Spatiotemporal Patterns at the Retinal Output

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INTRODUCTION

Our objective was to determine the patterns of activity elicited in a layer of retinal neurons by a flashed stimulus square subtending ~30° of visual angle. Recording simultaneously from the population of thousands of neurons in a given layer that respond to such a stimulus is not yet feasible, so we reconstructed the patterns by recording from a single cell and shifting the square to ~2,000 different positions in a 54 × 36-position grid with 25 µm spacing overlaid on that cell. A pattern representing the simultaneous activity of all the cells within the scanning area in the layer, at the resolution of the grid, can then be generated when the ensemble of recordings is played back simultaneously, each at its appropriate position in the grid. Each recording takes 1 s, so the entire pattern can be obtained in <1 h. For this technique to be valid, the recorded cell, in addition to being part of a spatially invariant population, must have no long-term memory (that is, its response must not vary significantly between presentations of an identical stimulus). The primary cause of occasional significant response variations that we observed over the course of an experiment appeared to be light adaptation in the photoreceptors; thus care was taken to set a constant adaptation point by controlling the background light level and to allow the retina to reach a steady state before commencing with the experiment.

METHODS

Extracellular recording and pattern reconstruction

Recordings were made from single ganglion cells in whole-mounted tiger salamander (Ambystoma tigrinum) retina using single tungsten electrodes (impedances between 1 and 10 MΩ). The retina was continuously superfused with oxygenated N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) Ringer solution. Pharmacological agents (picrotoxin, 250 µM; bicuculline, 250 µM) were added to the Ringer without substitution and delivered via superfusion. The cathode ray tube–generated stimulus (a positive or negative contrast square of intensity ranging from 0.5 to 5 µW/mm² white light superimposed on a background in the same scotopic intensity range, flashed on for 1,000 ms, and then off for 1,000 ms) was presented in each of 2,052 positions over a 1,500 × 1,000–µm² grid centered on the recorded cell, and spikes were threshold or window discriminated and binned with the use of a 50-ms interval. By simultaneously replaying all of the sequences of activity, each in a position that corresponds to where the stimulus was presented, a reconstruction can be obtained of the pattern of activity over a putative uniform layer of cells with identical response characteristics.

Intracellular recording

Recording was made from a single bipolar cell in whole-mounted tiger salamander retina using a glass intracellular electrode (impedance 1.000 MΩ). The retina was continuously superfused with oxygenated HCO₃⁻ Ringer solution. The stimulus protocol used was the same as described above, except that in the absence of spikes, graded potentials were averaged over 50-ms windows.

In 50 experiments, we extracellularly recorded the spike rate of ganglion cells in the light-adapted tiger salamander (Ambystoma tigrinum) retina while applying the flashed square stimulus in a raster configuration as described above. Most (34/50) of the cells could be classified according to their response to full-field illumination as ON/OFF-transient (generating a spike burst in response to increase or decrease of illumination); a smaller number (10/50) of somewhat more sustained OFF-only and even fewer (6/50) sustained ON-only cells were also observed (Wunk and Werblin 1979).

The activity evolves in both space and time and is best expressed as a movie. We represent it here in a series of snapshots as well as an x–t plot taken from a representative section through the pattern.¹

¹ Movies of these results are available in MPG, AVI, and Quicktime formats on the website: http://retina.berkeley.edu/patterns or via anonymous ftp from retina.berkeley.edu, directory /pub/patterns.
FIG. 1. Patterns of activity at salamander ON-OFF-type ganglion cell layer reconstructed from a single-cell recording in response to a 300 × 300 μm² bright square flashed onto a dark background. A: full 2-dimensional pattern is shown at 3 representative points in time. B: evolution of the ridge pattern over a time course of 300 ms following presentation of the stimulus. One-dimensional cross-sections, taken through the center of the 2-dimensional pattern obtained after presentation of a square stimulus, are “cascaded” to show the time course. The intersecting plane (IP) in A represents the location of the cross-section. The representation of the square is well-developed by 160 ms. As the response distribution spreads laterally, the central region of activity is suppressed by feedback inhibition (200 ms, 240 ms) forming “ridges” that represent the boundary of the stimulus square. Response decays completely by 300 ms.

