The Functional Organization of Ganglion Cells in the
Salamander Retina

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Running Head: Oversampling by Retinal Ganglion Cells

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ABSTRACT

Recently, we reported a novel technique for recording all of the ganglion cells in a retinal patch and showed that their receptive fields cover visual space roughly 60 times over in the tiger salamander. Here, we carry this analysis further and divide the population of ganglion cells into functional classes using quantitative clustering algorithms that combine several response characteristics. Using only the receptive field to classify ganglion cells revealed six cell types, in agreement with anatomical studies. Adding other response measures served to blur the distinctions between these cell types rather than resolve further classes. Only the biphasic OFF type had receptive fields that tiled the retina. Even when we attempted to split these classes more finely, ganglion cells with almost identical functional properties were found to have strongly overlapping spatial receptive fields. A territorial spatial organization, where ganglion cell receptive fields tend to avoid those of other cells of the same type, was only found for the biphasic OFF cell. We further studied the functional segregation of the ganglion cell population by computing the amount of visual information shared between pairs of cells under natural movie stimulation. This analysis revealed an extensive mixing of visual information among cells of different functional type. Together, our results indicate that the salamander retina uses a population code in which every point in visual space is represented by multiple neurons with subtly different visual sensitivities.

Keywords: retina, oversampling, tiling, ganglion cell, receptive field, multi electrode array
INTRODUCTION

In now classic work, Wässle and colleagues showed that the alpha ganglion cell in the cat, which is distinguished by its large soma size, formed two plexuses with their dendritic arbors – one with ON-type light responses, the other with OFF-type – that precisely covered visual space (Wässle and Boycott, 1991). The somas of neighboring cells tended to be spaced one dendritic diameter apart from one another, the tips of their dendrites just barely touching. A similar territorial organization and dendritic coverage factors close to one have been found in other, prominent morphological types of ganglion cells both in the monkey and the rabbit (Dacey, 1993; Vaney, 1994). Tight correspondences between anatomy and function have also been found (Wässle and Boycott, 1991), along with an increasingly intricate functional segregation of axons and dendrites in the inner plexiform layer (Sterling, 1983; MacNeil et al., 1999; Roska and Werblin, 2001; Pang et al., 2002; Wassle, 2004; Zhang et al., 2004). For all these reasons, tiling has come to be regarded as a fundamental principle of retinal organization.

However, this simple picture of efficient tiling does not hold for all of the ganglion cells. Several other anatomical types have coverage factors greater than two (Wässle and Boycott, 1991; Rodieck, 1998), and even as large as six (Pu et al., 1994; Berson, 2003). In a recent anatomical classification of rabbit ganglion cells, Rockhill et al. found that their 13 morphological types needed to have an average coverage factor of 3.2 in order to account for the ganglion cell density that they measured (Rockhill et al., 2002). Interestingly, it has been suggested that a coverage of roughly 3 is necessary for avoiding aliasing (Wassle and Boycott 1991). Furthermore, the area of the receptive field measured by physiology can be significantly larger than the receptive field measured anatomically (Peichl and Wassle, 1979, 1983).

While anatomical techniques have made the systematic study of entire neuronal populations possible, the results of electrophysiology have been more limited. Several multi-electrode array studies have recorded simultaneously from many ganglion cells, reporting that brisk transient ganglion cells in the rabbit (DeVries and Baylor, 1997) and parasol ganglion cells in the monkey (Frechette et al., 2005) show convincing tiling. However, these methods may not record from a large fraction of the ganglion cells over the array. This is important, because such methods may then sample only a few cell types that tile and overlook other cell types with different properties. We have
developed a new method of multi-electrode recording and spike sorting that allow us to record from all or nearly all of the ganglion cells in a small patch of the salamander retina (Segev et al., 2004), a species that is technically advantageous. Here, we perform a systematic study of the functional organization of this ganglion cell population. We use several quantitative clustering algorithms to resolve the ganglion cells into functional types and explore the spatial organization of their receptive fields. We also use a more general, information-theoretic method to assess the functional similarity between ganglion cells. Our results indicate that only one functional type tiles visual space in the salamander, and that there is extensive mixing of visual information among cells of different functional type.

**MATERIALS AND METHODS**

**Recording.** Experiments were performed on the larval tiger salamander (*Ambystoma tigrinum*). Retinas were isolated from the eye in darkness and peeled from the pigment epithelium. Retinas were then placed with the ganglion cell layer facing a multi-electrode array (Multichannel systems, Germany), and superfused with oxygenated Ringer's medium at room temperature. Two array geometries were used: a hexagonal layout with 19 electrodes and 28 µm spacing between electrodes, and a rectangular array (6 lines and 5 rows) with 30 µm spacing. For both arrays the electrode diameter was 10 µm. Extracellularly recorded signals were digitized at 10 kSamples/s on a personal computer and stored for offline analysis. We used a total of 6 retinas from 6 different animals. Our rectangular array had two separate regions of 30 densely spaced electrodes, which allowed us to record from two small patches in each retina. These two patches each had an area ~ 0.2 mm² and were 500 µm apart. In some cases, only one patch yielded good signals, because of the curvature of the tissue. In our analysis, we combined the data from several experiments to obtain larger populations of ganglion cells. Prior to combining experiments, we checked that the overall firing rates and response latencies were the same. We also verified *a posteriori* that cell types identified by our clustering algorithm were not dominated by cells from a single patch. For the clustering analysis which combined four different response characteristics, we could use only three retinas because we did not have PSTH data for the other three retinas.
**Multi-Channel Spike Sorting.** The spike sorting procedure was previously described in detail (Segev et al., 2004). Briefly, using the peak voltage on 30 electrodes, we identified examples where a ganglion cell fired an action potential without overlapping spikes from other cells. Such instances are easy to find, although they represent only a fraction of all spikes produced by a given cell. These isolated spikes were averaged together to form a 3.2 ms template of the voltage activity on the array. Templates having a range of time shifts were matched against putative spike patterns in the raw data using mean-squared error as a measure of goodness of fit. Typically, the 3 or 4 smallest values of mean-squared error resulted from the same template with successive time shifts, indicating that matches have a temporal precision of ~0.1 ms. We subtracted the best fitting template from the raw data and repeated this procedure on that residual until the mean-squared error for the cumulative fit no longer decreased. Most spike waveforms had a peak amplitude larger than 100 µV on a single channel and some were as big as 500 µV, while the root-mean-squared electrical noise was about 10 µV. Typically, the amplitude of the spike waveform from a single ganglion cell was larger than the electrical noise on 5-10 channels. Due to the high signal-to-noise ratio of signals on many channels, this iterative procedure robustly found the optimal match.

We studied the retina of the salamander, a species that is especially well-suited for recording from all of the ganglion cells for several reasons: its ganglion cells are large, they form a monolayer in the ganglion cell layer, and they occur at a moderate areal density that is uniform across the retina. When we applied our method to recordings from the salamander retina, we found that we could match spike patterns unambiguously to all of the signals significantly above the noise. Comparing the density of ganglion cells over the array using retrograde labelling with the number of isolated cells obtained from the spike sorting revealed that we have recorded from a large fraction of the ganglion cells over the array in the salamander retina – at least 80% – and the data are consistent with recording from 100% of the overlying cells (see (Segev et al., 2004) for details). For comparison, previous methods that use a more widely spaced array of electrodes can only record from ~10% of the cells over the array (Meister et al., 1994; Schnitzer and Meister, 2003).

