Receptive Field Mosaics of Retinal Ganglion Cells Are Established Without Visual Experience

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INTRODUCTION

A key aspect of sensory systems is the existence of “sensory maps,” a term used to describe an orderly set of neuronal connections representing the sensory space. In the visual system, sensory maps are observed at many levels starting with the retina, where retinal ganglion cells (RGCs) form several separate feature maps. Different types of RGCs encode different features of the visual scene. Each RGC type covers the retina in a regular and uniform fashion, ensuring that each part of the retina is sensitive to all visual features.

Such regular organization of RGC types, which resembles the tiling of a mosaic, has been described both anatomically and functionally. Anatomical mosaics are characterized by the regular spacing of cell bodies of the same RGC type and a homogeneous distribution of the dendrites (Dacey 1993; Yang 1994; for review, see Wassle 2004; Wassle et al. 1981). This anatomical organization is also reflected at a functional level: the receptive fields (RFs) of the same RGC type form mosaics that uniformly sample the visual scene and provide a regular map of visual space (Devries and Baylor 1997; Field et al. 2007; Peichl and Wassle 1979). Although much progress has been made in understanding the formation of anatomical mosaics (for review, see Poche et al. 2008), how the remarkable regularity of RF organization emerges during development is not known.

Factors that control the formation of RF mosaics may differ from those mediating the formation of anatomical mosaics. The formation of the anatomical regularity is thought to rely on interactions between neighboring cells (Fuerst et al. 2008; Reese 2008; Reese and Galli-Resta 2002). However, the RGC receptive fields are determined not only by the location of somas and the extent of dendrites, but by a number of additional factors including the distribution of bipolar and amacrine cell inputs, the relative strength of excitation and inhibition, the electrotonic properties of cells, and the presence of active conductances in dendrites. Indeed, the relationship between the receptive and dendritic field of a RGC can vary depending on the RGC type. For example, in primates, the dendritic trees of neighboring midget cells barely touch and overlap significantly less than parasol cell dendritic trees, which extend to the somas of neighboring parasol cells (Dacey and Brace 1992; Dacey and Petersen 1992). However, the degree of the RF overlap in midgets and parasols is very similar (Gauthier et al. 2009). This evidence points to a possibility that even though molecular mechanisms govern soma positioning and dendritc patterning, additional factors may be needed for the formation of RF mosaics that underlie sensory encoding.

Neural activity driven by visual experience could be an important factor influencing the formation of RF mosaics. Vision is critical for the normal development of horizontal and bipolar cells (Bayley and Morgans 2007; Lee et al. 2008), and there are indications that it is also needed for the normal maturation of RGC dendrites (Sernagor and Mehta 2001; Tian and Copenhagen 2003; Wingate and Thompson 1994; Wong et al. 1991). However, many aspects of RGC development are immune to alterations in neural activity (Daw and Wyatt 1974; Elstrott et al. 2008; Kerschensteiner et al. 2009; Lau et al. 1990). The role of vision in the formation of RF mosaics is not known.

Here we use a large-scale multielectrode array to determine whether visual experience is required for the mosaic coverage of visual space by RGC RFs.

METHODS

Animals

Adult and juvenile [postnatal days 13–14 (P13–P14)] Long Evans rats were used for the recordings. The juvenile rats had either eyes completely closed or one eye partially open. The average weight of...
FIG. 1. Two ON and two OFF retinal ganglion cell (RGC) types were most readily identified in adult rats and at the age of eye opening. Shown are the results from 1 adult and 1 P12 rat. A: top: spatial RFs of all OFF cells and all ON cells identified simultaneously in the same retina. Middle and Bottom: spatial RFs of the 2 ON and 2 OFF RGC types that were reliably distinguished across preparations, separately in each age group. Overlaid is a rectangular outline of the multielectrode array. B: overlaid spike-triggered average (STA) time courses for all cells of each identified RGC type. The vertical axis is normalized for each cell so that the area under each STA curve equals 1. A higher degree of the response transience is indicated by a time course with more symmetric positive and negative deflections. A 0 crossing closer to the time of spike (time 0) indicates faster response kinetics (see Fig. 2 and Table 1 for quantitative cross-type comparisons). C: overlaid autocorrelation functions for all cells of each identified RGC type. The vertical axis is normalized for each cell so that the area under each autocorrelation curve equals 1. The difference in the shape of autocorrelation functions between cell types reflects differences in intrinsic firing properties.
juvenilerats was 35 g, and the weight of adults varied between 230 and 560 g. All procedures were approved by the University of California, San Diego Institutional Animal Care and Use Committee and conformed to the Salk Institute Guidelines for the care and use of animals.

