration and inhibitory index.

Direction selectivity index D

The measure of directional tuning was calculated from the cells’ responses to the moving grating protocol, as described above. We calculated the average firing rate for each of the eight equally separated directions. D was defined for the action potential discharges as suggested by Taylor & Vaney (2002)

\[ D = \sum_{i=1}^{N} \frac{\bar{n}_i}{n_i} \]

where \( n_i \) are vectors pointing in the direction of the stimulus and having length \( r_i \), equal to the number of spikes recorded during the stimulus presentation. The direction selectivity index ranges from 0, when the average firing rates are equal in all stimulus directions, to 1, when a cell response is measured for one stimulus direction only.
Bias index and response duration

These parameters were calculated from the cells’ responses to an optimal spot in the area–response protocol, as described by Carcieri et al. (2003). For each cell we selected the spot size that gave the largest response. The maximal response amplitude $A_1$ (spikes/20 ms interval) to this spot was compared with the maximal response $A_2$ after the spot presentation, which was a homogeneous gray background. The bias index is then defined as $(A_1 - A_2) / (A_1 + A_2)$. For ON–OFF cells the two values are similar and the bias index is close to 0. For ON cells the bias index is positive, whereas for OFF cells it is negative. This holds true for stimuli brighter than background. The response duration is similar and the bias index is close to 0. For ON cells the bias index is positive.

Inhibitory index

Previous studies have shown that local edge detectors are much more strongly inhibited by large bright spots than are sustained cells (Zeck et al., 2005; van Wyk et al., 2006). In contrast, it has been reported that uniformity detectors are not inhibited by stimuli exceeding their receptive field center (Levick, 1967). The effect of stimuli larger than optimal can be quantified by an inhibitory index calculated by a linear approximation to the tail distribution in the area–response curves. If the averaged firing rate (number of spikes/2 s) decreases by a factor of 2 for a spot stimulus twice as large in diameter as optimal the inhibitory index is −1.

As the four indices differ in their units and range each value was normalized for the whole cell population to a mean of 0 and SD of 1.

Cluster analysis was performed using a Gaussian mixture model (Duda & Storck, 2000). In this approach the probability density function $f$ of a ‘data point’ $x$ (e.g. the vector $x$ represents one cell parameterized by the abovementioned four indices) is modelled as a sum of Gaussian functions $f(x \mid j)$.

$$f(x) = \sum_{j=1}^{k} f(x \mid j) \times P(j)$$

with

$$f(x \mid j) = \frac{1}{\sqrt{2\pi}\sigma_j} \times \exp \left( -\frac{(x - \mu_j)^2}{2\sigma_j^2} \right)$$

the Gaussian probability distribution of parameter $x$, mean $\mu_j$ and SD $\sigma_j$. For the four-dimensional case $\sigma$ in Eqn 3 becomes the covariance matrix and the mean $\mu_j$ a four-dimensional vector. $K$ is the number of components in the mixture models and $P$ is the mixture proportion of component $j$.

In this study $K$ is the (optimal) number of clusters and the mixture proportion $P(j)$ denotes the percentage of cells in cluster $j$. By definition, $\sum_{j=1}^{K} P(j) = 1$. To estimate the optimal parameters $\mu_j, \sigma_j$ and $P(j)$ we minimize the (negative log-) likelihood, defined as

$$L(\mu_j, \sigma_j, P(j)) = -\sum_{x} f(x)$$

where $f$ depends on $\mu_j$ and via $\sigma_j$ Eqs 2 and 3 and the summation is performed for all data points $x$.

A solution can be derived by means of the expectation maximization (EM) algorithm (Dempster et al., 1977). We further introduce a partial membership $p_{mi}(j)$ of a data point $x_i$ belonging to cluster $j$ given the current parameters $\mu_j, \sigma_j$ and $P(j)$. (The partial membership is $\sim 1$ if a data point can be assigned with high confidence to one cluster and 0.5 if the data point lies exactly between the centers of two clusters.) Once the partial memberships are known the mixing proportion for each cluster is given by

$$P(j) = \frac{\sum_{i} p_{mi}(j)}{N}.$$ 

In the maximization step we fix the partial memberships and update the parameters $\mu_j$ and $\sigma_j$. In the expectation step we update the partial membership $p_{mi}(j)$ for each data-point (E-step) in each constituent distribution $f$ again by minimizing Eqn 4, keeping $\mu_j$ and $\sigma_j$ constant.

For the implementation of the EM algorithm see Duda & Storck (2000). As a side note we mention that, in the case of binary partial memberships ($pm = \{0, 1\}$), the presented algorithm would be equivalent to K-means clustering.

To ‘visualize’ this brief description of the EM algorithm applied to parameter estimation for a Gaussian mixture model we present in Fig. 1 the histogram of the direction-selective indices $D$. The probability distribution $f$ of the DS indices can be written as the sum of three Gaussian distributions, with means of −0.3, 0.6 and 0.2, respectively.

In our clustering approach each cell was represented by four indices as described above. The initial conditions from which the clustering started were randomly chosen, with the boundaries given by the extreme values of the above-measured indices. The range for the direction selectivity indices is the interval [0 1] for the bias index [−1 1], response duration [10–2000 ms] and inhibitory index [−4 0], respectively. It should be noted that the EM algorithm for the Gaussian mixture model is not convex and that it is therefore possible to end up in a local, rather than a global, minimum of the log-likelihood. To rule out this possibility, we repeated the clustering

$$f(x) = \sum_{j=1}^{k} f(DS\text{index} \mid j) * P(j)$$

![Fig. 1](image-url)
using multiple different random initial conditions and selected those cluster means with the lowest clustering cost, i.e. with the minimal log-likelihood (Eqn 4).