When observing ganglion cells with very similar function and nearly concentric receptive fields, we need to make sure that this is not the result of spike sorting errors.
To this end, we checked that each sorted spike train did not have spikes within the short refractory period of the ganglion cell spike generator. Typically, the fraction of spikes within a 2 ms interval of the preceding spike was less than 0.1% (Segev et al., 2004). This indicates that our sorted spike trains were isolated to spikes primarily from a single ganglion cell. Another possible spike sorting error is to split the spikes of a single ganglion cell into two sorted spike trains. We screened for this possibility with two tests. First, we combined pairs of sorted spike trains into a single spike train. If the two spike trains came from the same ganglion cell, then the combined spike train will still have a clean refractory period. Second, we checked whether the cross-correlation function between pairs of sorted spike trains had a clean refractory period, as this will only occur if the two spike trains are subsets of spikes from a single ganglion cell. This analysis was carried out for every pair of cells in each experiment (see figures 6 and 7 in (Segev et al., 2004) for more details), and whenever a pair failed this test, their spike trains were combined together.

**Stimulation.** Random checkerboard stimuli were displayed on a cathode ray tube monitor at a frame rate of 120 Hz. The image from the monitor was focussed onto the plane of the retina using standard optics (Meister et al., 1994). In this stimulus ensemble, visual space was divided into 50 µm squares on the retina, which allows many squares to fit inside the receptive field center of each ganglion cell. Within each square, the red, green, and blue levels of the monitor were randomly and independently turned on or off every 4 frames, resulting in a light intensity and color that flickered rapidly. In some experiments, we used a black & white checkerboard, in which the red, green, and blue guns were either all on or all off in each square. Directional selectivity was probed with drifting square-wave gratings of full contrast (wavelength 1.2 mm on retina, period 0.33 s) presented in 8 different directions.

In addition to artificial stimuli, we also used natural movie clips, which were acquired using a Canon Optura Pi video camera at 30 frames per second. Movies were taken of woodland and urban scenes and included several qualitatively different kinds of motion, such as optic flow and simulated saccades. Individual retinas were stimulated with a 16 min clip that was repeated several times. Further details about the statistics of these movie clips can be found in reference (Puchalla et al., 2005). The mean intensity of the monitor was 12 mW/m².
The stimulus sequence started with about 5 minutes of spatially uniform light steps – 1 second ON and 1 second OFF – in order to adapt the retina to the light intensities of the monitor. This part of the stimulus was not included in the analysis. Then, we used the flickering checkerboard for 60-120 minutes, followed by another 5 minutes of ON/OFF light steps. The second ON/OFF stimulus was used for our clustering analysis. In some of the experiments, we next stimulated the retina with natural movie clips, in order to calculate the shared information. Our results did not change at all if we instead used the first segment of light ON/OFF rather than the second. Furthermore, our results did not change at all if we used only the first half or only the second half of the flickering checkerboard data. This indicates that retinal adaptation and other forms of non-stationarity were not significant in this study.

**Receptive field analysis.** The receptive field of each cell was mapped by calculating the average stimulus pattern preceding a spike under random checkerboard stimulation. This spike-triggered average (STA) is a function of spatial coordinates \( x \) and \( y \) (50 µm bin), time before the spike \( t \) (8.33 ms bin), and color index \( l \) (red, green, or blue). The central region of the receptive field was identified by finding all the squares with a time course of the same shape as the square with the maximal response. The STA in the central region was closely approximated as the product of three functions: the temporal dynamics \( A(t) \), spatial profile \( B(x,y) \), and chromatic sensitivity, \( C(l) \) (Schnitzer and Meister, 2003). The chromatic sensitivity was defined as the amplitude of the STA for each of the red, green, and blue gun (\( l = 1, 2, \) and 3) of the computer monitor. For convenience, each of the three functions, \( A \), \( B \), and \( C \), was separately normalized to have unit length (Schnitzer and Meister, 2003). The spatial profile, \( B(x,y) \), was fit using a 2-dimensional Gaussian, giving center coordinates, \( \bar{x} = (x_0, y_0) \), one-sigma radius \( \sigma = \sqrt{\sigma_x \sigma_y} \), and area \( A = \pi \sigma_x \sigma_y \). The eccentricity was \( \varepsilon = \sqrt{1 - \sigma_{\text{min}}^2/\sigma_{\text{max}}^2} \), where \( \sigma_{\text{max}} \) and \( \sigma_{\text{min}} \) are the major and minor radii, respectively.

The normalized receptive field distance between cells \( i \) and \( j \) was defined by:

\[
D'_{ij} = \frac{\bar{x}_i - \bar{x}_j}{\sqrt{(\sigma_i + \sigma_j)}}.
\]

**Classification of ganglion cells.** We used a broad stimulus ensemble – spatial, temporal, and chromatic flicker – to classify cells into types having systematically different visual sensitivities (Meister et al., 1994; DeVries and Baylor, 1997; Schnitzer and Meister, 2003; Puchalla et al., 2005). In addition to the spatio-temporal receptive
field, we supplemented our characterization of ganglion cell function with the firing rate in response to diffuse steps of light (Hartline, 1938; DeVries and Baylor, 1997; Burkhardt et al., 1998; Carcieri et al., 2003) as well as with responses to gratings drifting in different directions (Barlow and Levick, 1965; Amthor et al., 1989). The functional similarity between two cells $i$ and $j$ was initially quantified by computing the mean-squared difference between the temporal dynamics of the centers of their receptive fields, $A_{ij} = \sum_t [A_i(t) - A_j(t)]^2$, normalized by the median mean-squared difference between all pairs of single cells, $a_i = A_i / \text{med} \left\{ A_i \right\}$. With this normalization, the typical mean-squared difference between two cells was roughly one, and differences much greater than one indicated that the cells were functionally very dissimilar.

We included three other response characteristics to our quantitative measure of functional similarity. The difference between the receptive field size of two cells was measured by computing $B_{ij} = [\sigma_i - \sigma_j]^2$, where $\sigma_i$ is the radius of the receptive field center for cell $i$. Again, we normalized by the median difference between all cells, $b_i = B_i / \text{med} \left\{ B_i \right\}$. The difference between two cell’s auto-correlation functions $F(t)$ (calculated up to 20 ms with a 1 ms time bin) was measured by $f_{ij} = \sum_t [F_i(t) - F_j(t)]^2$ and normalized by the median difference between all cells to obtain $f_{ij}$. We use the checkerboard stimulus for calculation of $F(t)$. The difference between two cell’s firing rate in response to steps of light $R(t)$ was measured by $r_{ij} = \sum_t [R_i(t) - R_j(t)]^2$ and again normalized in the same fashion to obtain $r_{ij}$. We combined multiple measures of functional similarity by averaging the normalized values together; for instance, combining all four measures gives $D_{ij} = \frac{1}{4} (a_{ij} + b_{ij} + f_{ij} + r_{ij})$. Notice that combinations are formed using normalized measures of function difference, such as $a_i$ not $A_i$. Normalization is necessary because individual measures have different units, such as spikes/s for $R_{ij}$ and microns for $B_{ij}$. We used the median for our normalization in order to reduce the susceptibility to the outliers of the distribution. Because each measure was normalized, the resulting average effectively weighted each response characteristic equally. We experimented with changes in the relative weight of each
response characteristic, but did not find any significant changes in the resulting functional classification.

We did not include the difference in cell’s chromatic profiles, as the chromatic sensitivity was found to be essentially identical for every cell, or the directional index, as salamander ganglion cells did not show significant directionality. In addition, the surround was left out of the classification process because anatomical studies use only the shape of the dendritic tree to classify cells. Furthermore when we do measure the temporal dynamics of the receptive field surround, we find that its dynamics closely reflect the dynamics of the centre. This implies that adding the temporal dynamics of the surround to our classification would not change the resulting clusters.

**Broad cell types:** Functional classification was carried out using the well-known method of agglomerative clustering, which is an iterative algorithm (Duda et al., 2000). At the outset, each cell formed its own cluster. First, we found the pair of clusters that had the smallest mean-squared difference, $D_i$. Then, these clusters were merged into a single cluster by averaging all of their properties, weighted by the number of cells in each cluster. This procedure was repeated until all cells were merged into a single cluster. The significance of each merger was evaluated using the merger score, which is the functional difference between the two clusters that were merged together. By looking at the merger score as fewer and fewer clusters remain (fig. 1C, fig. 2, fig. 3), we can find that the differences between clusters suddenly become large, which indicates that these clusters are significant.