Acute retina preparation

All dissections were performed using dim red ambient light and infrared illumination to prevent photopigment bleaching. Animals were dark adapted for ≥40 min before the experiment and anesthetized with an intraperitoneal injection of a ketamine/xylazine cocktail before decapitation. After enucleation, an eye was transferred to oxygenated Ames medium and hemisected posterior to the ora serrata. The cornea, lens, and vitreous were removed. Blood vessel landmarks visible through the retina on the choroid were used to mark the orientation of the eyecup before removing the pigment epithelium. The isolated retina was hemisected along the nasal-temporal axis. For all recordings, retina from the dorsal region of the right eye was used to minimize variability across preparations.

Multielectrode array recordings

Recordings were made using a custom multielectrode array (MEA) comprised of 512 extracellular electrodes spaced at 60 μm on a hexagonal grid (Elstrott et al. 2008; Field et al. 2007; Frechette et al. 2005; Litke et al. 2004; Petrusca et al. 2007; Shlens et al. 2006). The retinal area covered by the MEA was ~2 mm². Once a piece of retina had been isolated, it was placed ganglion cell side down onto the array. A dialysis membrane was used to hold the retina in place on the electrode. Light stimuli were attenuated using neutral density filters to low photopic light levels (average intensity = 1,623 491-nm equivalent photons/μm²/s). To determine RFs of RGCs, we used a white noise stimulus—a flickering black-and-white checkerboard—where at every stimulus frame the intensity of each checker was drawn from a binary distribution.

Light stimulation

The photoreceptor layer of an isolated piece of retina was stimulated from above with the optically reduced image of a CRT display refreshing at 60 Hz, focused with a microscope objective, and centered on the array. Light stimuli were attenuated using neutral density filters to low photopic light levels (average intensity = 1,623 491-nm equivalent photons/μm²/s). To determine RFs of RGCs, we used a white noise stimulus—a flickering black-and-white checkerboard—where at every stimulus frame the intensity of each checker was drawn from a binary distribution.

RFs and intrinsic firing properties

The spatial and temporal components of RFs were characterized using a reverse correlation analysis. For a given cell, the spike-triggered average (STA) was obtained by computing the average stimulus present on the display in the 500 ms preceding a spike. At the time of maximum deflection of the STA time course from the mean threshold was represented by its waveform, a vector of voltage values recorded in a 3-ms period centered at the time of threshold crossing. For each electrode, principal component analysis (PCA) was performed on the collection of all waveforms recorded on that electrode. An expectation maximization algorithm was used to identify clusters in the PCA space based on a mixture of Gaussians model (Litke 2004). All resulting clusters were inspected manually. To verify that each cluster contained spikes from a single cell, the rate of refractory period violations was estimated using the number of interspike intervals between 0.5 and 1.2 ms. Only the clusters for which refractory period violations did not exceed 10% were used in subsequent analysis.

Several neighboring electrodes often picked up spikes from the same cell. In this case, voltage waveforms recorded on the adjacent electrodes were also included in the PCA (Field et al. 2007), providing additional information for spike sorting and maximizing the number of single cells identified per unit area.

Duplicate recordings of the same cell were identified using a temporal cross-correlation and removed. Cells with an average firing rate of <1 Hz were also excluded.
intensity, a two-dimensional Gaussian was fit to the spatial profile. The position of the RF center was estimated by the center of the Gaussian fit. An elliptical boundary of the RF was drawn at 1 SD of the Gaussian fit. The receptive field radius was computed as the geometric mean of the major and minor axes of the ellipse.

Cell polarity (+1 for ON, −1 for OFF) was determined by the sign of the amplitude of the primary peak of the STA time course (the peak closest to the time of the spike). Response kinetics were summarized for each cell by the time of zero crossing that occurred between the maximum and the minimum of the STA time course. This corresponded to the time of maximum response to a step change in illumination within the RF (assuming the response was linear). The degree of response transience was computed from the STA time course of each cell as \(1 - |(M - |m|)/(M + |m|)|\), where \(M\) and \(m\) refer to the maximum and minimum values of the time course. A symmetric, biphasic time course would have the transience value equal to 1, whereas a time course with only one lobe would have the transience value equal to 0.