RESULTS

The vast majority of the ON-OFF cells exhibited reconstructed activity patterns typified by the sequence shown in Fig. 1. A representation of the square, carried by spiking ganglion cells within the area of the square, develops within the first 150 ms, and the interior portion of the representation drops out by 200 ms; the remaining “ridges” then continue to expand in space, slowly decaying, until ~350 ms after stimulus presentation after which all activity ceases. A similar response pattern was measured at light OFF. ON-Only and OFF-only cells did not exhibit this complex behavior: the activity was more sustained, and there was no dropout of the interior portion of the figure.

The pattern of “expanding ridges” exhibited by the ON-OFF cells in response to a flashed square is especially intriguing because it is not accounted for by the classical lateral-inhibition models of the retina, which predict that a stationary representation of the edges would develop as a result of horizontal cell-mediated lateral inhibition (Werblin 1991). Nor has the pattern been observed previously through the receptive-field mapping techniques (e.g., scanning with a small spot) conventionally used with single-cell recording.

The synaptic sites mediating the pattern formation can be located in the retina by using pharmacological methods to differentiate between known inhibitory pathways. Inhibitory interactions are mediated in the outer retina by horizontal cells, which feed back to cones, and in the inner retina by several types of amacrine cell, which feed back to bipolar cell terminals or forward to ganglion cells. Feedback from the amacrine cells at the bipolar terminals is mediated primarily at γ-aminobutyric acid-C (GABA<sub>C</sub>) receptors (Dong and Werblin 1997; Roska and Werblin 1997), whereas feed-forward to ganglion cells is mediated at GABA<sub>A</sub> receptors and (in a separate population of amacrine cells) by glycine (Yang et al. 1991). Neither strychnine, which blocks glycinergic inhibition, nor bicuculline, which blocks only GABA<sub>A</sub> receptors (Feigenspan and Bormann 1994), significantly changed the form of the “expanding ridges” pattern (Fig. 2, A and B). This would appear to rule out GABA<sub>A</sub> or glycine feed-forward from amacrine to ganglion cells or GABA<sub>C</sub> feedback from amacrine to bipolar cells (Lukasiewicz and Werblin 1994a,b) or GABA<sub>A</sub> feedback from horizontal cells to cones or feed-forward from horizontal to bipolar cells (Wu 1992) as the crucial inhibitory interaction. Under picrotoxin, however, which blocks both GABA<sub>A</sub> and GABA<sub>C</sub> receptors, the “hole” in the expanding representation failed to develop, suggesting that the inhibitory interaction that suppresses the interior of the square is mediated by GABA<sub>C</sub> receptors (Fig. 2C), which are present primarily at the bipolar cell terminals. The fact that picrotoxin prevents the hole from developing also rules out the hypothesis that the effect might be due to non-GABAergic feedback from...
horizontal cells to cones, which has been proposed to mediate center-surround antagonism at the outer retina (Wu 1992), because this interaction is not blocked by GABA or its antagonists (Hare and Owen 1996).