The Gaussian mixture model groups the data in a pre-determined number of clusters. To estimate the reasonable number of clusters \( K \) we selected the best overall clustering from the best clustering for each possible number of clusters. Our strategy was to optimize the average silhouette value for all points (Kaufman & Rousseeuw, 1990). The silhouette value \( s \) for a single point \( i \) in cluster \( K \) is defined as:

\[
S(i) = \frac{\min_{j \neq k} b(i,j) - a(i)}{\max_{j \neq k} \min \{ b(i,j), a(i) \}}
\]

where \( a(i) \) is the average distance from point \( i \) to all other points in cluster \( j \) and \( b(i,j) \) is the average distance from point \( i \) to all other points in cluster \( j \) excluding cluster \( k \). This is a ratio of the closeness of a point to the other points in its own cluster vs. the points in the nearest cluster.

We calculated the mean silhouette value for all cells after grouping them in a number of clusters between 4 and 12. It is widely accepted that in the rabbit retina there are at least four different cell types. The number of 12 cells may seem conservative but is mainly attributed to the restricted number of 81 cells. A higher number of clusters often results in one-cell clusters.

We found a peak in the mean silhouette value at eight clusters (Supplementary material, Fig. S1, a). We also show the silhouette value for each cell at the optimal cluster number of eight clusters (supplementary Fig. S1, b). Small silhouette values do not necessary mean a reduced cluster tendency but can also be attributed to different cluster sizes.

The above-described clustering algorithm using eight clusters gave correct estimates for the direction-selective cells, ON vs. OFF, transient vs. sustained, even if some cells had small silhouette values. The 'partial membership' was close to one for each cell, i.e. the parameters of each cell identified this cell unambiguously to its own cluster. Forcing the algorithm to produce a smaller number of clusters resulted in clusters containing both ON and OFF cells. We double-checked the polarity (ON vs. OFF) calculating the receptive field calculated by reverse correlation, as described above. Note that all of these eight cell types have been previously reported. Here we provide a quantitative parameterization.

We classified ON–OFF direction-selective cells (\( n = 22 \)) as cells that had DS indices \( \sim 0.5 \), a low bias index \( \sim 0 \) and a short response duration of \( < 100 \text{ ms} \). The average DS index of 0.58 ± 0.10 agrees well with reported values (Taylor & Vaney, 2002). [We also noted the selectivity for anterior or posterior movement. Fifteen additional cells sensitive for superior or inferior direction were also recorded but not considered here.]

ON direction-selective cells (\( n = 7 \)) were those cells with lower direction selectivity indices (0.31 ± 0.1; mean ± SD). In contrast to ON–OFF DS cells, these cells responded mainly to light onset (bias index 0.63 ± 0.18) with a relatively long response duration (426 ± 250 ms).

Transient cells had DS indices \( < 0.1 \) and a short response duration of \( \sim 60 \text{ ms} \). ON transient cells (\( n = 13 \)) were separated from OFF transient cells (\( n = 7 \)) by the opposite bias index (see Table 1).

Similarly we distinguished between ON sustained (\( n = 8 \)) and OFF sustained (\( n = 6 \)) cells. These cells had small DS indices (\( < 0.1 \)), long response durations (> 500 ms) and opposite bias indices (0.5 vs. \( \sim 0.6 \)). Two other sustained cell classes were characterized mainly by their response properties to full-field bright stimuli. Local edge detectors (\( n = 13 \)) are known to show a steep response drop for stimuli larger than their receptive field center (Zeck et al., 2005; van Wyk et al., 2006), evident in their inhibitory index of \( \sim 1.8 \). In contrast, the response properties of uniformity detectors (\( n = 5 \)) change little depending on the stimulus size (Levick, 1967; Caldwell & Daw, 1978) (inhibitory index \( \sim -0.37 \pm 0.1 \)). Local edge detectors and uniformity detectors had small DS indices (\( < 0.1 \)), low bias and long response duration.

Table 1 summarizes these mean indices for each cell type. These values also represent the centers of Gaussian distributions modelling each cell type. Prior to the clustering each value was normalized for the whole parameter vector (e.g. DS index) to a mean of 0 and SD of 1.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>DS index</th>
<th>Bias index</th>
<th>Duration (ms)</th>
<th>Inhibitory index</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON–OFF DS</td>
<td>0.58 ± 0.10</td>
<td>−0.05 ± 0.17</td>
<td>70 ± 26</td>
<td>−1.8 ± 0.3</td>
</tr>
<tr>
<td>ON DS</td>
<td>0.31 ± 0.04</td>
<td>0.63 ± 0.18</td>
<td>426 ± 250</td>
<td>−0.9 ± 0.3</td>
</tr>
<tr>
<td>ON transient</td>
<td>0.04 ± 0.02</td>
<td>0.72 ± 0.14</td>
<td>55 ± 20</td>
<td>−0.8 ± 0.2</td>
</tr>
<tr>
<td>OFF transient</td>
<td>0.04 ± 0.02</td>
<td>−0.62 ± 0.15</td>
<td>58 ± 26</td>
<td>−0.8 ± 0.2</td>
</tr>
<tr>
<td>ON sustained</td>
<td>0.02 ± 0.01</td>
<td>0.62 ± 0.25</td>
<td>549 ± 304</td>
<td>−1.4 ± 0.5</td>
</tr>
<tr>
<td>OFF sustained</td>
<td>0.05 ± 0.02</td>
<td>−0.53 ± 0.22</td>
<td>910 ± 416</td>
<td>−1.1 ± 0.4</td>
</tr>
<tr>
<td>Local edge detector</td>
<td>0.04 ± 0.02</td>
<td>−0.11 ± 0.19</td>
<td>521 ± 160</td>
<td>−2.4 ± 0.5</td>
</tr>
<tr>
<td>Uniformity detector</td>
<td>0.02 ± 0.01</td>
<td>0.25 ± 0.09</td>
<td>1389 ± 273</td>
<td>−0.4 ± 0.1</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. Based on these four indices 81 cells could be clustered. The cluster centers correspond to the mean index parameters.

