M and L cones in macaque fovea connect to midget ganglion cells by different numbers of excitatory synapses

David J. Calkins, Stanley J. Scheln*, Yoshihiko Tsukamoto† & Peter Sterling

Department of Neuroscience, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA
* Department of Psychology, University of California, Los Angeles, California 90024, USA
† Department of Anatomy, Hyogo College of Medicine, Nishinomiya, 663 Hyogo, Japan

VISUAL acuity depends on the fine-grained neural image set by the foveal cone mosaic1-3. To preserve this spatial detail, cones transmit through non-divergent pathways: cone→midget bipolar cell→midget ganglion cell. Adequate gain must be established along each pathway; crosstalk and sources of variation between pathways must be minimized. These requirements raise fundamental questions regarding the synaptic connections: (1) how many synapses from bipolar to ganglion cell transmit a cone signal and with what degree of crosstalk between adjacent pathways; (2) how accurately these connections are reproduced across the mosaic; and (3) whether the midget circuits for middle (M) and long (L) wavelength sensitive cones are the same. We report here that the midget ganglion cell collects without crosstalk either 28±4 or 47±3 midget bipolar synapses. Two cone types are defined by this difference; being about equal in number and distributing randomly in small clusters of like type, they are probably M and L.

The non-divergent circuit from each foveal cone actually involves two midget bipolar cells4. Their axons descend in parallel to different levels5,6 of the inner plexiform layer where they contact the dendritic arbors of single 'off' and 'on' midget ganglion cells7,8. We reconstructed such paired midget bipolar and ganglion cells from electron micrographs of serial sections (Fig. 1a). Studying 95 off and 91 on midget bipolar axon terminals, we found that each contacted one midget ganglion cell exclusively without contributing even a single synapse to a neighboring midget ganglion cell. Thus, in macaque fovea the midget bipolar pathways from adjacent cones neither diverge nor converge. This has also been reported for four midget bipolar cells in human parafovea9.

We quantified the bipolar synapses to the midget ganglion cells. Each synapse was identified by a ribbon pointing between a pair of postsynaptic processes (‘dyad’10). Most dyads comprised a midget ganglion cell dendrite and an amacrine process (88%); however, some dyads comprised two amacrine processes (9%) or two ganglion cell dendrites (1%). Occasionally, one postsynaptic element of a dyad was a non-midget ganglion cell dendrite (2%). The off and on bipolar terminals connected to the same cone had similar numbers of ribbon synapses. For example, the left pair in Fig. 1b had 25 and 32 ribbons, and the right pair had 46 and 52 ribbons. Figure 1c shows this strong association for 56 such pairs (Spearman rank correlation coefficient11 r_s = 0.76, P<0.01).

When we enumerated the ribbon synapses of every complete off and on midget bipolar terminal in the tissue, two non-overlapping distributions emerged: one with 32±3 ribbons and one with 49±3 ribbons (means±s.d.; Fig. 1d). The Kruskal ‘dip intensity’ statistic12 for this distribution is 4.0, indicating strong bimodality (P<0.01, n=145). The number of bipolar synapses to the ganglion cells differed correspondingly: 28±4 versus 47±3 synapses. Each midget ganglion cell also received amacrine cell synapses about equal in number to the ribbon synapses: 30±3 versus 45±3 synapses. Equal amacrine input was also reported for midget ganglion cells in human parafovea9. The bipolar terminals with more synapses had greater membrane surface areas, 103±10 μm² as opposed to 79±9 μm², and so did the corresponding ganglion cell dendrites, 105±23 μm² versus 81±7 μm². These differences were significant for both the bipolar terminals (Kruskal-Wallis11 H = 8.31, P<0.005, d.f. = 1) and the ganglion cells dendrites (H = 4.33, P = 0.04, d.f. = 1). The off and on bipolar-to-ganglion cell junctions from the same cone had the same membrane surface areas for both bipolar terminals (H = 0.23, P = 0.66, d.f. = 1) and for ganglion cell dendrites (H = 0.02, P = 0.90, d.f. = 1; compare ref. 13). Occasionally, a midget ganglion cell received a synapse from a diffuse bipolar cell terminal (see also ref. 9).
In retrospect, Polyak's classic drawings of Golgi-impregnated cells in rhesus monkey and also in chimpanzee suggest two sizes of dendritic arbor (Figs 70, 74 in ref. 7). Also in retrospect, the four midget ganglion cells reconstructed from human parafovea also had small and large junctions: 55 and 57 versus 71 and 81 bipolar synapses. Thus, the two sizes of midget junction observed here in Macaca fascicularis may be general in Old World primates.

Fifty-nine cones in this tissue were associated with small junctions and 45 cones were associated with large junctions, yielding a ratio of 1.3. The ratio in the population represented by the sample lies between 0.9 and 2.1 with 95% confidence.