An alternative way to set the significance threshold is by looking at the histogram of the merger score. In this manner, one can identify all of the outlier values of the merger score and set the number of clusters as the maximal number that includes all these outliers. For example in figure 2A left panel, we identify 5 such outliers, which include the first four mergers and also the tenth merger. Since we take the number of clusters as the maximal number of all these outliers, in this case the number is the tenth merger, which implies 11 clusters. From these 11 clusters, 5 contain only 1 or 2 cells and therefore we regard these cells as unclassified. The rest form 6 broad classes of cells.
Our choices of significance threshold are in every case indicated as a dashed line on plots of the merger score versus number of clusters (figs. 2 and 3). Further discussion of the issue of choosing the number of significant clusters in a data set can be found in several interesting books and articles (Jain et al., 1999; Duda et al., 2000; Kleinberg, 2002). For broad functional types, the algorithm was applied to all of the cells recorded from multiple retinas.

**Similarity matrix:** In order to visualize the patterns of functional segregation present in the ganglion cell population, we generated a similarity matrix between cells. In this matrix, each element is the similarity $D_{ij}$ between cells $i$ and $j$, as defined above for each response characteristic or for averages over several response characteristics. Initially, the cells were in a random order, and therefore no features can be observed in the matrix (fig 2A, middle). After applying the clustering algorithm, we reorganized the matrix such that neurons that belong to the same class were grouped together in adjacent rows/columns (fig 2A, right). Since the cells that belong to a single group are functionally similar, they should have small difference values between all pairs in the group. This will appear as a block of low difference values (shown by colors close to red) in the reordered similarity matrix. Conversely, the regions that correspond to ganglion cells from different functional types should all have high difference values (shown by colors close to blue). Therefore, if the ganglion cell population exhibits clustering into distinct functional types, the reordered matrix will have a clear block structure. If instead the functional distinctions within the population are mostly subtle, then the reordered matrix will not show clear block structure.

**Fine cell types:** In order to split the ganglion cell population into as many types as could be justified by the data, we used K-means clustering to define cluster boundaries, as this method is known to be biased towards forming extra clusters when used on data with relatively few examples (Duda et al., 2000). In K-means clustering, we first decide how many clusters the data will be divided into and randomly assign one ganglion cell to each cluster. All remaining ganglion cells are assigned to the nearest cluster, based on the (normalized) mean-squared difference between cell $i$ and cluster $k$, $a_{ik}$. For this analysis, we used only the temporal dynamics of the receptive field center, $A_{ij}$. Next, the cluster waveform is computed by averaging the temporal waveforms of all members. At this point, a goodness-of-fit measure is obtained by
calculating the total mean-squared difference between all cells and their respective clusters, $\bar{a} = \sum_i a_{ik}$. The algorithm iterates by starting with the new cluster waveforms and reassigning all ganglion cells to the nearest cluster. This iteration is continued until the total difference between cells and clusters, $\bar{a}$, no longer decreases. Because the resulting cluster structure depends on the choice of initial clusters, we repeated this algorithm with 1000 different random choices of initial cluster definitions for each value of $K$ and selected the final cluster partition that had the smallest total difference, $\bar{a}$.

The number of clusters $K$ is a parameter of this algorithm, and as more clusters are used to describe the population, the total difference $\bar{a}^{(k)}$ must decrease. In order to determine what value of $K$ resolves significant clusters, we plotted the decrease in the total difference as new cluster were added, $\Delta^{(k)} = \bar{a}^{(k)} - \bar{a}^{(k-1)}$. When this decrease is large, clusters are significant, and when the decrease is small, the new clusters resolve only minor details in the ganglion cell population (fig. 6A).

**Coverage factor.** The coverage factor of each cell type was defined as $C = A_{\text{type}} \rho_{\text{type}}$, where $A_{\text{type}}$ is the average area within the one-sigma receptive field radius of all cells of a given type, and $\rho_{\text{type}}$ is the density of that cell type. Cell type density was determined by multiplying the total ganglion cell density by the fraction of all recorded cells from that cell type. For salamander, we assumed a ganglion cell density of 1400 cells/mm$^2$ in this calculation (Segev et al., 2004). The average eccentricity of ganglion cell receptive fields was $\varepsilon = 0.46$; the minimal and maximal eccentricities were 0.09 and 0.81 respectively. Our definition of the coverage factor is an average that does not reflect the detailed orientation of each cell’s receptive field.

**Shared information between ganglion cells.** In order to measure the functional similarity between ganglion cells in a manner that does not rely on prior functional classification or on strong assumptions about how ganglion cells encode visual scenes, we computed the shared information $I(R_1;R_2)$ between the responses $R_1$ and $R_2$ of two ganglion cells. This quantity is an upper bound on the redundancy between cell pairs (Schneidman et al., 2003) and has values that quite closely correspond with the redundancy. We estimated the shared information using a procedure very similar to that described in (Puchalla et al., 2005) for the redundancy. In brief, the response of
each cell was mapped into spike words by binning the spike train in 10 ms time windows and concatenating $K$ time bins to form word, $W$ (Strong et al., 1998; Puchalla et al., 2005). The joint distribution of spike words $(W_1, W_2)$ for each cell pair was compiled during the entire experiment and used to evaluate $I(R_1; R_2) = H(R_1) + H(R_2) - H(R_1; R_2)$. The entropy of each cell's response is given by $H(R_i) = - \sum_{w_j} p_i(w_j) \log p_i(w_j)$, where $p_i(w_j)$ is the probability of spike word $j$ for cell $i$, and the joint entropy of both cell's responses $H(R_1; R_2)$ has an analogous formula using the probability distribution of joint spike words. The firing rates of individual ganglion cells varied by a factor of over 100 within the population, and the shared information depended strongly on this firing rate. So to put all cell pairs on a single, comparable scale, we normalized by $\min\{H(R_1), H(R_2)\}$. This normalization factor is an upper bound on the mutual information, so that the normalized shared information, $\Omega$, ranges between 0 and 1. Cells that are completely independent in their function have zero shared information, and two cells that are identical have $\Omega = 1$. Note that this normalization is different from that used in (Puchalla et al., 2005), so that the value of the shared information is not necessarily smaller than the redundancy. A comparison of the two quantities indicated that they correspond quite closely: cells with large redundancy had large shared information, and cells with zero redundancy also had zero shared information.

Correction for finite data size was made by extrapolating the trend in spike train entropies from 1/4 of the data set to all of the data. To best capture the effect of correlations between successive time bins, the entropy trend was extrapolated from words of $K=3$ digits up to $K=6$ digits. A final bias correction was made by randomly shifting one cell’s spike train relative to the other cell. The shared information between shifted spike trains should be zero, but instead had values ranging up to ~3% for strongly correlated cell pairs because of finite sampling. We subtracted the value for shifted spike from the value for the original spike trains. As a test of our bias correction, we note that ON/OFF cell pairs from different retinal patches (with spacing $D' > 2$) had an average shared information of $\Omega = 0.0004 \pm 0.002$ ($n = 62$ pairs). Because such cell pairs are expected to have zero shared information, this result implies that our estimate has a remaining upward bias of 0.04% and a random error of 0.2%. The overall
pattern of shared information within the population (fig. 9) was not qualitatively changed either by this normalization or by our bias correction.

RESULTS
To understand the manner in which ganglion cells collectively represent a visual scene, we measured the spatio-temporal receptive field of every recorded ganglion cell in a small patch of the retina. As shown in figures 1A and 1B, the receptive field was factored into spatial, temporal, and chromatic components (see Methods). Because our recordings had very little sampling bias, we could estimate the total coverage of visual space by all of the ganglion cells, which is the average receptive field area times the total ganglion cell density (see Methods). This coverage was 59 ± 5 (mean ± S.E.M., n = 3 retinas, 103 cells) in the salamander under these visual conditions, indicating either that there are ~60 functional types assuming all cell types tile or that there are several types that have significant overlap.