The intrinsic firing properties of different cells were distinguished by their temporal autocorrelation functions, which showed the probability of firing over time relative to a spike at time 0. The steady rate of the autocorrelation function was determined as the mean value of the autocorrelation function between 50 and 100 ms after a spike.

A two-tailed \(t\)-test and a two-sided rank sum test were used to compare the receptive field radius, time of zero crossing, degree of transience, and autocorrelation steady rate between different cell types and ages.

**Table 1. Spatiotemporal receptive field and functional mosaic properties for the RGC types identified in the retinas of adult rats and juvenile rats at the time of eye opening**

<table>
<thead>
<tr>
<th>Type</th>
<th>(n)</th>
<th>Time to Zero Crossing, ms</th>
<th>Degree of Transience</th>
<th>Normalized Autocorr. Rate, (\times 10^4)</th>
<th>RF Radius, (\mu m)</th>
<th>NND, (\mu m)</th>
<th>Normalized NND</th>
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<tr>
<td>Rat 1</td>
<td>52</td>
<td>180 ± 23</td>
<td>0.42 ± 0.11</td>
<td>4.9 ± 0.6</td>
<td>101 ± 11</td>
<td>130 ± 16</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Rat 2</td>
<td>54</td>
<td>171 ± 8</td>
<td>0.54 ± 0.10</td>
<td>6.1 ± 0.12</td>
<td>96 ± 9</td>
<td>150 ± 15</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Rat 3</td>
<td>54</td>
<td>126 ± 8</td>
<td>0.61 ± 0.10</td>
<td>4.6 ± 0.5</td>
<td>127 ± 12</td>
<td>162 ± 19</td>
<td>1.3 ± 0.1</td>
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<td>Total</td>
<td>160</td>
<td>163 ± 22</td>
<td>0.57 ± 0.17</td>
<td>5.1 ± 0.9</td>
<td>107 ± 12</td>
<td>147 ± 16</td>
<td>1.4 ± 0.1</td>
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<tr>
<td>Rat 1</td>
<td>40</td>
<td>196 ± 11</td>
<td>0.58 ± 0.07</td>
<td>1.9 ± 1.1</td>
<td>111 ± 10</td>
<td>144 ± 12</td>
<td>1.3 ± 0.1</td>
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<td>Rat 2</td>
<td>38</td>
<td>224 ± 21</td>
<td>0.55 ± 0.08</td>
<td>1.7 ± 0.7</td>
<td>113 ± 10</td>
<td>162 ± 20</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Rat 3</td>
<td>40</td>
<td>145 ± 10</td>
<td>0.61 ± 0.05</td>
<td>2.1 ± 0.4</td>
<td>153 ± 10</td>
<td>188 ± 17</td>
<td>1.3 ± 0.1</td>
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<tr>
<td>Total</td>
<td>118</td>
<td>193 ± 32</td>
<td>0.60 ± 0.08</td>
<td>2.0 ± 0.4</td>
<td>130 ± 30</td>
<td>174 ± 37</td>
<td>1.3 ± 0.1</td>
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<tr>
<td>Rat 1</td>
<td>62</td>
<td>170 ± 6</td>
<td>0.69 ± 0.08</td>
<td>1.3 ± 0.5</td>
<td>82 ± 9</td>
<td>134 ± 16</td>
<td>1.5 ± 0.2</td>
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<td>Rat 2</td>
<td>62</td>
<td>198 ± 17</td>
<td>0.68 ± 0.08</td>
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<td>85 ± 8</td>
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<td>Rat 3</td>
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<td>134 ± 4</td>
<td>0.79 ± 0.05</td>
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<td>119 ± 9</td>
<td>160 ± 13</td>
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<td>Total</td>
<td>180</td>
<td>174 ± 22</td>
<td>0.73 ± 0.09</td>
<td>1.3 ± 0.1</td>
<td>99 ± 27</td>
<td>142 ± 25</td>
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<td>Rat 1</td>
<td>57</td>
<td>179 ± 8</td>
<td>0.67 ± 0.10</td>
<td>1.8 ± 0.7</td>
<td>55 ± 6</td>
<td>115 ± 14</td>
<td>2.1 ± 0.2</td>
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<tr>
<td>Rat 2</td>
<td>47</td>
<td>204 ± 9</td>
<td>0.65 ± 0.06</td>
<td>1.4 ± 0.7</td>
<td>55 ± 6</td>
<td>116 ± 20</td>
<td>2.1 ± 0.4</td>
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<td>Rat 3</td>
<td>42</td>
<td>142 ± 8</td>
<td>0.70 ± 0.10</td>
<td>1.3 ± 0.7</td>
<td>64 ± 8</td>
<td>147 ± 14</td>
<td>2.4 ± 0.3</td>
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<tr>
<td>Total</td>
<td>156</td>
<td>183 ± 20</td>
<td>0.70 ± 0.07</td>
<td>1.4 ± 0.4</td>