The mosaic formed by small and large junctions represents the arrangement of cone outer segments at 1° (Fig. 1c). This is so because the axons of adjacent cones do not cross17, nor do those of adjacent midget bipolar cells. The mosaic contains small clusters of like type. Such clusters would be expected from a random distribution. This we verified by measuring, along each axis of the triangular lattice, the number and lengths of unbroken sequences (strings) of like cone type. The number of strings summed over all three axes was not different from that expected from a random mosaic (Z = 0.88, P = 0.38)12. The lengths of strings, 2.0 ± 1.3 cones for cones with small midget junctions and 1.4 ± 0.7 for cones with large junctions, were not different from those calculated for five random mosaics with the same number and ratio of cones (Fig. 2).

The finding of two cone types, about equal in number and random in distribution, matches closely the pattern of M and L cones shown by in situ measurements of spectral sensitivity18. Therefore, we speculate that the two cone types identified here by differences in midget circuitry are M and L; indeed, we can.

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FIG. 1. a, Midget circuit from one cone. Cone terminal (orange) connects via paired midget bipolar cells (off, cyan; on, violet) to paired midget ganglion cells (off, pink; on, yellow); the cone also connects to diffuse bipolar neurons not shown. Off and on regions of the inner plexiform layer5,9 are demarcated. b, Left, paired midget pathways for one cone. Bipolar terminals contained 25 and 32 ribbon synapses (white rectangles); corresponding midget ganglion cells received 22 and 31 bipolar synapses (white circles). Right, midget pathways from a neighbouring cone. Bipolar terminals contained 46 and 52 ribbons; corresponding midget ganglion cells received 43 and 51 bipolar synapses. Some synapses are hidden. c, Off and on midget bipolar terminals connected to the same cone have similar numbers of ribbons. d, Number of midget bipolar ribbon synapses formed two non-overlapping distributions. e, Inferred mosaic of 108 cones at 1° nasal fovea. S cones, identified by their lack of an on midget circuit12,18 and by connections to on 'blue cone bipolar'21,22 cells, are coloured blue. Cones connected to small midget junctions are red (N = 59). Cones connected to large midget junctions are green (N = 45). This mosaic shows the putative distribution of M and L cones, but the colour that actually represents M or L could not be established.

METHODS. A retina was obtained from an adult male Macaca fascicularis and prepared for electron microscopy23. Consecutive sections (319) were cut radially at 90 nm through the nasal fovea along the horizontal meridian and photographed at 2,000 × magnification, yielding a patch 30 × 130 μm, with midget circuits at 510–640 μm eccentricity and the cone inner segments that serve them at 0.8–1.2°. Neurons were reconstructed by tracing their successive profiles onto mylar sheets. The tracings were stacked by computer24 and surface-rendered (a, b). For 43 midget pathways 1:1 correspondence between bipolar terminal and ganglion cell was assessed directly by tracing both cells. Fifty-five cones were represented in the series only by their midget cell pathways. These were quantified as described and projected by interpolation onto the mosaic in e.

FIG. 2 a, b and c. Distribution of lengths of unbroken sequences (strings) of cones connected to large midget circuits (in a, N = 88 strings) and to small midget circuits (in b, N = 92 strings). Smooth curves are calculated from the simple binomial model, given by (a, p) = Np(N−1)p−1, where N is the number of strings, i is string length and p is the best-fitting probability of success parameter. With N fixed, the sum of squares for error was minimum with p = 0.52 (a) and 0.50 (b), suggesting the possibility that the cone sample was drawn from a population with equal numbers of M and L cones. Five random mosaics with the same number and ratio of cones were generated; the distributions of string lengths for the two cone types in the inferred mosaic (Fig. 1e) were not statistically different from those of the random mosaics (x^2 = 0.02, P = 0.90, d.f. = 1).
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think of no plausible alternative. Whether M corresponds to the large midget junctions and L to small junctions, or vice versa, we cannot yet say, but because primate ganglion cells can now be identified following in situ spectral measurements, this issue can be addressed.

Selective pressure is likely to minimize the number of synapses employed in transferring the neural image from the cone mosaic to the ganglion cells. Therefore, the 28 ± 4 synapses from bipolar to ganglion cell (glutamatergic and therefore excitatory) is probably irrefutable. Also, it is likely that there is pressure to minimize the variation between off and on midget pathways leading from the same cone (Fig. 1b, c) in order that each point in the neural image be represented equally well for signals dimmer than or brighter than the mean. For the midget junctions serving cones of the same type, variation is equally small (s.d. is about 10% of the mean). This, too, seems critical in order that neighbouring points in the natural image be represented equally well. Such constancy of synaptic connections for neurons of the same type has been demonstrated for many other cell types in mammalian retina.

It might also have been expected that the midget circuits leading from M and L cones use the same number of synapses, but apparently one type employs about 50% more excitatory synapses than the other. This difference is proving helpful in determining the spectral characteristics of other retinal circuits, but why it should be so is an enigma.

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