How Müller Glial Cells in Macaque Fovea Coat and Isolate the Synaptic Terminals of Cone Photoreceptors

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ABSTRACT

A cone synaptic terminal in macaque fovea releases quanta of glutamate from ~20 active zones at a high rate in the dark. The transmitter reaches ~500 receptor clusters on bipolar and horizontal cell processes by diffusion laterally along the terminal’s 50 μm² secretory face and ~2 μm inward. To understand what shapes transmitter flow, we investigated from electron photomicrographs of serial sections the relationship between Müller glial processes and cone terminals. We find that each Müller cell has one substantial trunk that ascends in the outer plexiform layer below the space between the “footprints” of the terminals. We find exactly equal numbers of Müller cell trunks and foveal cone terminals, which may make the fovea particularly vulnerable to Müller cell dysfunction. The processes that emerge from the single trunk do not ensheathe a single terminal. Instead, each Müller cell partially coats two to three terminals; in turn, each terminal is completely coated by two to three Müller cells. Therefore, the Müller cells that coat one terminal also partially coat the surrounding (~ six) terminals, creating a common environment for the cones supplying the center/surround receptive field of foveal midget bipolar and ganglion cells. Upon reaching the terminals, the trunk divides into processes that coat the terminals’ sides but not their secretory faces. This glial framework minimizes glutamate transporter (EAAT1) beneath a terminal’s secretory face but maximizes EAAT1 between adjacent terminals, thus permitting glutamate to diffuse locally along the secretory face and inward toward inner receptor clusters but reducing its effective spillover to neighboring terminals. J. Comp. Neurol. 453:100–111, 2002.

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The synaptic terminals of vertebrate photoreceptors release the neurotransmitter glutamate at a high tonic rate in the dark. To support this release rate, the cone terminal houses ~20 active zones, each served by a synaptic ribbon with a reservoir of ~100 tethered, synaptic vesicles. In the retina and elsewhere, when a synaptic vesicle releases its transmitter, the concentration in the synaptic cleft rises quickly and then decays in time and space (Clements, 1996; Rao-Miroznik et al., 1998; Bergles et al., 1999). The details of this decay are important, because postsynaptic receptors near a release site, 10 nm across the synaptic cleft, would see high concentrations for a short time and ought to have low affinity and fast unbinding, whereas receptors farther from a release site would see lower concentrations for longer times and ought to have high affinity and slow unbinding (Rao-Miroznik et al., 1998; DeVries, 2000).

Geometry is critical because glia may physically isolate one synapse from another within a single terminal or the...
synapses of one terminal from those of another. In the
cerebellum (Lehre and Danbolt, 1998), a single Bergman
glial cell wraps the entire dendritic tree of a single Pur-
kine cell and coats every synapse (Ramón y Cajal, 1892;
Spacek, 1985). By contrast, many glial processes wrap a
hippocampal pyramidal cell and leave certain synapses
naked (Ventura and Harris, 1999). Numerical relation-
ships and glial-synaptic geometries are therefore speci-
cific for each brain region.

Such relationships have yet to be clarified in primate
retina at the elaborate synaptic terminal of the cone pho-
toreceptors, which is enveloped by glia called Müller cells
(Müller, 1851; Ramón y Cajal, 1892; Dogiel, 1893). There,
a quantal pulse of glutamate from the cone terminal dif-
fuses 1–2 μm both laterally and inward to ~500 sites
where horizontal and bipolar cell dendrites express clus-
ters of glutamate receptors (e.g., Schein et al., 1996; Herr
et al., 1997; DeVries and Schwartz, 1999; DeVries, 2000; Haverkamp et al., 2000, 2001). The many pulses spread
and merge to form a veritable river of glutamate whose
flow ought to be primarily in the inward dimension from
the secretory face into the outer synaptic layer. Here, we
show that there is one Müller glial cell for each foveal cone
terminal and describe quantitatively how Müller cells coat
and isolate cone terminals, thus promoting flow primarily
in the one dimension.