Results
In each experiment we recorded about a dozen ganglion cells from a patch of the rabbit retina in the mid-periphery, using a multielectrode array with dense electrode spacing. This relatively low number of recorded cells results from the small area sampled by the dense array (two patches of \( \sim 0.04 \text{ mm}^2 \) per recording) but is similar to numbers reported for the salamander retina using the same array (Segev et al., 2004). We report here data from 81 functionally identified cells. They were classified into the following cell types: ON and OFF transient cells; ON and OFF sustained cells; uniformity detectors; ON direction-selective cells; local edge detectors and the four ON–OFF direction-selective cell subtypes (one for each of the four cardinal preferred directions). This classification followed previous reports and was formalized here on the basis of a statistical cluster approach as described in Materials and methods.

Definition of coding events and spike patterns
Retinal ganglion cells signal a sparse code (Berry et al., 1997; Reich et al., 1997; Keat et al., 2001; Nirenberg et al., 2001; Koch et al., 2004; Uzzell & Chichilnisky, 2004), i.e. use short trains of action potentials separated by long periods of silence (Fig. 2a). The first step towards a quantitative analysis of spike trains was to identify putative coding epochs within the spike train. This was done using a spike analysis formalism introduced by Rodieck et al. (1962) and further developed to characterize bursting activity in the thalamic nuclei (Reinagel et al., 1999). We define in analogy to a thalamic burst a discrete 'coding event' of a ganglion cell. An action potential can be classified as the first (cardinal) spike of a coding event if it is preceded and if the threshold \( T \) we define the coding event as completed. A convenient way to visualize the coding events is to plot a two-dimensional joint
ISI map (Fig. 2b), where each spike is represented as a function of both the preceding and the following ISI. As an example, Fig. 2b plots the spike train of an ON–OFF DS anterior cell recorded in response to spatial white noise. The lower right quadrant of the joint ISI plot represents the cardinal spikes. The shallow shape of this area shows that the second spike in an event usually occurs very soon after the cardinal spike. The lower left quadrant represents those spikes that occurred between the cardinal spike and the final spike of the coding event (burst spikes). There are many coding events with three or more spikes. (If there were only two-spike coding events this area would be empty.) The upper left quadrant represents the terminal spike in a coding event and must, by definition, contain the same number of action potentials as the cardinal spike cluster (lower right quadrant). The spikes in this area are more widely distributed than the cardinal spikes.

The upper right quadrant represents solitary spikes, where both the time before a spike and after the spike is long. The solitary spikes will be ignored in this study. They usually constitute less than 10% of all of the spikes (supplementary Fig. S2) as long as the threshold $T$ is set to more than 25 ms. (For two cell types, ON direction-selective cells and local edge detectors, the percentage of solitary spikes was higher than 10%). Variation of the threshold value $T$ between 25 and 100 ms has little effect on the percentage of solitary spikes. We do not know the role of the solitary spikes but it seems unlikely that they would be part of a cell-specific recognition sequence. Among other things, solitary spikes are less likely than bursts to elicit a thalamic spike (Usrey et al., 1999).

These plots are not unique to the spatial white-noise stimulus or to the rabbit retina. We also studied recordings in response to temporal white noise (full-field flicker), moving gratings, flashed spots and a ‘natural’ movie, as well as spontaneous activity in dim light. The maps for all retinal ganglion cells ($n = 81$) encountered in our recordings could be separated into four distinct areas. A similar two-dimensional ISI pattern was observed for mouse retinal ganglion cells ($n = 19$, two retinas). We conclude that these four-quadrant maps are a generic representation of retinal ganglion cell activity (supplementary Fig. S3).

Once the threshold $T$ is set to 25 ms, the coding events can be extracted individually. A raster plot of the six-spike events extracted from the ISI plot of Fig. 2b is shown (for the same cell) in Fig. 2c. All events are aligned to the cardinal spike at time zero. Subsequent spikes occur at precise times visible as bands. The total event length rarely extends for more than 50 ms, even though each action potential in an event was allowed to be separated from its neighbor by 25 ms and thus the total event length would have been allowed to extend to more than 150 ms. The percentage of events shorter than 50 ms was $91.5 \pm 1.5\%$, considering all cells except the local edge detectors in response to spatial white-noise stimuli. In the following section we ask how robust is the distribution of ISIs, which will be referred as ‘spike

![Fig. 2. Retinal ganglion cell activity can be described by burst-like coding events.](image)
patterns’, in these 50 ms segments, i.e. if they reproducibly maintain their characteristics under varying conditions.