<td>66 ± 18</td>
<td>143 ± 46</td>
<td>2.2 ± 0.1</td>
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<td>Rat 1</td>
<td>42</td>
<td>101 ± 5</td>
<td>0.79 ± 0.07</td>
<td>2.7 ± 0.1</td>
<td>103 ± 9</td>
<td>194 ± 22</td>
<td>1.8 ± 0.2</td>
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<td>Rat 2</td>
<td>52</td>
<td>98 ± 7</td>
<td>0.85 ± 0.08</td>
<td>3.5 ± 0.8</td>
<td>102 ± 10</td>
<td>159 ± 17</td>
<td>1.5 ± 0.2</td>
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<td>40</td>
<td>103 ± 6</td>
<td>0.86 ± 0.05</td>
<td>2.8 ± 0.7</td>
<td>108 ± 6</td>
<td>182 ± 20</td>
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<td>100 ± 2</td>
<td>0.83 ± 0.04</td>
<td>3.0 ± 0.4</td>
<td>104 ± 3</td>
<td>178 ± 18</td>
<td>1.6 ± 0.1</td>
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<td>Rat 1</td>
<td>27</td>
<td>102 ± 7</td>
<td>0.65 ± 0.12</td>
<td>4.4 ± 0.5</td>
<td>107 ± 12</td>
<td>177 ± 34</td>
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<td>Rat 2</td>
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<td>95 ± 8</td>
<td>0.77 ± 0.14</td>
<td>4.6 ± 0.3</td>
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<td>Rat 3</td>
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<td>0.70 ± 0.12</td>
<td>4.6 ± 0.5</td>
<td>111 ± 14</td>
<td>163 ± 23</td>
<td>1.5 ± 0.3</td>
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<td>Total</td>
<td>87</td>
<td>100 ± 5</td>
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<td>4.5 ± 0.1</td>
<td>108 ± 3</td>
<td>163 ± 14</td>
<td>1.5 ± 0.1</td>
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<tr>
<td>Rat 1</td>
<td>27</td>
<td>105 ± 8</td>
<td>0.88 ± 0.07</td>
<td>2.6 ± 0.6</td>
<td>91 ± 7</td>
<td>171 ± 20</td>
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<td>Rat 2</td>
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<td>112 ± 8</td>
<td>0.91 ± 0.06</td>
<td>2.3 ± 0.4</td>
<td>91 ± 9</td>
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<td>90 ± 3</td>
<td>168 ± 5</td>
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<td>Rat 1</td>
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<td>118 ± 15</td>
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<tr>
<td>Rat 2</td>
<td>12</td>
<td>100 ± 7</td>
<td>0.60 ± 0.14</td>
<td>4.9 ± 0.4</td>
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<td>194 ± 18</td>
<td>1.7 ± 0.2</td>
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<td>Rat 3</td>
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<tr>
<td>Total</td>
<td>43</td>
<td>100 ± 1</td>
<td>0.63 ± 0.05</td>
<td>5.0 ± 0.7</td>
<td>117 ± 3</td>
<td>185 ± 12</td>
<td>1.6 ± 0.2</td>
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Values are mean ± SD for NND and normalized NND, the mean was computed for the densest 70% of the distribution. RGC, retinal ganglion cell; NND, nearest neighbor distance.
Functional cell types

Because cells of the same functional type are expected to have a similar preferred stimulus and firing properties, and hence a similar RF size, STA time course, and autocorrelation function, we performed cell classification sequentially using the following procedure. First, direction selective ganglion cells were identified using responses to a drifting grating stimulus as previously described (Elstrott et al. 2008) and excluded from the subsequent classification. Second, ON and OFF RGCs were separated based on their polarity. Third, for the cells of a given polarity, PCA was performed separately on the set of all STA time courses and the set of all autocorrelation functions. Fourth, clusters were identified in a series of six two-dimensional scatter plots that showed the weights of the first three principal components of the STA time course and the first three principal components of the autocorrelation function as a function the RF size. For each cluster, we repeated all of the above steps starting with the PCA, further classifying cells based on their autocorrelation and RF properties.