MATERIALS AND METHODS

This investigation used the same set of serial thin sec-
tions of the foveal retina of an adult male monkey (Macaca
fascicularis) as was used in Tsukamoto et al. (1992), which
gives details of preparation for electron microscopy. A
series of 319 consecutive thin sections (90 nm thick) was
cut vertically through the nasal fovea, parallel to the
horizontal meridian, at a region connected to foveal cone
outer segments at ~1 degree eccentricity (Tsukamoto et
al., 1992). Sections were stained with uranyl acetate and
lead citrate. The region spanning 480 μm to 640 μm from
the foveal center was photographed at 400×, 2,000×,
5,000×, and 10,000×. From electron photomicrographs
like the one shown in Figure 1, we determined the X (horizon-
tal) and Y (vertical) coordinates of the center of
each cone terminal. Electron photomicrographic negatives
and positive prints were scanned with an Agfa scanner,
and the tiff files were adjusted for brightness and contrast
with Adobe Photoshop 5.0. No retouching or other image
manipulation was performed.

The center points were plotted from 400× photomicro-
graphs onto clear plastic sheets and digitized by using the
Montage software package (Smith, 1987). The schematic
in Figure 2A shows how the terminals would appear in
horizontal section. The Z coordinate of each center point

Fig. 1. Müller cell (MC) somas (+), typically polygonal in cross-
section, are present with approximately the same incidence as the
overlying cone terminals (CT). Vertical section through the macaque
monkey retina centered at 560 μm along the horizontal meridian,
connected to nasal foveal receptors at ~1 degree eccentricity (Tsuka-
moto et al., 1992). HF, Henle fibers; OPL, outer plexiform layer; INL,
inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell
layer. Scale bar = 50 μm.
The tissue had been cut slightly off the vertical; in addition, the photomicrographs were taken without de-epithelialization. Consequently, we rotated the monolayer of terminals into the horizontal plane as follows: To correct for the angle off vertical, we best fit a line to the points projected into the YZ plane, computed the angle of that line, and rotated the cloud of points (about the X axis) by the opposite angle. Then, to correct for the angle off horizontal, we best fit a line to the new points projected into the XY plane, computed the angle of that line, and rotated the cloud of points (about the Z axis) by the opposite angle. The same cone-terminal-based rotations were applied to the coordinates of Müller cell somas and of Müller cell trunks.

RESULTS

Identical spatial densities of Müller cells and cone terminals

To compare the spatial densities of Müller cells and cone terminals, we first counted Müller cell nuclei and cone terminals. However, this method is only approximate because in the fovea Müller cell nuclei are displaced laterally from the cone terminals, which can cause large errors in calculating spatial densities (Schein, 1988). Also, we found that the array of outer midget bipolar nuclei did not replicate the array of cone terminals, so the mapping of Müller cell nuclei to their trunks in the outer retina may be similarly disorderly.

By tracing processes of 12 Müller cells at the level of the cone terminals, we found that each Müller soma emits a single trunk that ascends through the OPL (Fig. 3; also see Polyak, 1941; Robinson and Dreher, 1990; Dreher et al., 1992; Reichenbach et al., 1995). These trunks provide a good marker of each Müller cell at the same level as the cone terminals. Because the cone terminals and the Müller cell trunks are in the same layer, they could be mapped and counted in the same physical space.

We first determined the largest box that fully sampled the point patterns representing the centers of cone terminals (Fig. 4A) and Müller cell trunks (Fig. 4B). This box enclosed 101 cone terminals and 100 Müller cell trunks, suggesting that their densities are very similar. However, this method of analysis depends somewhat on the size of the box and its precise placement.

For greater accuracy, we counted the numbers of points representing either cone terminals or Müller cell trunks in boxes of increasing area. Then, plotting number of points vs. box size, we computed the best fitting line through the origin (Fig. 4C,D). This method smoothed the variation in measured density that arises from the discontinuous entry of points into expanding boxes. When box area increased beyond the larger box in Figure 4B, whose area was 3,813 \( \mu m^2 \), the counts fell below the line in Figure 4D, indicating the limit of the area in which we had fully sampled Müller cell trunks. We therefore fit the points in Figure 4C,D up to the area of that box. The slopes of these lines are the densities: 26,961 terminals \( mm^{-2} \) and 26,959 trunks \( mm^{-2} \). The densities of terminals and trunks thus appear to be identical.

To further show that the similarity of the densities was not due to choice of the size of the largest box, we also graphed the number of trunks vs. the number of terminals contained in each counting box (Fig. 4E,F). With increasing area, the boxes first contain more terminals than trunks, then more trunks than terminals, and so forth. The best fitting line (Fig. 4F), however, had a slope of 0.999, confirming that the densities are the same.