**Spike timing patterns are robust**

The ISI distributions were calculated for the first six intervals in a coding event for each of the 81 cells examined. Figure 3 shows typical examples for each cell type (to conserve space only the first four intervals are shown) in response to a spatial white-noise stimulus. The normalized probability for each interval was calculated separately. The main characteristics of these distributions are: a gap at small intervals, a peak at a preferred interval and a tail for long intervals. The gap at small intervals is mainly due to the cell’s refractory period. (This also affirms that we correctly sorted the action potentials recorded on the dense microelectrode array.)

In the following we describe the spike patterns for each cell type. The name of the cell type always refers to the conventional classification presented in Materials and methods. ON ($n = 13$) and OFF ($n = 7$) transient cells had narrow ISI distributions with similar values for each consecutive interval (Fig. 3b and c; width at half maximum 0.6 ms). Translating this firing pattern to the frequency domain, transient cells responded on average with a 400–500 Hz pulse in the first tens of milliseconds of firing. No other cell type had such short, regular bursts.

A small but notable difference was the longer tail in the interval distribution for ON transient cells, which will be exploited later. For the so-called uniformity detectors ($n = 5$; Levick, 1967; Caldwell & Daw, 1978), only the first two interval distributions are sharp (width at half maximum 1.1 ms), whereas subsequent intervals broaden but without a change in median interval (width at half maximum 2.4 ms). This leads to a shift in the median interval.

ON–OFF DS cells coding for anterior movement ($n = 8$) had narrow distributions (precise timing) of the first and second interval (Fig. 3e). Cells responsive to posterior movement ($n = 14$) had much broader ISI distributions (Fig. 3f), with similar shapes but slightly increasing median ISI values. ON–OFF DS cells coding for inferior ($n = 9$) and superior ($n = 6$) movement had intermediate ISI distributions (first ISI with sharp peak, all following distributions broadened; data not shown). These 15 cells are not included in the

![Fig. 3. Probability distributions for consecutive interspike intervals (ISIs) in a coding event are cell type specific. (a) Schematic spike train, illustrating the segregation of coding events and the numbering of ISIs within them. This example shows three coding events and two solitary spikes. (b–j) ISI distributions for nine cells of different types. For each spike in a coding event the distance to the next spike, the ISI, was calculated. The ISI probabilities for the first four intervals in a coding event are largely cell type specific. The ISI probability distributions shown here were normalized so that the area under each curve equals 1. Binning for the ISI distributions was 0.2 ms. The distributions shown in (g) to (j) were smoothed with a Gaussian window using a width of 0.8 ms.](image)
number of 81 cells that we report because of the low number of spikes recorded.

For ON and OFF sustained cells (Fig. 3h and i) the distribution of the first intervals peaked at ~5 ms but was followed by a prominent shoulder, indicating a less regular bursting behavior.

The two cell types with considerably longer ISIs are the ON DS cells ($n = 7$) and local edge detectors ($n = 13$). The median intervals for ON DS cells increased slightly from ~10 ms for the first interval to ~12 ms for the last interval (Fig. 3g). Local edge detectors (Fig. 3j) show the largest distance between two neighbor spikes (~15 ms).

To begin characterization of the spike patterns, we extracted for each cell the median values of each of the first six intervals. Figure 4 shows the characteristic response pattern (to spatial white noise) for each cell type, classified by independent criteria described earlier (see Materials and methods). For all except the local edge detectors cells, the first intraevent interval was the shortest interval, usually less than 10 ms. The greatest change in interval duration was measured for ON–OFF DS anterior cells (from 2 to 3 ms for the first interval to ~10 ms for the last interval) and the least for transient cells (the difference between the first and sixth interval was ~1 ms in all examples). To varying degrees, the spike timing patterns are different for each cell type (these will be quantitatively compared below). For cells with relatively long intervals the duration of the last intervals (i.e. intervals 5 and 6) varies considerably and will not be considered further. These median intervals are usually calculated from a reduced number of intervals because the mean spike number in a coding event is around four. However, for any particular cell type the spike timing pattern of the first four intervals was very reproducible from cell to cell.

How much do the spike timing patterns change under different stimulus conditions? We repeated the analysis for spike trains in response to temporal white noise, a moving grating, flashed spots and spontaneous activity in dim light (Fig. 5). For 29 cells we presented a natural movie (van Hateren & Ruderman, 1998). The mean spike patterns and mean event length for this presentation were virtually the same as for the presentation of moving gratings and will not be discussed separately.

The fundamental result was that the spike timing patterns were robust under these various conditions, in the sense that the characteristic features of the timing pattern changed rather little under the changing stimulus conditions. We quantified the similarity of the first intervals recorded in response to spatial white noise to the first intervals calculated for all other stimuli using the Wilcoxon rank sum test. The higher the $P$-values of this test the closer are the two distributions under consideration. The $P$-values for the different cell

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**Fig. 4.** The patterns of interspike intervals are robust among different cells of the same type. The median interval of each interval distribution (Fig. 3) for the first six intervals was plotted vs. the interval number. Each curve shows data from one individual ganglion cell in response to spatial white noise. Each subplot refers to a different cell type, which has been pre-classified as described in Materials and methods. All examples of a particular cell type show a similar pattern of spike timing.
types were (ON–OFF DS posterior, 0.88; ON–OFF DS anterior, 0.56; ON DS, 0.49; ON transient, 0.98; OFF transient, 0.83; ON sustained, 0.87; OFF sustained, 0.17; uniformity detector, 0.93; local edge detectors, 0.30). Qualitatively we observed the following changes in the interval distributions. The distributions narrowed slightly in response to stimuli that are close to optimal (e.g. an optimally moving grating for a DS cell). This effect was most pronounced for cell types with broad interval distributions; for the sustained cells (both ON and OFF) and the ON–OFF DS posterior cells the median intervals were on average 1 ms shorter (Fig. 5e). For ON–OFF DS cells sensitive to anterior movement, the interval difference is visible in the higher ISIs only (Fig. 5d). For cells with narrow interval distributions, such as the