Classification of Ganglion Cells
Previous studies have classified ganglion cells into functional types using a variety of methods, relying on different measures of functional similarity and on different ensembles of visual stimuli (Lettvin et al., 1959; Maturana et al., 1960; Grusser-Cornehls and Himstedt, 1973; Cleland and Levick, 1974b, 1974a; Hochstein and Shapley, 1976; Caldwell and Daw, 1978; Stone, 1983; Roth, 1987; DeVries and Baylor, 1997; Carcieri et al., 2003; Schnitzer and Meister, 2003). Our objective here was to use quantitative clustering techniques along with a very broad set of stimuli, so that our results better reflect the information encoded by ganglion cells under natural visual conditions. As there exists no a priori method of functional classification, we have made several choices of stimulus set and clustering method in order to divide the ganglion cells into broader or finer groupings.

Broad types
Following previous studies (DeVries and Baylor, 1997; Schnitzer and Meister, 2003), we initially used the temporal dynamics of the receptive field center to classify ganglion cells into functional types. We formalized classification using an iterative algorithm that at each step merged the most functionally similar cells into the same cluster and averaged their receptive fields together; similarity was judged by the mean-squared
difference between the receptive field center dynamics of two cells, normalized by the median difference between all cells (see Methods). With this normalization, the typical difference between cells was 1. By examining the similarity of the clusters that are merged at each step of this algorithm, we can assess the significance of the merger: when two clusters are very similar, their merger score will be close to zero, and when two clusters are very different, their score will be greater than one.

Figure 1C shows the results of this clustering algorithm when applied to 103 ganglion cells recorded from 3 retinas in the salamander, whose temporal profiles are shown in figure 1D. There was a clear break in the similarity of cell clusters, shown by a dashed line. At this point, there were six distinct clusters with multiple members – fast ON, slow ON, biphasic OFF, monophasic OFF, medium OFF, and slow OFF (shown in colors) – as well as six cells that belonged to their own cluster (shown in grey). The distinct clusters had members recorded from all three retinas used in this study and were routinely observed in other experiments, so we treated them as broad types. Unique cells were observed in a single retinal patch and were not commonly seen in other experiments, so we treated them as unclassified. The classification of cells into six broad types is a robust property of the temporal dynamics of the receptive field center. When we used a different measure of the similarity of ganglion cells – the overlap between two cell’s temporal profile rather than the mean-squared difference – the same six classes emerged from our clustering algorithm (Puchalla et al., 2005).

Other response measures
There are many other aspects of a ganglion cell’s light response upon which to base functional classification. To explore whether other response measures could help resolve additional functional classes, we added to our analysis the radius of the spatial profile of the receptive field center, the auto-correlation function measured during random flicker stimulation, and the firing rate during diffuse ON and OFF steps of light (see Methods). These same three measures were used to resolve functional types in a systematic, multi-electrode array study of ganglion cells in the rabbit retina by DeVries and Baylor (DeVries and Baylor, 1997). For each measure, we calculated the mean-squared difference between ganglion cells and normalized by the typical distance, as we had for the temporal dynamics of the receptive field center. Individual measures were averaged together to give a total difference score between ganglion cells (see labels on each panel of figures 2 and 3), and the same agglomerative clustering
algorithm described above was used to assign cells to functional classes (see Methods).

Figure 2 shows the results of this clustering analysis on a different set of 99 ganglion cells from three retinas, where we measured multiple response characteristics. On the left is the merger score as a function of the number of clusters, and on the right is the matrix of similarities between all pairs of cells both before and after the clustering process (see Methods). When using only the temporal dynamics of the receptive field center (fig. 2A), the post-clustering matrix was clearly organized into 5 or 6 large blocks, where all the members of the block have high similarity with each other and lower similarity with cells in other blocks. Each block corresponded with a distinct, significant cluster found by the clustering algorithm. If we take the number of broad types to be six, those types are the same as those illustrated in figure 1: fast ON, slow ON, biphasic OFF, monophasic OFF, medium OFF, and slow OFF.

When clustering was performed with the size of the receptive field center, 5 clear blocks were evident. However, membership in these clusters did not always correspond with membership in the clusters formed by the receptive field dynamics. (We return to this point in more detail in fig. 4). When using the auto correlation function for clustering (fig 2B), we found 3 main blocks corresponding to 3 classes, along with many outliers. However, one class contained almost all of the cells. Again, the correspondence between membership in classes based on the auto-correlation function and the previous classes was not precise (see fig. 5). Finally, when we clustered using the response to diffuse steps of light, we detected again 3 classes. One class was dominant and would be classically defined as an OFF class according to its response to diffuse flash and two would be defined as ON/OFF classes. Again, these classes did not always correspond with the previous classes, and we also saw response patterns more complex than typically described (see fig. 6).

When the spatial extent of the receptive field center was combined with the temporal profile (fig. 3B), we were surprised to see that no additional subtypes were resolved. Although these measures each divided the population into 5 or 6 types by themselves, the combined clustering only revealed 4 broad types. Furthermore, an examination of the similarity matrix shows that two the major clusters formed using the receptive field dynamics alone actually added new members when the receptive field size was
simultaneously included. Adding in the autocorrelation function also did not resolve any further subgroups (fig. 3C). The resulting membership in the 4 major clusters was not changed, but the degree of functional similarity within these clusters was reduced. Thus, inclusion of the auto-correlation function in our combined cluster analysis served to blur the boundaries rather than resolve new subtypes. Finally, adding the response to steps of light further blurred these categories to the point that the two largest clusters from panel 3C were no longer distinct (fig. 3D). Thus, this response characteristic also does not resolve any more subgroups.

Why do these additional response characteristics blur the distinctions between broad functional types formed using the receptive field dynamics alone? First, each of these response characteristics had great diversity within the ganglion cell population, such that relatively few broad classes could be defined using each characteristic individually. This diversity is perhaps better described as a functional continuum than as several distinct classes. Second, the classes defined by the clustering algorithm did not fall precisely within the classes defined by the temporal dynamics of the receptive field. For instance, the distribution of receptive field radii had only three, extensively overlapping peaks (fig. 4). While both slow ON and slow OFF cells tended to have large receptive fields (fig. 4A), the monophasic OFF cells had both small and medium sized receptive fields (fig. 4B), and the medium OFF cells could have any size of receptive field (fig. 4C).

By itself, the auto-correlation function split the ganglion cells into only three broad types: very bursty cells (fig. 5A) with a short refractory period (1-2 ms) under our stimulus conditions and an abundance of inter-spike intervals around 2-3 ms; bursty cells (fig. 5B) with a longer refractory period and many intervals around 5-10 ms; sustained cells (fig. 5C) with a much longer refractory period and relatively few short inter-spike intervals. Again, it should also be kept in mind that both the bursty and sustained classes possessed considerable diversity. Very bursty cells were often fast ON, but could also be OFF-type (fig. 5A). Most cells fell into the bursty category, making this a group quite diverse. Bursty cells tended to come from three of the broad types – biphasic OFF, monophasic OFF, or medium OFF – but the correspondence was not exact. For example, figure 5B shows four cells with identical auto-correlation functions; two of them are monophasic OFF and two are slow OFF. Sustained cells were often slow OFF or slow ON, but figure 5C again shows cells with nearly identical
auto-correlation functions, where one of them is medium OFF. As a result of this inconsistent correspondence between types of auto-correlation functions and types of receptive field dynamics, the auto-correlation function tended to blur the functional categories. The reader might be tempted to think that the data of figure 5B and 5C provide evidence that our six broad types have clear subtypes. This is not the case, because there are many intermediate cases that are not shown in the figure.