As noted above, the density of Müller cells at the level of their nuclei gives only an approximate measure of the density of Müller cells at the level of the cone terminals. Nonetheless, we applied the same expanding box method as used for the Müller cell trunks (Fig. 4D) to the Müller cell nuclei and obtained a density of 27,163 \( mm^{-2} \) \( (r^2 = 0.989) \), essentially equal to that of the Müller cell trunks and cone terminals.

How Müller cells coat the cone terminals

If each Müller cell completely ensheathed one cone terminal, the space between adjacent terminals would al-
Fig. 3. Each Müller cell emits a single trunk that ascends through the neuropil below the region where cone terminals abut; upon reaching the terminals, it avoids their secretory faces. **A:** Vertical section through three cone terminals. The center terminal is flanked on the left by one Müller cell trunk (+) and on the right by a second trunk (*). The + trunk coats the right side of the left-hand terminal exclusively and the middle terminal on its left side and top. This + trunk then descends to coat the right side of the center terminal and is met by the ascending * trunk of the neighboring Müller cell (boxed region shown at higher magnification in **D**). **B:** Full thickness of the left Müller cell trunk (+) is captured three sections forward from **A**. The right Müller cell trunk (*) has disappeared, replaced by neuropil, so transmitter may diffuse in the direction of the arrow to the neuropil underneath the adjacent cone terminal. **C:** Full thickness of the right Müller cell trunk (*) is captured six sections back from **A**. The left Müller cell trunk (+) has disappeared, replaced by neuropil, so transmitter may diffuse in the direction of the arrow. **D:** Boxed region from **A** at higher magnification. The arrows point to the membranes between the two (+ and *) Müller cell trunks that isolate the middle and right terminals, respectively. Scale bars = 3 μm in **A**, 2 μm in **B,C**, 0.5 μm in **D**.
Fig. 4. Three methods of data analysis demonstrate equal numbers of cone terminals and Müller cell trunks. A,B: The centers of cone terminals (A) and of Müller cell trunks (B) after rotation of their raw coordinates (Fig. 2B) into the horizontal plane (see Materials and Methods section). Scales differ for X and Z coordinates. The large boxes extend 64.00 µm in the +X and −X directions from the center and 14.89 µm (0.2327 times as far) in the +Z and −Z directions. The small boxes extend 10.00 µm in the +X and −X directions and 2.0327 µm in the +Z and −Z directions. This aspect ratio (0.2327) and the center (5.370 µm, −8.700 µm) were determined from the maximum sampling area among the trunks. The larger box contains 101 cone terminals and 100 Müller cell trunks. C,D: The expanding box method demonstrates equal densities of cone terminals (C) and Müller cell trunks (D). The counting box was expanded from the smaller to the larger boxes (see A and B) in 1.0000 µm steps for X and 0.2327 µm steps for Z. Slopes of the best fitting lines forced to go through the origin gave densities of 26,961 terminals/mm² ($r^2 = 0.996$) and 26,959 trunks/mm² ($r^2 = 0.995$). E,F: Number of Müller cell trunks (filled diamonds in E) equals the number of cone terminals (open circles in E) in the expanding box. The slope of the best fitting line forced to go through the origin (F) is 0.999 with an $r^2$ of 0.994.
ways be occupied by two different Müller cells, as shown in Figure 5A. In an alternative model, shown in Figure 5B, the space between adjacent terminals would mostly be occupied by one Müller cell process. We found examples of both descriptions, with adjacent terminals separated by the processes of two Müller cells (right side of the center terminal in Fig. 3A, magnified in Fig. 3D) or by the processes of one (left side of the center terminal in Fig. 3A). Thus, neither of the models in Figure 5 correctly describes the actual arrangement.

To determine the arrangement, we reconstructed the coating around the bases of 10 terminals, composed of two groups of contiguous terminals and a single terminal. We show these coatings in the horizontal plane at the bases of the terminals (Fig. 6A–C). Each Müller cell trunk has its own color. Where a process of one trunk separates adjacent terminals, thick lines of the same color mark both terminals’ perimeters. Where the processes of two trunks separate adjacent terminals, two medium lines of different colors mark the two terminals’ perimeters. Finally, where we identified one Müller cell trunk coating a terminal but did not know whether there was another one between that terminal and its neighbor, we used a thin line of the color of the identified trunk to mark the one terminal’s perimeter. (The thin lines are mainly at the tops and bottoms of terminals.) There is a small “hole” in the coating between terminals #46 and #60 and between #52 and its right-hand neighbor; these fenestrations allow gap junctions between adjacent terminals (Tsuchamoto et al., 1992).