Mosaic characterization

The density recovery profile (normalized spatial autocorrelogram of RF centers) was generated by accumulating the RF position of all RGCs of a given type relative to the position of all other cells of the same type. Distances between cells were binned (bin size = 40 μm) and the cell count in each bin was normalized to the total number of cells and to the area of a 40-μm-wide annulus with a radius given by the bin center. To account for boundary effects, compensation factors were calculated for each bin, assuming similar and uniform conditions in the unsampled regions of the retina (Rodieck 1991).

The nearest neighbor distance (NND) distribution was constructed in the same manner; however, the position of only the nearest cell was used for each reference cell. A normalized NND distribution was obtained by dividing the distance separating each pair of nearest neighbors by the average RF radius computed for this pair. The typical values for NND and normalized NND were estimated by the mean of the densest 70% of the corresponding distributions.

RESULTS

Two OFF and two ON RGC types are reliably identified in rats at the age of eye opening

To assess the presence of functional RGC types in a retina with no visual experience, we examined rats shortly before eye opening, which occurs around P14, and compared them with adult rats. RGC activity was recorded using a large-scale multielectrode array (MEA) while stimulating the retina with a white noise light stimulus. Spatiotemporal RFs of individual RGCs were identified using a spike-triggered average (STA) (see METHODS). In both age groups (adult and eye opening), 300–500 RGCs with RFs were detected in the 2-mm² retinal area recorded by the MEA (Fig. 1A). The RFs overlapped to cover the recorded area with no gaps, as could have been seen if we overlaid all ON and all OFF RFs in the same retina (Fig. 1A, top row). Distinct RGC groups were distinguished based on their RF size; light response polarity (ON or OFF) and response kinetics (as characterized by the STA time course; Fig. 1B); and intrinsic firing properties (as characterized by the autocorrelation function; Fig. 1C) (Carcieri et al. 2003; Chichilnisky and Kalmar 2002; Kerschenstein et al. 2008; Segev et al. 2004).

In each preparation, we typically found two distinct types of ON and two distinct types of OFF cells that together accounted for 30–60% of all recorded RGCs. The differences between RGC

A Cell density and nearest neighbour distance

B Normalized nearest neighbour distance

FIG. 3. Mosaic properties of juvenile retinas with no visual experience are comparable to the properties of mature mosaics. A: the density recovery profile shows a normalized spatial autocorrelogram of RF centers (open bars). Dotted horizontal line represents uniform cell density that would result from a random distribution. Overlaid in gray is a distribution of the nearest neighbor distances. Arrowheads mark a typical distance between the nearest neighbor RFs computed as a mean of the densest 70% of the distribution (Table 1). B: the distribution of normalized nearest neighbor distances in the units of RF radius. Arrowheads mark a typical normalized nearest neighbor distance (NND) as a mean of the densest 70% of the distribution (Table 1). For ON-2E, an asterisk above the last bin indicates that all values of NND > 5 have been also included into that bin. In both A and B, the results are from 1 adult and 1 P12 rat (same animals as in Figs. 1 and 2A).
types were evident despite the variability in cell properties within each type (Fig. 2A; Table 1). It is likely that the same RGC types were identified across different retinas in the same age group, because the relative differences between the four identified cell types within each retina remained consistent (Fig. 2B). Although the composition and properties of the other RGCs varied from preparation to preparation, the four cell types consistently observed in adult and the four cell types consistently observed in juvenile rats were likely favored by the MEA recordings because of their morphological or electrophysiological properties. The observation that some cell types were more readily identified than others was similar to observations in the macaque retina (Chichilnisky and Kalmar 2002). However, we do not have evidence that the four types readily detected at eye opening (referred to as OFF-1E, OFF-2E, ON-1E, and ON-2E in the figures) are the same cell types that develop into the four types readily detected in the adult (OFF-1A, OFF-2A, ON-1A, and ON-2A). Our ability to track the same functional RGC type through development was limited by the immaturity of functional properties at the age of eye opening. Therefore it is possible that the four cell types reliably recorded in young rats are, indeed, the same as the four reliably recorded types in the adult—only their properties have changed. Alternatively, because we do not have a complete understanding of what makes a given type to be readily detected by an MEA, it is possible that, in mature retina, the cell types that are most readily recorded are different from the cell types that have been most readily recorded at younger ages.