The light step response showed a broad continuum of ratios between the number of spikes elicited by ON steps versus OFF steps. While most biphasic OFF, monophasic OFF, and medium OFF cells responded to both ON and OFF steps to some degree, the pattern was not precise. Figure 6A shows an example of three biphasic OFF cells; two of them responded transiently to both ON and OFF steps (red), while the other responded only to OFF steps (black). The population also had a range of responses that were more transient (fig. 6A) versus those that were more sustained (fig. 6B). Transient cells tended to come from biphasic and monophasic OFF-types, while sustained cells tended to be slow or medium OFF-type. Again, many exceptions existed. Figure 6B shows an example of four ganglion cells with similar, sustained responses to steps of light; two cells are slow OFF-type, but the other two are monophasic OFF-type. In addition, some ganglion cells had more complex responses, showing multiple bursts of firing (fig. 6C). As a result of the great diversity of responses to steps of light, this response measure significantly blurred the functional classes identified using other response characteristics.

We conclude that for the salamander, the response characteristic that divides the population into the most clear functional classes is the temporal dynamics of the receptive field center, $A(t)$. This measure resolves six broad functional types: fast ON, slow ON, biphasic OFF, monophasic OFF, medium OFF, and slow OFF. Adding more response characteristics tended to blur these classes rather than resolve clear subclasses, so we treat the clusters resolved by the receptive field center dynamics as the six broad functional types of ganglion cells in the salamander.

**Fine types**
Because agglomerative clustering algorithms lump the ganglion cell population into no more than six broad functional types, we wanted to explore other clustering schemes that might resolve more types. Our approach was motivated by the observation that for
data recorded from a single patch of the retina, where ganglion cells presumably shared inputs from some of the same presynaptic neurons, we often found several ganglion cells with exceptionally similar functional properties. Building on this observation, we used a fine classification scheme, where we considered only cells from a single retinal patch, and used only the receptive field dynamics, as other response characteristics did not help to resolve more classes. In order to divide the population into the maximal number of cell types allowed by the data, we used K-means clustering to define cluster boundaries (see Methods). This algorithm is known to be biased towards forming extra clusters when used on data with relatively few examples (Duda et al., 2000). As a result, our definition of fine functional types probably split the ganglion cell population into too many types. Over-splitting will lead to a decrease in the coverage factor and a greater chance of observing territoriality, but it will also give rise to cells of different type that encode very similar visual features.

Figure 7 shows examples of fine types formed from ganglion cells recorded in two different retinal patches (left column, 21 cells; right column 29 cells). As more clusters were formed, the total difference between the temporal profile of individual ganglion cells and cluster averages decreased (fig. 7A-B). For the first retinal patch (left column), this decrease $\Delta^{(k)}$ was large when the number of clusters was 10 or less, and dropped significantly when more than 10 clusters were formed. As a result, we divided this group of 21 ganglion cells into 10 fine types (fig. 7C, color). For the second retinal patch, a transition in the clustering score $\Delta^{(k)}$ was found after 7 clusters. Consistent results were found for other retinal patches, with a total number of fine types ranging up to 12, depending in part on how many cells were recorded in a single patch. Our fine classification scheme was consistent with the broad scheme: fine functional types were either the same as a broad type or they were subtypes within a single broad type; the fine types never combined cells from different broad types. Ganglion cells of only three of the broad types – monophasic OFF, medium OFF, and slow OFF – were resolved into multiple categories by our fine clustering. Biphasic OFF cells emerged as a single, clear fine type. Both fast ON and slow ON cells tended to fall into a single fine type, although these classes might split into two or more fine types if we could record more examples in a single retinal patch.
Coverage and Territoriality of Receptive Fields

Tiling consists of two different properties. First, ganglion cells of each class should be territorial, meaning that the location of each cell’s soma avoids other cells of the same type (Wässle and Boycott, 1991). Second, the coverage factor of each type of ganglion cell should be roughly equal to one. When we compared the spatial profiles of ganglion cells recorded from the same patch and corresponding to the same fine type, we often found extensive overlap. Several examples are shown in figures 7E and 7F with cells of the same fine type displayed in the same color. To quantify the territorial organization of each cell type, we calculated the distance between ganglion cells and normalized that distance by the sum of their receptive field radii; this measure gives a value of 1 when adjacent receptive fields just barely touch (see Methods). The distribution of normalized receptive field distances $D'$ was statistically the same between cells of the same fine type as between cells of different fine type (fig. 8A). This indicates that most ganglion cell types in the salamander retina do not have a territorial spatial organization. However, we did find one exception. The biphasic OFF cell had a low coverage factor, and its receptive fields just barely touched in most cases (figs. 8B and 8C). In addition, these cells had the largest action potentials recorded and appeared in our measurements with a fraction (~7%) close to the fraction of myelinated axons in the optic nerve. These data suggest that the biphasic OFF cell may be analogous to the alpha ganglion cell found in the mammalian retina, a cell type that has been shown to have a territorial organization (Wässle and Boycott, 1991).

When we estimated the coverage factors of different cell types, we found values considerably greater than one. This result comes as no surprise, since the total coverage of the ganglion cell population was ~60 and many fewer functional types could be resolved. For the broad types, the coverage factors were: fast ON 5.1, slow ON 5.6, biphasic OFF 2.5, monophasic OFF 13, medium OFF 21, slow OFF 10 (see Methods). Dividing cells in the last three types finely rather than broadly still resulted in coverage factors of ~2.5 to 5 for each fine type, although such fine types did not necessarily correspond from one retinal patch to the next. Therefore, regardless of what method of classification we used to decompose the ganglion cell population into cell types, extensive overlap of receptive fields was found for cells of the same functional type. This indicates that within each ‘channel’ of visual information, defined as all of the ganglion cells of the same functional type, multiple cells sample the same features of a visual scene.
**Shared Information between Ganglion Cells**

The definition of coverage that we have discussed so far is completely dependent on the results of our functional classification. As there are many possible algorithms one might use to perform functional classification as well as many possible response characteristics, we would like to have an assessment of functional overlap that is less dependent on such arbitrary choices. To this end, we used information theory to quantify the functional similarity between ganglion cells. We calculated the shared information, which is closely related to the redundancy between two cells (Schneidman et al., 2003). Because of the wide variety of average firing rates found in the ganglion cell population, we normalized the shared information, $\Omega$, to have a value between zero for independent cells and one for identical cells (see Methods).

The shared information can be evaluated between cells of either the same or different functional type, and it does not rely on making assumptions about the neural code, such as that a ganglion cell’s function is completely described by its classical receptive field or that each spike conveys the same visual message. In addition, the shared information can be calculated during stimulation with long sequences of natural movies (see Methods), allowing us to evaluate functional similarity under the most realistic operating conditions possible. Natural stimuli may evoke different patterns of functional overlap between ganglion cells both because they contain visual features, like wide field motion or optic flow, not contained in simpler, artificial stimulus ensembles as well as because they have complex statistics that can evoke different mechanisms of retinal adaptation, which alter a ganglion cell’s spatio-temporal receptive field and could lead to differences in the outcome of a cluster analysis based on the receptive field.

We can use the shared information to evaluate how sharply the ganglion cell population clusters into distinct functional types and to test whether more functional types can be resolved. If two cells with overlapping receptive fields encode completely independent visual features, than those cells will have zero shared information. In this case, the neurons should clearly belong to different functional types. However, we might also want to assign cells to different functional types if a less extreme condition is met. Cells of the same functional type should at least share more visual information, at equivalent spatial overlap, than pairs formed from cells of different functional types. This is fundamentally what it means to assign cells to distinct functional types: namely, that cells of the same type have greater functional similarity than cells of different type.
The shared information is a much more abstract quantity than the similarity between receptive fields. In order to gain intuition about it, we show four specific examples of the shared information between ganglion cells and compare it with the cross-correlation function. For cells of the same functional type and having large spatial receptive field overlap, there was often a prominent peak in the cross-correlation function near zero time lag, and the shared information was relatively high (fig. 9A, two monophasic OFF cells, \(\Omega = 19\%\)). For pairs formed by one ON cell and one OFF cell, there was no peak in the cross-correlation function, and the shared information was close to zero (fig. 9B, \(\Omega = 0.5\%\)). But the pattern of information sharing in the ganglion cell population was quite complex. In some cases, cell pairs formed from very different function types had high shared information (fig. 9C, biphasic OFF/slow OFF, \(\Omega = 14\%\)). In other cases, cell pairs of the same functional type and large spatial overlap had much lower values of the shared information (fig. 9D, two monophasic OFF cells, \(\Omega = 7.5\%\)).