Fig. 5. The patterns of interspike intervals are largely stimulus independent. The median intervals were calculated in response to different stimuli. The mean values for each stimulus presentation are shown. In each of the nine graphs data from one cell type are shown: (a) ON transient cells; (b) OFF transient cells; (c) uniformity detectors; (d) ON–OFF direction-selective cells for anterior movement; (e) ON–OFF direction-selective cells for posterior movement; (f) ON direction-selective cells for anterior movement; (g) ON sustained cells; (h) OFF sustained cells; (i) local edge detectors. The stimuli were spatial white noise, temporal white noise, moving grating, flashed spots and spontaneous activity. Each set of points represents the mean value for the specified cell type when tested with the stimulus indicated in the key. Numbers in parentheses indicate the number of cells studied. The error bars represent the SEM.
transient cells, the median interval in response to flashed spots was ~0.5 ms shorter than for spatial white noise or moving gratings/movie (Fig. 5a).

The median intervals were slightly larger during spontaneous firing than during stimulation (change in interval duration: transient cells, 0.3 ms; uniformity detectors, 0.7 ms; DS anterior, 0.5 ms). However, the more pronounced differences that appeared in the spontaneous activity were shorter coding events and about a twofold increase in solitary spikes. For ON–OFF DS cells the percentage of solitary spikes increased from 12% (n = 22 cells) during spatial white-noise stimulation to 27% (n = 17) during spontaneous activity in the dark. A similar trend was observed for transient cells (11% solitary spikes during spatial white-noise stimulation and 18% during spontaneous activity).

**Length of coding event can signal the trigger stimulus**

If the firing pattern does not change very much under different stimulation conditions, how can the cell signal its response to different stimuli? Although there were the small differences in the median ISI just described, the more pronounced effect was upon the mean number of spikes per coding event (Fig. 6). We calculated the mean number of spikes per event for all cell types under the various stimulus conditions. During spontaneous activity, there were many short events (mean ± SEM: ON transient, 3.5 ± 0.4 spikes/event; uniformity, 3.0 ± 0.2; DS cells, 3.4 ± 0.4; local edge detectors, 3.65 ± 0.5). Full-field stimulation with temporal white-noise statistics had different effects; ON–OFF DS cells responded with short events only (~2.8 spikes/event), whereas transient cells on average responded with 5.4 spikes. This may reflect different inhibitory surround effects for each cell type (local edge detectors did not respond at all to full-field flicker stimuli). In contrast, stimuli closer to the traditional ‘preferred stimulus’ (a grating moving in the DS cell’s preferred direction, a flashed spot on a transient cell or a local edge detector) elicited long events, on average six spikes per event for the DS cells, 10 spikes per event for the transient cells and five spikes per event for the local edge detector, respectively. These represent increases of about 100% for the DS cells and transient cells, and about 50% for the local edge detector. Note that the stimuli were not tailored to the spatial receptive field of any individual cell and were thus substantially less than optimal. The local edge detector, for example, is known to respond poorly to any full-field stimulus (Levick, 1967; Roska & Werblin, 2001; Zeck et al., 2005).

The number of spikes per event could signal more subtle modulations of the cell’s response, of the sort expected to occur in viewing complex stimuli. This was tested in an experiment where the stimulus to the receptive field center was held constant but its spatial extent was varied, for the specific case of the ON–OFF DS cells (n = 6). A grating moving in the preferred direction and confined to the cells’ receptive field center (500 μm diameter) was compared with the same grating stimulus extending into the surround. Previous studies have shown that small stimuli crossing the receptive field of ON–OFF DS cells stimulate the cells more strongly than stimuli extending into the surround (Barlow & Levick, 1965; Werblin, 1972; Wyatt & Daw, 1975). We found that the distribution of intervals within a coding event was little affected by the changing stimulus (Fig. 7a).

The number of long intervals increased slightly, leading to a somewhat larger median interval value Fig. 7b. However, the distribution of spikes per event was markedly different for the two conditions; spatially restricted stimuli elicited notably longer coding events (Fig. 7c). Thus, the spike pattern (the temporal signature of a cell type) can remain the same while still transmitting a stimulus-dependent variation.

Were other spike train parameters modulated? The event frequency, a second putative coding event parameter, was calculated for the ON transient cells and ON–OFF direction-selective cells. The event frequency is the number of events averaged over time or simply the frequency of cardinal spikes (Fig. 2b). Both cell types showed a large increase in the event length in response to trigger stimuli (Fig. 6) but there was little conclusive change of the event frequency. This measure increased by about a factor of 2 for direction-selective cells; 4.1 events/s in response to a moving grating in the preferred direction (i.e. the trigger stimulus for these cells) compared with 2.2 events/s for recordings in response to all other stimuli. However, the event frequency did not change for ON transient cells (2.5 events/s for flashed spots compared with 2.2 events/s for all stimuli). Thus, the duration of the coding events was the more reliable indicator of the presence of a cell’s ‘optimal’ stimulus.