RF mosaics at the age of eye opening are similar to the mature mosaics of adult rats

If mosaic formation requires visual experience, the retinas that had never experienced normal vision would be expected to exhibit an irregular spacing of RF centers and/or a significant and irregular overlap of the spatial extent of the RFs. Instead, each of the cell types distinguished at eye opening formed an orderly RF mosaic covering the same retinal patch (Fig. 1A). The gaps that occasionally interrupted this regular structure appeared most likely in place of the cells whose firing we could not reliably record with the MEA or could not separate from the firing of other cells recorded on the same electrode (see METHODS). To quantitatively compare the RF mosaics in P13–P14 and adult rats, we considered two separate aspects of mosaic organization.

First, we compared the regularity of RF center positions by computing the average number of RFs per unit area found at a given distance from each cell. The resulting density recovery profile (Cook 1996) showed that, at the age of eye opening, exclusion zones had already formed around RF centers similar to the exclusion zones observed in adults (Fig. 3A). To quantify the size of the exclusion zones, we computed an average distance to the nearest neighbor in each mosaic (NND; Fig. 3A, arrowheads). The NND values were generally higher in adult ($P < 0.01$, across all 4 cell types, $n = 3$ animals at each age) but showed a considerable variation across cell types so that one of the juvenile types (OFF-2E) had a higher NND than two adult types (OFF-2A and ON-1A; Table 1).

Second, we assessed the degree of overlap between neighboring RGC RFs at eye opening and in adult by examining the NND normalized to the RF radii (Fig. 3B; Table 1). The normalized NND allows for a direct comparison of mosaic properties independent of the size of any given RGC type. The normalized NND showed less variability compared with the nonnormalized NND and stayed close to 2.0 for all cell types (range: 1.3–2.2; Table 1). A normalized NND of exactly 2.0 would mean that the RF centers of neighboring cells were separated, on average, by the distance equal to two RF radii (Devries and Baylor 1997; Field et al. 2007; Peichl and Wassle 1979). In other words, the RF outlines of these cells, which were drawn at SD of the two-dimensional Gaussian fits to their spatial RFs (Fig. 1), would touch without overlap. Although the extents of the RFs that go beyond SD do overlap considerably, the narrow range of the observed normalized NND values emphasizes the regularity in the degree of this overlap. There was no significant difference in normalized NND between ages ($P > 0.05$, across all 4 cell types, $n = 3$ animals at each age), indicating that the degree of overlap in RGC mosaics at eye opening is similar to that observed in adult.

Light response properties of individual RGCs are not mature at the age of eye opening

The above results show that at least some functional RGC types and corresponding RF mosaics are present in rats at the age of eye opening. However, previous studies indicate that, at this stage of development, several aspects of retinal circuits remain immature, including the intrinsic firing properties of RGCs (Myhr et al. 2001; Qu and Myhr 2008; Wang et al. 1997), synaptic inputs to RGCs (Tian et al. 1998), and RF structure (Bowes-Anders et al. 1975; Musland 1977; Rusoff and Dubin 1977; Tootle 1993). To test whether the RF mosaics...
observed at eye opening were accompanied by mature light responses, we compared the RGC light response properties with those observed in adult retinas.

A comparison of STA time courses (Figs. 1B and 4B) showed two aspects of the light response that remained immature at the age of eye opening. First, we compared light response kinetics characterized by the time to zero crossing of the STA time course. The times were averaged in each retina over all recorded RGCs and separately across RGCs belonging to each one of the identified functional types. All RGC types identified at the age of eye opening had significantly slower light responses compared with all RGC types identified in adults (Fig. 4A; Table 1). The comparison including not just the identified types, but all recorded RGCs, showed that the light response kinetics were significantly slower at the age of eye opening compared with adult (Fig. 4B; 1,023 cells at eye opening and 962 cells in adult from 3 animals at each age; \( P < 0.001 \)). Second, we compared light response transience (see METHODS) and found that the light responses of RGCs recorded at eye opening were significantly less transient than those recorded in adult (\( P < 0.001 \); Fig. 4C). The lesser degree of transience at eye opening was consistent with our recordings in younger rats (P10–P12; data not shown) where light responses became progressively more transient with age. Together, these results suggest that the establishment of distinct type-specific functional properties of RGCs occurs before the complete maturation of the light response circuits.