In all cases, there was a correspondence between the value of the shared information and the cross-correlation function: cell pairs with high shared information had a large peak in the cross-correlation function near zero time lag (although this peak varied in width, shape, and exact time lag), and cell pairs that shared no visual information had no such peak. This comparison indicates that the dominant form of correlation between ganglion cells is a tendency to fire spikes synchronously, as described in many previous studies (Mastronarde, 1989; Meister et al., 1995; Brivanlou et al., 1998; DeVries, 1999), and that this correlation is closely related to the functional similarity between ganglion cells (Puchalla et al., 2005). Anti-correlation was found only very rarely, presumably because salamander ganglion cells have very sparse spike trains (Berry et al., 1997).

To study how visual information is distributed within the entire population, we plotted the shared information \(\Omega\) versus the receptive field distance \(D'\) between 604 pairs of ganglion cells recorded from two retinas under naturalistic visual stimulation (fig. 10A). Pairs formed from ganglion cells of the same broad functional type based on their receptive field center dynamics are shown in color corresponding to their functional type, and cell pairs of different type are shown in grey. Shared information values were larger for nearby ganglion cells and decayed to zero for separations greater than \(D' \approx 2\) receptive field spacings, indicating that ganglion cells were systematically independent.
at this distance apart. Under natural visual conditions, the stimulus has long-range spatial correlations and lots of wide-field motion. However, ganglion cells manage to reduce these long-range correlations and act independently when they are far apart. One should keep in mind that independence of neurons activity leads to independence of the information being conveyed by these cells. This is an indication that ganglion cells represent information only about a restricted spatial region. Biphasic and monophasic OFF pairs tended to share more information than medium or slow OFF cells, but exceptions to this pattern were found. Most notably, many cell pairs of the same functional type shared less information than pairs of different type at similar spacings (color vs. grey). Figure 10B shows a specific example of this mixing of information between different broad functional types: pairs of monophasic OFF cells (yellow dots) had values of shared information comparable to that of pairs formed from one monophasic OFF cell and one medium OFF cell (green diamonds). Similar results were found for other combinations of functional types; similar results also were found under stimulation with flickering checkerboards (data not shown).

Some functional types were distinct: pairs formed from one ON- and one OFF-type cell were found to be systematically independent (fig. 10C). Thus, these two cell populations form truly distinct channels of visual information (Puchalla et al., 2005). In addition, the largest values of the shared information were only ~25%, implying that cells of the same functional type still had subtle but significant functional differences. Together, the analysis of shared information confirms and strengthens the conclusions of our functional classification, namely that multiple ganglion cells with subtly different properties jointly participate in encoding the same features in a visual scene.

DISCUSSION
We conclude that many functional types of ganglion cells in the salamander have coverage factors much larger than one, and that most lack a territorial organization. This was true even when we split the population more aggressively by forming fine functional types. Our study used a new multi-electrode technique that allowed us to record from all or nearly all of the ganglion cells in a small patch of the retina (Segev et al., 2004). We relied on quantitative methods of functional classification, involving several choices of clustering algorithm as well as several choices of response characteristic. Because any method of functional classification requires some arbitrary
choices, we supplemented this approach by analyzing the shared information between ganglion cells, a measure of functional similarity that can be calculated during stimulation with natural scenes and makes minimal assumptions about how ganglion cell spike trains encode the visual world. These two approaches gave consistent and mutually reinforcing results: namely, that the ganglion cell population in the salamander can only be resolved into a small number of functional types, and all but one have highly overlapping receptive fields. Our data even suggest that these six broad functional types are not completely distinct from each other (Schneidman et al., 2002).

**Functional Classification**

Our classification of salamander ganglion cells into six broad functional types (4 OFF-types, 2 ON-types) is in good agreement with the number of cell types defined in previous classifications performed in the salamander. Physiological classification studies using either simple stimuli (Grusser-Cornehls and Himstedt, 1973) or white noise (Warland et al., 1997) have described four functional types (3 OFF-types, 1 ON-type). The slow ON cell type that we observed has not been previously reported, so the only difference with these studies is whether there are 3 or 4 OFF-types, which is somewhat ambiguous in our data as well. Quantitative anatomical studies have classified salamander ganglion cells into 5 morphological types (Toris et al., 1995; Costa Lda and Velte, 1999), which agrees with our number of broad types. In this work, the primary feature that distinguished anatomical types was the size of the dendritic field, which fell into three groups. Similar to their results, we found three broad classes of receptive field size (fig. 4). However, it is not clear how these morphological types map onto our functional types, in particular because receptive field size did not resolve more types than were found using the temporal dynamics of the receptive field center.

Burkhardt classified salamander ganglion cells into three main types based on the response to steps of light – ON, OFF, and ON/OFF (Burkhardt et al., 1998). When we looked at the responses to diffuse steps of light, these three categories were evident, but each contained a great variety of different responses. This variety included a continuous range of response latencies as well as examples of cells with two or three peaks of firing in response to a single step of light (fig. 6c). Almost all of the cells of biphasic OFF-, monophasic OFF-, and medium OFF-type, in fact, had responses to both the onset and offset of light. This large group comprised 76% of all retinal ganglion cells in the salamander, which is consistent with Burkhardt’s finding that 68%
of all cells were ON/OFF and Toris’s finding that 80% of all cells had dendrites that were bistratified in the inner plexiform layer (Toris et al., 1995). Interestingly, the response of salamander bipolar cells to steps of light exhibits considerable variety (Burkhardt and Fahey, 1998; Pang et al., 2004). Presumably, this great functional diversity is the basis for much of the diversity we observe at the level of the ganglion cells.

Many studies have used anatomy or a combination of anatomy and physiology to classify retinal ganglion cells. However, it is important to keep in mind that anatomical and functional classification are distinct projects that need not give the same results. For instance, ganglion cells with nearly identical dendritic morphologies may in fact receive synapses from different bipolar and amacrine cells or may express different balances of ionic conductances. Furthermore, ganglion cells with subtle but distinguishable dendritic morphology (e.g. curved vs. straight dendrites) may actually instantiate virtually the same function. Despite the impressive evidence in favor of a close relationship between structure and function (Sterling, 1998; Masland, 2001; Wassle, 2004), this correspondence has not fully converged, as seen in recent, systematic recent studies carried out in the rabbit retina (DeVries and Baylor, 1997; Roska and Werblin, 2001; Rockhill et al., 2002). Although all these studies found between 10 and 13 ganglion cell types, they do not actually agree on what those cell types are, nor are they completely consistent with classic studies (Cleland and Levick, 1974a, 1974b; Caldwell and Daw, 1978). The existing data do not rule out the possibility that retinal ganglion can be resolved into more distinct classes based on their anatomy than based on their responses to light. While anatomy might thus provide a more satisfying basis for a descriptive characterization of variety in the ganglion cell population, one must keep in mind that a characterization of the similarities and differences in function within the population is ultimately more fundamental for understanding how the system works.

An important component in our analysis is the ability to combine data from several preparations, which may introduce additional variability in the response properties of ganglion cells that might not be present within a single retina. However, all of the qualitative results that we report here – the lack of correspondence of different response characteristics within functional types (figs. 4, 5, and 6), the overlap and lack of territoriality for most fine functional types (figs. 7 and 8), and the mixing of visual
information among broad functional types (figs. 9 and 10) – were observed among ganglion cells recorded from the same retinal patch. Therefore, prep-to-prep variability cannot explain any of these results. We also note that many of the classic studies of retinal function have relied on methods that record only a few ganglion cells per animal. Our study, where we have been able to record 30-40 ganglion cells per animal, represents a marked improvement.