**Spike pattern clusters are largely cell type specific**

The data of Figs 3–5 provide evidence that consecutive intervals in a coding event are cell type specific. In the following we ask to what extent stimulus-independent spike train patterns can be grouped together and to what extent do these groups correspond to the clusters (pre-)defined by the conventional criteria described in Materials and methods.

Starting points are the median values of the first four consecutive intervals in a coding event. Intervals of the local edge detectors show a large absolute variability (but similar to the other cells with ~10% relative change) and were grouped in different clusters. To avoid this effect we calculated the logarithmic values of the median intervals and normalized each value for the whole cell population to a mean of 0 and SD of 1. Using the first four intervals of the coding events and the statistical clustering approach described in Materials and methods, some cell types (e.g. local edge detectors, ON DS cells or uniformity detectors) formed separate clusters, whereas other types could not be separated.

With the addition of a fifth parameter, a better correspondence of the spike train patterns with the conventional clusters was obtained. The
Fig. 7. Spatial information can affect the spike train patterns. A square-wave grating moving at 1 Hz was presented to ON–OFF DS cells. In the first condition (black) the grating extended into the cell’s surround (stimulus diameter 2000 μm). In the second condition the grating covered only the cell’s receptive field center (diameter 500 μm). (a) Representative interspike interval distributions; the graph shows the first and fifth intervals in the population of coding events generated by one representative cell under the two stimulus conditions. The interval distribution is similar, the exception being a few longer intervals during the stimulation by extended gratings. (b) Median values for six ON–OFF DS cells vary by only ~1 ms. [This was due largely to the few long intervals seen in (a)]. (c) The number of spikes per event for the example shown in (a) is larger when the stimuli are restricted to the receptive field center of the DS cell; thus, the stimulus that would be regarded as closer to the optimal stimulus caused an increase in the burst length. The inset shows the mean number of spikes per event calculated for six ON–OFF DS cells.

Fig. 8. Discrimination among cell types on the basis of spike timing patterns. (a) Cumulative distribution functions of the first interval (shown here for four cells) are used, together with the means of the first four intervals in an event to discriminate between cell types. The time where the cumulative interval distribution of the first interval in a coding event reaches 0.9 was introduced as an additional parameter to delineate cells. (The cumulative distribution function of a value x is the probability of observing any outcome less than or equal to x.) We only considered the cumulative probability distribution of the first interval in a coding event. (b) Mean partial memberships quantify to what degree cells are assigned to the correct (pre-classified) cluster. Spike train patterns of the burstiest cells (i.e. transient cells, uniformity detectors and ON–OFF DS anterior cells) and of the most sluggish cells (local edge detectors and ON DS cells) are assigned to the correct (pre-defined) cluster with a high probability. The cluster centers for three other cell types described by the interval parameter set are close to each other and spike train patterns from these clusters are considerably intermixed. (c) Examples of mean partial memberships of pre-defined cell types to the nine spike pattern clusters. Spike train patterns from ON transient cells were mostly assigned to cluster 1, whereas spike patterns from local edge detectors were assigned to cluster 9. Spike patterns from ON sustained cells were mostly assigned to cluster 3 (50.8%) but to a considerable extent to other clusters as well.
parameter chosen (among several possibilities) was the time when the cumulative probability distribution of the first interval reached 0.9 (Fig. 8a). This measure was taken because it captures the prominent tail in the interval distribution of ON and OFF sustained cells or even of ON transient cells as compared with OFF transient cells. Using this additional parameter we could separate the cells in nine clusters corresponding to the conventional cell types. We separated these two cells based on different median intervals for ON–OFF DS anterior and posterior cells. Each cluster is defined by a five-dimensional vector μj and a corresponding covariance matrix (Eqn 3). The spike train signatures, here the centers of the mixture of Gaussian distributions, for each cell type are given in Table 2. Cell types with short intervals of 2–3 ms are listed in the first rows.

Whereas the pre-classification of the cells in different types using conventional criteria resulted in clusters with a ‘partial membership’ value of −1 the values of this parameter were more distributed for the spike train patterns. We calculated a mean ‘partial membership’ for all cells (i.e. the cell-type-specific spike train patterns) of each type to the pre-defined cluster. Out of 13 (pre-classified) ON transient cells in our study, spike train patterns calculated from two of them in response to spatial white noise were misidentified as OFF transient cells (i.e. had a higher membership for cluster 2 containing OFF transient cells), whereas the other 11 cells had a partial membership of 1 for the ON transient cluster 1 (Table 2). The mean partial membership for ON transient cells was therefore \( \left( 1 \times 11 + 0 \times 2 \right) / 13 = 0.85 \). The mean partial membership values calculated for the presentation of spatial white noise and a summary of all stimuli are shown in Fig. 8b. To further illustrate the correspondence of conventional clusters and spike pattern clusters we present the percentage of pre-classified cells in each spike pattern cluster (Fig. 8c). For these plots all stimulus presentations were considered. Spike patterns calculated from ON transient cells were assigned with a probability of 77% to cluster 1 (see Table 2) and with a probability of 16% to cluster 2. Spike patterns from local edge detectors were assigned with a probability of 91% to cluster 9 and with a probability of 9% to cluster 8 containing mostly spike patterns from ON DS cells. Three other spike pattern clusters, containing mostly the sustained cells and the ON–OFF DS posterior cells, had mean partial membership values around 0.5. This low mean partial membership value reflects the fact that not all cells classified by conventional criteria into the same type form one single cluster using the spike train patterns. For instance, cluster 3 contains spike patterns recorded from ON and OFF sustained cells and, to a lesser degree, from ON–OFF DS posterior cells (Fig. 8c). It is worth noting that there is no misidentification of these cells as transient cells, ON–OFF DS anterior or local edge detectors.