DISCUSSION

We showed that at least some types of rat RGCs form RF mosaics before the onset of visual experience. Clear mosaic organization of spatial RFs was observed at the age when retinal circuits underlying RF structure were still immature. Indeed, at the time of eye opening, RGCs had significantly slower and less transient responses to light compared with adult (Fig. 4). These findings are consistent with several other studies that have shown that both excitatory and inhibitory synaptic inputs (Tian et al. 1998), as well as RGC firing properties (Myhr et al. 2001; Qu and Myhr 2008; Wang et al. 1997), do not mature until 2–4 wk after eye opening. A similar observation has been made in a recent study of mice that lack transcription factors critical for the development of particular bipolar cell types: despite significantly altered temporal RFs and contrast adaptation, the organization of spatial receptive fields was unaltered (Kerschensteiner et al. 2008).

These results support the hypothesis that the formation of RF mosaics is determined primarily by the formation of anatomical mosaics rather than the maturation of circuits presynaptic to RGCs or the physiological properties of RGCs. Anatomical mosaics, defined by the spacing of somas and uniform overlap of dendrites, are established early in development. It has been proposed that their formation depends on type-specific interactions between neighboring cells (Fuerst et al. 2008; Reese 2008; Reese and Galli-Resta 2002). In live imaging during development, it was observed that RGC dendrites actively avoid growing near RGCs of the same type (Lohmann and Wong 2001), indicating the presence of a repulsive interaction. In agreement with this hypothesis, removing a few RGCs during a critical period leads to an expansion of dendrites into the open space (Eysel et al. 1985; Kirby and Chalupa 1986; Perry and Linden 1982). These observations suggest that the spatial extent of processes is the result of an active dendritic growth restricted by homotypic interactions between cells. However, in a line of transgenic mice in which >95% of RGCs die, the dendrites of remaining RGCs do not expand to fill the entire space (Lin et al. 2004), indicating that the outgrowth of RGC dendritic trees is also limited by cell-intrinsic mechanisms.

Does retinal activity play a role in the development of mosaics?

Neural activity is essential for the formation of several circuits throughout the developing visual system. Visual deprivation alters the development of RGCs in turtles (Sernagor and Grzywacz 1996; Sernagor and Mehta 2001; Sernagor et al. 2001) and the lamination of RGC dendrites into ON and OFF sublamina in mice (Tian and Copenhagen 2003) (for review, see Tian 2008). Dark rearing has been shown to influence the maturation of synaptic circuits not only within the retina (Tian and Copenhagen 2001), but also in the lateral geniculate nucleus of the thalamus (Grubb and Thompson 2004; Hooks and Chen 2006, 2008). Indeed, even before eye opening, retinal activity in mice, rats, and ferrets is propagating up the visual pathway (Haglund et al. 2006) and can drive light-evoked responses in visual thalamus and cortex (Akerman et al. 2002; Krug et al. 2001). However, diffuse light stimulation through the closed eyelids for the few days before eye opening has not been implicated in the development of visual maps.

These findings imply that, at least for several RGC types, the spatial organization of RFs is established independent of visually induced neural activity. However, spontaneous neural activity such as retinal waves could play a role in mosaic formation. Retinal waves are detected for an extended period perinatally, which in mice lasts from 1 wk before to 2 wk after birth. Whereas retinal waves have been shown to play a role in refining retinal projections to the superior colliculus and visual thalamus into orderly retinotopic and eye-specific maps (Huberman et al. 2008; Torborg and Feller 2005), the role of waves in retinal development is less well explored (Bansal et al. 2000; Sernagor et al. 2001). Recently, it was reported that different types of RGCs have different participation in waves (Kerschensteiner and Wong 2008; Qu and Myhr 2008), indicating that waves could correlate the firing of cells within types differently than across types and contribute to mosaic formation.

In summary, we showed that the RF mosaics of at least some RGC types form early in development independent of vision. This is in contrast to other parts of the visual system, where visual experience plays a critical role in the establishment of sensory maps (for review, see White and Fitzpatrick 2007). We cannot exclude the possibility that the mosaics that are present in the retina at the age of eye opening get disassembled later in development, and the mosaics that we observe in adult develop later, potentially under the guidance of visual experience. However, our findings lead us to suggest that the formation of a sensory map in the retina relies primarily on the anatomical substrate and indicate the presence of a uniform spatial representation of at least some features of visual space at the onset of vision.
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