**Spatial Organization of Receptive Fields**

In the most detailed physiological study of the functional organization of the ganglion cells to date, DeVries and Baylor used a multi-electrode array to record many ganglion cells from the rabbit retina (DeVries and Baylor, 1997). They found extensive evidence of territoriality and a coverage factor of ~1.8 for the brisk transient cell type (using the same definition of coverage). We found similar results for the biphasic OFF cell type, which may be analogous to the brisk transient cell type. However, all other cell types lacked a territorial organization of their receptive fields. The DeVries-Baylor study differs from ours, in part because they recorded only a small fraction (≤15%) of all of the ganglion cells; in addition, their methods are likely to be biased in favor of cells with large amplitude spikes and non-overlapping receptive fields. In fact, when we used single channel spike sorting methods, we sometimes found that over half of the cleanly isolated cells in the salamander were of the biphasic OFF type, while our new multi-channel method reveals that the fraction of biphasic OFF cells is only ~7%. Another important difference is the species studied. We have identified only six cell types, while studies in the rabbit and other mammalian retinas have described 10-15 cell types (Masland, 2001; Roska and Werblin, 2001; Wassle, 2004). One possible explanation is that the mammalian retina is more precisely organized than the salamander.

Anatomical studies in the mammalian retina have demonstrated that several easily distinguishable anatomical types, such as the midget cell (Dacey, 1993), alpha cell (Wassle et al., 1981), or the bistratified DS cell (Vaney, 1994), clearly tile visual space with a coverage close to one. In agreement with anatomy, Frechette et al. used a multi-electrode array to record simultaneously from many parasol ganglion cells in the monkey and found clear tiling (Frechette et al., 2005). Parasol cells have dendritic coverage close to one (Dacey et al., 2003) and are hypothesized to be analogous to the alpha cell in the rabbit (Rodieck, 1998). However, many other cell types are not as
easily distinguished using anatomy. For the salamander, we find that one distinct cell

type, which may be an analog of the alpha cell, roughly tiles. However, the other
ganglion cells have a more diverse and distributed organization. This leads to the
speculation that many of less-well understood cell types in the mammal, variously
called W cells in the cat or sluggish cells in the rabbit, may also have extensive
functional overlap. Of course, significant differences may exist between the
organization of the salamander retina and mammalian retinas. This question can only
be resolved by further experiments.

**Shared Information**

We used the shared information between pairs of ganglion cells to augment the
classification we performed using simple, traditional stimuli. This allowed us to
measure the functional similarity between ganglion cells under natural stimulus
conditions and with minimal assumptions about the nature of the retinal code. The
degree of shared information between ganglion cells was closely related to the chance
that those cells fired spikes synchronously, as measured by their cross-correlation
function (fig. 9). Our analysis revealed extensive mixing of visual information between
functional types, such that cells of different type sometimes were functionally more
similar than cells of the same type with comparable spatial overlap (fig. 10).

Paralleling this analysis, we found that functional groupings made using one response
characteristic did not precisely correspond to groupings made using other response
characteristics. As a result, including additional response characteristics served to blur
functional groupings rather than resolve more clusters. We hypothesize that this
diverse pattern of response properties among the ganglion cells arises primarily from
connections to presynaptic circuitry that are somewhat more heterogeneous than
previously appreciated. However, we also observed a nearly continuous spectrum of
auto-correlation functions in the ganglion cell population. Since the tendency to burst is
strongly influenced by the intrinsic properties of a ganglion cell, we also speculate that
ganglion cells may express a continuum of different ratios of ionic conductances.

Only ON and OFF-type cells were functionally independent, indicating that the
salamander retina sends the brain just two truly independent channels of visual
information (Puchalla et al., 2005). This result is not as obvious as it might seem. ON
and OFF cells should tend to have opposite membrane fluctuations in response to the same visual stimulus, which should make their spike trains anti-correlated, rather than independent. We think that it is the combination of sparse firing with anti-correlated inputs that leads to independence, because sparse cells are silent both when their synaptic input is inhibitory and when their input is not sufficiently excitatory. Furthermore, the temporal profile of ON and OFF cells were not exactly mirror images of each other, so that their inputs may be only weakly anti-correlated. Interestingly, functional classification based on the temporal profile of the receptive field, $A(t)$, resulted in a categorical distinction between ON- and OFF-type cells, as was also found using the shared information. In contrast, classification based on the light step response, $r(t)$, revealed a continuum of responses ranging from ON-OFF to OFF. This comparison further strengthens our conclusion that the temporal dynamics of the receptive field center is the best response characteristic for the purposes of functional classification.

Within the two, broad channels of visual information, there is considerable functional diversity. Because the largest values of the shared information were only ~25%, cells of the same functional type still had significant functional differences (Schneidman et al., 2002). This argues against the view that ganglion cells of the same functional type are ‘nominally identical’. It is not clear whether this degree of cellular individuality benefits the animal. However, one possible advantage is that it allows neurons in downstream brain regions to achieve a huge variety of feature selectivity by forming combinations of single spikes from different ganglion cells, a simple feed-forward operation that can be computed rapidly (Schneidman et al., 2005). This possibility may be particularly important for the salamander, an animal that must make due with only ~45,000 ganglion cells per eye and with relatively simple visual centers in the brain (Roth, 1987; Segev et al., 2004).

Finally we would like to emphasis the difference between previous report by Puchalla et al. (Puchalla et al., 2005). The Puchalla paper dealt with the redundancy of the visual information encoded by ganglion cells, using sparser recordings from the ganglion cells based on single channel spike sorting. This work uses a dense array along with a multi-channel spike sorting algorithm to record all or nearly all of the ganglion cells in each retinal patch, thus achieving essentially complete and unbiased sampling of ganglion cell diversity. This paper then uses different quantitative methods
and multiple response characteristics to assign ganglion cells to functional classes. Finally, we explore the spatial organization of ganglion cells within each functional class, showing that most classes do not tile nor do they even exhibit territorial organization. These results challenge widely-held views about the spatial organization of the ganglion cells as well as their functional diversity, and thus constitute a finding of considerable interest.

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FIGURE CAPTIONS

Figure 1. Mapping the receptive field. A. Spatial profile of a ganglion cell, B(x,y) (color scale) with a 2D Gaussian curve fit to the center (black). B. Time course of the receptive field center – shown separately for the red, green, and blue gun of the computer monitor – did not depend on the color. The relative color sensitivity, C(l), is shown on the right. C. Merger score as function of number of clusters. Significance threshold (red dashed line) identifies 6 broad types and 6 unique cells. D. Temporal dynamics, A(t), of 103 ganglion cells measured from 3 retinas with broad type indicated by color. Cell types had the following frequencies: fast ON 7.8%, slow ON 4.9%, biphasic OFF 6.8%, monophasic OFF 30 %, medium OFF 35 %, slow OFF 8.7%, and unclassified 6.8%.

Figure 2. Classification based on a single response characteristic. A. Classification using the temporal dynamics of the receptive field center for 99 ganglion cells from 3 retinas. Left column: The merger score of iterative algorithm plotted versus the number of clusters. The decision about how many clusters are significant was made by the detection of the sudden drop in the merger score (red dashed line). Middle column: The initial organization of the similarity matrix. The order of the cells was random and therefore the matrix organization was also random. The experiment from which data was obtained is shown below the matrix. Right column: The similarity matrix after ordering according to the significant clusters found in the clustering algorithm. The matrix was organized into blocks that are associated with different clusters; blocks are denoted with labels below the matrix. B. The result of the clustering algorithm when the receptive field center size was used as the similarity measure between cells. Note the explicit definition of the pairwise similarity, D_{ij}. C. The result of the clustering algorithm when the auto correlation function was used as a similarity measure between cells. D. The result of the clustering algorithm when the response to diffuse steps of light was used as the similarity measure.