<table>
<thead>
<tr>
<th>Cluster number (conventional cell type)</th>
<th>1st interval (ms)</th>
<th>2nd interval (ms)</th>
<th>3rd interval (ms)</th>
<th>4th interval (ms)</th>
<th>CPD 90 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (ON transient cell)</td>
<td>2.4 ± 1.1</td>
<td>2.7 ± 1.1</td>
<td>3 ± 1.2</td>
<td>3.2 ± 1.2</td>
<td>8.3 ± 1.2</td>
</tr>
<tr>
<td>2 (OFF transient cell)</td>
<td>2.6 ± 1.1</td>
<td>2.8 ± 1.1</td>
<td>3 ± 1.2</td>
<td>3.1 ± 1.4</td>
<td>4.3 ± 1.4</td>
</tr>
<tr>
<td>3 (ON sustained cell)</td>
<td>5.8 ± 1.3</td>
<td>7.9 ± 1.2</td>
<td>7.1 ± 1.8</td>
<td>7.3 ± 1.2</td>
<td>16.8 ± 1.4</td>
</tr>
<tr>
<td>4 (OFF sustained cell)</td>
<td>7.1 ± 1.2</td>
<td>8.7 ± 1.1</td>
<td>8.2 ± 1.1</td>
<td>8.1 ± 1.3</td>
<td>15.3 ± 1.2</td>
</tr>
<tr>
<td>5 (uniformity detector)</td>
<td>3.1 ± 1.4</td>
<td>4.0 ± 1.3</td>
<td>4.6 ± 1.3</td>
<td>5.2 ± 1.2</td>
<td>7.5 ± 1.5</td>
</tr>
<tr>
<td>6 (ON–OFF DS anterior cell)</td>
<td>3.25 ± 1.1</td>
<td>4.3 ± 1.3</td>
<td>7.2 ± 1.1</td>
<td>8.8 ± 1.3</td>
<td>8.9 ± 1.2</td>
</tr>
<tr>
<td>7 (ON–OFF DS posterior cell)</td>
<td>5.3 ± 1.1</td>
<td>6.7 ± 1.2</td>
<td>6.7 ± 1.2</td>
<td>6.9 ± 1.4</td>
<td>12.2 ± 1.3</td>
</tr>
<tr>
<td>8 (ON DS cell)</td>
<td>9.5 ± 1.3</td>
<td>11.6 ± 1.2</td>
<td>11.9 ± 1.4</td>
<td>11.3 ± 1.6</td>
<td>19.0 ± 1.4</td>
</tr>
<tr>
<td>9 (local edge detector)</td>
<td>16.1 ± 1.2</td>
<td>15.1 ± 1.2</td>
<td>15.4 ± 1.1</td>
<td>14.8 ± 1.1</td>
<td>23.3 ± 1.8</td>
</tr>
</tbody>
</table>

These spike patterns mark, together with the 90% value of the cumulative probability distribution of the first interval (last column) (CPD 90), the centers of Gaussian distributions in the Gaussian mixture model approach. For each of the nine centers five Gaussian probability distributions demark a cell-type-specific region. The data are presented as means ± SDs, where the SDs calculated from the diagonal elements of the covariance matrices. For three dimensions each cell type would be described by an ellipsoid with the length of the axis given by the eigenvalues of the covariance matrix.

Discussion

High-frequency coding events followed by periods of silence are a generic feature of many sensory systems (Krahe & Gabbiani, 2004). ‘Peaky and sparse’ firing by retinal ganglion cells has been shown in the salamander (Berry et al., 1997), rabbit (Berry et al., 1997; Keat et al., 2001), mouse (Nirenberg et al., 2001), cat (Reich et al., 1997), guinea pig (Koch et al., 2004) and monkey (Uzzell & Chichilnisky, 2004). A major result of those studies is to show the precise timing of the coding event onset in response to repetitions of full-field flicker stimuli and most recently to movie sequences (Koch et al., 2006), and the great reliability of spike timing and number. The structure of coding events was modelled for the full-field stimulus by including a recovery function after each spike, mimicking the cell’s refractory period (Keat et al., 2001; Paninski et al., 2004). We confirm that ‘burstiness’ is a generic feature of every retinal ganglion cell’s activity in the rabbit retina (and for 19 tested mouse ganglion cells in two retinas). Here, we address two new questions, the robustness of the coding events’ microstructure in the face of different stimuli and the cell type specificity.

Microstructure of the spike timing patterns is probably cell intrinsic

The spike timing ranged from short, regular bursts of spikes (transient cells) to much more sluggish responses (local edge detectors), with instantaneous firing rates up to 500 Hz (5 spikes/10 ms) or as low as ~50 Hz (5 spikes/100 ms). These burst-like events lasted about 50 ms for most cells (up to 100 ms for local edge detectors). This characteristic value might well correspond to subthreshold depolarizations as inferred from intracellular recordings of Y-cells (Zaghloul et al., 2005) and subsequent modelling by a linear filter. To our knowledge no study predicts the robust high-frequency intervals within a coding event.