**DISCUSSION**

Circuitry that might underlie the expanding-ridges pattern is shown in Fig. 3A. The square stimulus is represented by a dynamic distribution of neural activity that, during the first several hundred milliseconds of the response, expands laterally across the ganglion cell layer. This expansion could be attributed to the gap junction coupling of ON bipolar cells (Borges and Wilson 1990; Owen and Hare 1989), which with a space constant of several hundred micrometers and a time constant of 50 ms fits much of our data, or else to activity spreading through the coupled horizontal cell network as read out by OFF bipolar cells, which would account for some recordings in which the extent of the expansion is very wide (>500 μm). The bipolar cells excite a population of GABAergic amacrine cells (Yang et al. 1991) with processes that span only 150 μm, less than the space constant for coupled bipolar cells (Maguire et al. 1989). These amacrine cells, which also represent the object as an expanding distribution of activity, feed back to GABA<sub>C</sub> receptors (Wells and Werblin 1995) at the bipolar cell terminals, truncating synaptic release there and thereby creating the ‘‘hole’’ in the representation. The inhibitory effect of these amacrine cells appears to be significantly delayed with respect to that of the bipolar cells (Dong and Werblin 1997; Roska and Werblin 1997), so the expanding representation at the amacrine cell layer lags that at the bipolar cell layer, and thus the outer margin of the expanding bipolar cell activity escapes the effect of feedback inhibition, forming the expanding ridges that are then ‘‘read out’’ by the ganglion cells.

We believe that the morphology of these patterns and the pharmacological evidence together imply that classical models of lateral inhibition at the outer retina (resulting in a horizontal-cell–mediated antagonistic surround at the bipolar cells) are insufficient to account for the dynamic representation of the stimulus’ edges that we observe at the ganglion cell layer. A properly scaled antagonistic surround would lead to an image of the stimulus square in which the center indeed drops out (Fig. 3B), but the dynamic expansion of the edges characteristic to our result would be miss-
FIG. 3. A: proposed circuitry mediating the pattern observed in on-off ganglion cells. The electrically coupled (EC) bipolar cells (top) drive (FF) a class of amacrine cells (middle) with narrow-ranging processes, which in turn feed back (FB) to inhibit bipolar cells via GABA<sub>C</sub> receptors at their synaptic terminals. This feedback truncates release from the bipolar cell terminals after about a 40- to 80-ms delay. The output of bipolar cell terminals is “read out” by retinal ganglion cells (bottom). B: theoretical result of convolving a 300 × 300-μm<sup>2</sup> square stimulus with an antagonistic-surround receptive field with dimensions: center 20 μm, surround 50 μm. C: theoretical result of convolving the same stimulus with a 20-μm center, 400-μm surround receptive field. D: steady-state pattern of activity at salamander ON bipolar cell layer in response to a 300 × 300-μm<sup>2</sup> bright square flashed onto a dark background. The bipolar cell pattern, like the pattern predicted in C based on horizontal cell and cone space constants, fails to exhibit enhancement of the figure’s edges.

...ing. Furthermore, the space constant of tightly electrically coupled horizontal cells in tiger salamander is very large (∼400 μm) compared with that of the weakly coupled cones (∼20 μm). Thus we would expect that the image of the square at the bipolar cell layer would not exhibit significant edge extraction (Fig. 3C). Indeed, a pattern reconstructed from an intracellular recording in an ON bipolar cell (Fig. 3D) using a 300 × 300 μm<sup>2</sup> square (with the same stimulus protocol used in the ganglion cell experiments) conformed to this expectation. Finally, the fact that bicuculline does not affect the expanding ridges, whereas picrotoxin abolishes them, implies that the interaction responsible for the effect is GABA<sub>A</sub>ergic but not mediated by GABA<sub>A</sub> receptors, which would rule out known GABA<sub>A</sub> as well as potential non-GABA<sub>A</sub>ergic inhibitory interactions at the outer retina, but is consistent with localization to the GABA<sub>C</sub> receptors at the bipolar cell terminals.

Does the complex pattern of expanding ridges evoked at the output of the tiger salamander retina by a spatially extensive stimulus contribute to object recognition in that organism? It appears plausible that this effect could serve to enhance detection of the edges of objects impinging upon the visual field. Physiological studies at more central areas of the visual system would help to determine the function of the effect, and because the dynamics of the response to a single stimulus change are relatively long lasting (several hundred milliseconds), behavioral investigation to determine salamander response time to behaviorally relevant, spatially extensive stimuli is also indicated.

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REFERENCES