Figure 3. Classification based on multiple response characteristics. A. The result of the clustering algorithm using the temporal dynamics of the receptive field center for same 99 cells as in figure 2. Left column: The merger score of iterative algorithm plotted versus the number of clusters (dots), with the significance threshold (red dashed line). Middle column: The initial organization of the similarity matrix. Right column: The similarity matrix after re-ordering according to the significant clusters. B.
The result of the clustering algorithm when the temporal dynamics and the size of the receptive field center were averaged together in the merger score. Note the explicit definition of the pairwise similarity, $D_{ij}$. C. The result of the clustering algorithm when the autocorrelation function of the cells was added to the receptive field dynamics and size. D. The result of the clustering algorithm when the response to diffuse light steps was added to the merger score together with all of the characteristics used for C. See Methods for a description of how we chose the significance thresholds.

Figure 4. Distribution of receptive field sizes. A. Frequency of receptive field radii for either slow OFF or slow ON cells (purple, 14 cells) versus all 103 ganglion cells (grey). B. Frequency of receptive field radii for either biphasic or monophasic OFF cells (orange, 38 cells) versus all ganglion cells (grey). C. Frequency of receptive field radii for medium OFF cells (green, 36 cells) versus all ganglion cells (grey).

Figure 5. Types of auto-correlation functions. A. Auto-correlation function for three 'very bursty' ganglion cells (left column) along with the temporal dynamics of the receptive field center for the same cells (right column). B. Auto-correlation function (left column) and temporal profile (right column) for four 'bursty' ganglion cells. C. Auto-correlation function (left column) and temporal profile (right column) for three 'sustained' ganglion cells.

Figure 6. Types of responses to diffuse steps of light. A. Firing rate for three 'transient' ganglion cells (left column, below) during diffuse steps of light (left column, above) compared with the temporal dynamics of the receptive field center for the same cells (right column). B. Firing rate during diffuse steps of light (left column) versus temporal profile (right column) for four 'sustained' cells. C. Firing rate during diffuse steps of light for three 'rebound' ganglion cells.

Figure 7. Overlapping receptive fields. A. Change in the mean-squared difference between cluster centers and individual cells $\Delta^{(K)}$ plotted as a function of the number of clusters $K$ for one retinal patch with 21 cells (left) and another with 29 cell (right). Clusters were defined using K-means clustering (see Methods). The decrease in mean-squared difference dropped sharply after (10/7) clusters were defined (left/right) and reached a similar small value, indicating that these subsequent distinctions were not significant (dashed red line). B. Temporal profile of 21 cells simultaneously
recorded from one retinal patch (left) and 29 cells from another retinal patch (right). Cells are divided into fine types, shown by their color. Bold lines are cells featured below. C. Examples of the 1-σ contour of the spatial receptive field profile of ganglion cells. Colors match the temporal profiles above (red: biphasic OFF; yellow: monophasic OFF; green: medium OFF; magenta: slow OFF). Spatial profiles of each fine functional type are offset for clarity; dots represent a common point on the multi-electrode array.

Figure 8. Territoriality. A. Histogram of receptive field distances \( D' \) between cell pairs. Solid grey is for cells of the same fine functional type (excluding biphasic OFF cells; \( n = 105 \) cell pairs); black line is for cell pairs of different functional type (\( n = 1118 \) cell pairs) B. Histogram of receptive field distances \( D' \) between pairs of biphasic OFF cells (red); black line is for cell pairs of different fine functional type. C. Spatial profile (1-σ contour) of the receptive fields of 7 biphasic OFF cells measured from one retinal patch (left) and 4 biphasic OFF cells measured from a second patch (right), showing a territorial organization for cells of this type.

Figure 9. Examples of shared information. A. Cross-correlation function between two monophasic OFF cells (left) along with the 1-σ contour of the spatial receptive field profile of both ganglion cells (right). The shared information, \( \Omega = 19\% \), is shown above. B. Cross-correlation function (left) and spatial profile (right) between a monophasic OFF cell (yellow) and a fast ON cell (blue). C. Cross-correlation function (left) and spatial profile (right) between a biphasic OFF cell (red) and a slow OFF cell (purple). D. Cross-correlation function (left) and spatial profile (right) between two monophasic OFF cells.

Figure 10. Shared information within the ganglion cell population. A. Shared information \( \Omega \) plotted versus receptive field spacing \( D' \) for 604 ganglion cells under natural movie stimulation. Color dots show pairs of same-type cells, grey dots are for pairs of different-type cells. B. Shared information versus receptive field spacing (subset of panel A) highlighting monophasic OFF pairs (yellow dots) and pairs formed from one monophasic OFF cell and another medium OFF cell (green diamonds). C. Shared information versus receptive field spacing (same data as in A) highlighting pairs with one ON cell and one OFF cell (blue).
BIBLIOGRAPHY


Figure 1 (Berry)

A

Distance on Retina (mm)

B

Spike-Triggered Average

Color Ratio

C

Merger Score (norm.)

significance threshold:
6 broad types
6 unclassified cells

D

Spike-Triggered Average (Normalized)

Number of Clusters

Time before Spike (s)

fast ON
slow ON
biphasic OFF
monophasic OFF
medium OFF
slow OFF
unclassified

Number of Clusters
Figure 2 (Berry)

A

Receptive Field Dynamics

Number of Clusters

Merger Score

Significance threshold:
6 broad types
7 unclassified cells

B

Receptive Field Size

Number of Clusters

Merger Score

Significance threshold:
5 broad types
1 unclassified cell

C

Auto-Correlation Function

Number of Clusters

Merger Score

Significance threshold:
3 broad types
9 unclassified cell

D

Response to Steps of Light

Number of Clusters

Merger Score

Significance threshold:
3 broad types
1 unclassified cell
Figure 3 (Berry)

**A. Receptive Field Dynamics**

Significance threshold: 6 broad types
7 unclassified cells

**B. RF Dynamics and RF Size**

Significance threshold: 4 broad types
2 unclassified cells

**C. RF Dynamics, RF Size, and Auto-Correlation Function**

Significance threshold: 4 broad types
4 unclassified cells

**D. All Response Included**

Significance threshold: 3 broad types
5 unclassified cells

\[ D_{ij} = a_{ij} \]

\[ D_{ij} = \frac{1}{2} (a_{ij} + b_{ij}) \]

\[ D_{ij} = \frac{1}{3} (a_{ij} + b_{ij} + f_{ij}) \]

\[ D_{ij} = \frac{1}{4} (a_{ij} + b_{ij} + f_{ij} + r_{ij}) \]
Figure 4 (Berry)

A

Receptive Field Radius (µm)

Frequency

B

Receptive Field Radius (µm)

Frequency

C

Receptive Field Radius (µm)

Frequency
Figure 5 (Berry)

A

very bursty cells

B

bursty cells

C

sustained cells

Spike-Triggered Average

Time before Spike (s)

Spike Probability in Cell A

Time Relative to a Spike in Cell B (ms)

Spike Probability in Cell A

Time Relative to a Spike in Cell B (ms)

Spike Probability in Cell A

Time Relative to a Spike in Cell B (ms)

Spike Probability in Cell A

Time Relative to a Spike in Cell B (ms)
Figure 6 (Berry)

A

Firing Probability

Time (s)

transient cells

Spike-Triggered Average

Time before Spike (s)

B

Firing Probability

Time (s)
sustained cells

Spike-Triggered Average

Time before Spike (s)

C

Firing Probability

Time (s)

rebound cells
Figure 7 (Berry)

A

Change in MSE (%)

Number of Clusters Formed

B

Change in MSE (%)

Number of Clusters Formed

C

Spike-Triggered Average

Time before Spike (s)

D

Spike-Triggered Average

Time before Spike (s)

E

F

100 µm

100 µm
Figure 9 (Berry)

A

\[ \Omega = 19\% \]

Time Relative to Spike in Cell A (sec)

B

\[ \Omega = 0.5\% \]

C

\[ \Omega = 14\% \]

D

\[ \Omega = 7.5\% \]

Firing Rate Cell B (Hz)

Time Relative to Spike in Cell A (s)