It seems most likely that the biophysical properties of the ganglion cell, the collection of ion channels and conductances expressed, determine the detailed (fast time scale) spike timing patterns. It is easy to imagine how they would control the timing of burst events in the cell, each in a distinctive way, and there is direct evidence that each type of retinal ganglion cell has its own collection of channels and receptors, a biophysical signature for each cell (Ishida, 1995; O’Brien et al., 2002). The difficulty of distinguishing between ON transient and OFF transient cells in our stimulus-independent approach supports this hypothesis, if one assumes that the major difference in the two types is not the intrinsic physiology of the ganglion cell but the fact...
that ON and OFF pairs have different pre-synaptic partners, ON or OFF bipolar cells and their associated amacrine cells. ON and OFF sustained cells were also difficult to distinguish by the five-parameter spike train patterns. These cells have a strong tendency to fire doublets of action potentials (~50% of all spikes occur as doublets) followed by a variable interval length of ~10–30 ms. These longer intervals change the median intervals considerably and lead to cell type mixing.

The obvious alternative explanation would be that the spike timing is controlled instead by synaptic inputs from different bipolar and amacrine cells, which must also be distinctive for the different physiological types of retinal ganglion cell, but the spike timing patterns occur on a time scale (fractions of ms) shorter than the times of synaptic events (several ms) and the integration times of the cells. It seems unlikely, even if not entirely impossible, that synaptic inputs could be coordinated on such a fast temporal scale. Much evidence from other types of neurons is consistent with this conclusion (Krahe & Gabbiani, 2004). This is not to say that the responses are determined entirely Y-cell intrinsic mechanisms; as noted above, pre-synaptic inputs may control the average event length and they are certainly a cause of the selectivity for trigger stimuli.

Twenty-two DS cells sensitive to either anterior or posterior movement were studied and these cells always had distinctive spike timing patterns. DS cells preferring upward or downward movement \((n = 15)\) had a third spike timing pattern, which in our analysis was similar for both the upward and downward directions. We do not know whether this apparent inconsistency among the four directional subtypes of DS cell is real or if there is a spike timing pattern that would distinguish upward from downward DS cells, were a better sample of spike train patterns available.

**Spike timing can be used experimentally to distinguish ganglion cell types**

As the distinction among cells is substantially independent of stimulus conditions, this analytical method should be useful in identifying the many types of retinal ganglion cells, present in most mammalian retinas, that remain uncharacterized (Pu et al., 1994; Rockhill et al., 2002; Carcieri et al., 2003; Dacey et al., 2003; Kong et al., 2005). The approach presented here relies on an alternative to estimating the traditional trigger stimuli. The required steps are the analysis of the joint ISI plot (to segregate the coding events) followed by a formal cluster analysis of selected parameters as presented here. It is quite likely that other parameters of the spike train would need to be considered in order to achieve reliable clustering of the dozen or so types that make up the whole complement of ganglion cells. Once an initial segregation of the types had been accomplished by this means, it could be verified by correlation with dendritic morphology (Roska et al., 2006), with responses to specifically designed images or stimulus sets, and ultimately with cell-type-specific expression of genes or combinations of genes. In this context it should be noted that, although we could recover similar spike patterns for all stimuli, the signatures would not require a one-to-one representation of the retinal ganglion cell activity. All that would be required is a stereotyped response of the ganglion relay neuron to repetitions of the same retinal spike pattern. This has indeed been confirmed in dual recordings across the retinogeniculate synapse of the cat in vivo (Kara et al., 2000) and in a mouse ganglionic slice preparation (Blitz & Regehr, 2003). Thus, it seems likely that some reflection of the coding pattern of a retinal ganglion cell is transmitted, in its original form or a reliably transformed form, by the thalamic relay. The question is experimentally testable; one could inject the temporal patterns observed here into single axons of the optic nerve while recording from thalamic relay neurons.

There is much evidence in higher visual areas (lateral geniculate nucleus and visual cortex) for burst length as a signal for stimulus tuning and it is not difficult to imagine how synaptic summation would make long bursts more effective in driving a post-synaptic cell than short bursts (Cattaneo et al., 1981; Martinez-Conde et al., 2002). A more difficult question is whether the internal structure of the burst, the identity signature of the ganglion cell type, could serve a useful function. Here, a central fact is that a fairly large sample of spikes is required to establish the identity of the cell. Although we cannot rule out that the brain uses the coding events to identify cell types ‘on the fly’ (this might be done by some sort of population-based averaging), it seems more likely that they would be used for a purpose not requiring rapid discrimination among stimuli; the fact that the brain would need to accumulate a sample of tens or hundreds of coding events to establish the identity of a single cell would suggest a role other than one requiring rapid decision-making. One such function is the control of neuronal connectivity, either during its post-natal development or its maintenance in the mature animal. A final possibility is that the characteristic structures of the coding events are related to the (still poorly understood) mechanisms of temporal integration within synapses comprising particular circuits of the lateral geniculate body, superior colliculus and other optic nerve targets.

**Supplementary material**

The following supplementary material may be found on www.blackwell-synergy.com

Fig. S1. The optimal number of clusters can be roughly estimated from the mean silhouette values.
Fig. S2. The threshold time $T$ between spikes does not affect the percentage of solitary spikes.

Fig. S3. All retinal ganglion cell types show similar bursting activity, irrespective of the stimulus.

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Abbreviations

DS, direction-selective; EM, expectation maximization; ISI, interspike interval.

References


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