Measurements from these data revealed that each Müller cell coated an average of $38\% \pm 24\%$ (mean $\pm$ SD) of a terminal’s perimeter (Fig. 7A). (Inversely, a given terminal was coated by an average of $2.6 \pm 0.7$ Müller cells.) The processes of just one Müller cell occupied the space around an average of half (48%) of a terminal’s perimeter (Fig. 7B), and the processes of two Müller cells occupied the space around the remainder (52%).

Figure 6D–F is a schematic version of the reconstructions in Figure 6A–C. In the schematics, wide, filled blocks correspond to regions between adjacent terminals occupied by one Müller cell; medium-wide, filled blocks correspond to regions occupied by two. Where the number of contributing trunks separating adjacent terminals is not known, as indicated by the thin lines in the reconstructions, the possible second trunk is represented by an open block. Finally, black blocks represent trunks that did not coat a reconstructed terminal but nonetheless occupied space between that terminal and its adjacent terminals.

Each schematic terminal has two numbers within it. The first number indicates how many trunks coat it. The second number indicates how many trunks tile the space around it, that is, its “neighborhood”. Each + associated with the second number tallies an open block around that terminal in the schematic, where there may be an additional trunk in the neighborhood. For example, in Figure 6C, terminal #52 is coated by two Müller cells (red and green); the schematic in the corresponding Figure 6F indicates that two more Müller cells (black) occupy the neighborhood of terminal #52. In addition, the open block above and the open block below the terminal represent two more Müller cells that may be present in the neighborhood of this terminal. The numbers in terminal #52 thus read as follows: 2 for the coatings, 4 for the Müller cells that are definitely in the neighborhood of the terminal, and ++ for up to two more Müller cells that may be in the neighborhood. Clearly, more Müller cells tile the neighborhood of a terminal than actually coat that terminal.

Assuming that half of the uncertain neighbors (+) are true neighbors, the average number of Müller cells in the neighborhood of each of the 10 cone terminals in Figure 6 is the total number of definite neighbors (41) plus half of the total number of possible neighbors (14/2 = 7), divided by the number of terminals: 48/10 = 4.8. Conversely, because there are equal numbers of Müller cells and terminals, each Müller cell must be in the neighborhood of 4.8 terminals. This finding raises a caution: by light microscopy a fully stained Müller cell, although actually coating just two to three terminals, is likely to be in the neighborhood of twice as many terminals and thus appear to coat many more than two to three of them.

Clearly, equal densities do not require a simple 1:1 ensheathing as in Figure 5A or the simple tiling pattern as in Figure 5B. The actual tiling is more complex, as illustrated by the models in Figure 6G,H. The tiles in the models have the following properties that closely reflect the quantitative results: (1) Our results show that the numbers of cone terminals and Müller cells are equal. This property requires that each Müller cell coat an amount of perimeter equal to that of one whole terminal. (2) In Figure 6G, where terminals pack squarely, each Müller cell coats 2 terminals; in Figure 6H, where terminals pack triangularly, each Müller cell coats 3 terminals. In a mixture of geometries, each Müller cell would coat an average of 2.5 terminals. Our results show 2.6. (3) In turn, each terminal is coated by 2 (Fig. 6G) or 3 (Fig. 6H) Müller cells, so in a mixture of geometries, each terminal would be coated by an average of 2.5 Müller cells. (4) The space around half of a terminal’s perimeter is tiled by one Müller cell, half by two. Our data indicate 48%/52%. (5) Each terminal has 4 (Fig. 6G) or 6 (Fig. 6H) Müller cells in its neighborhood, so in a mixture of geometries, an average of 5 Müller cells tile the space between terminals. Our data show 4.8.

How Müller cells partition the OPL

Müller cells partition the underlying neuropil differently from the way they partition cone terminals. Indeed,
Fig. 6. Divergence of each Müller cell onto cone terminals and convergence of Müller cells onto each cone terminal, reconstructed from serial sections. A–C: Reconstructions of the Müller cell coatings of six, three, and one terminals whose locations are mapped in Figure 2A. The coating patterns were reconstructed at the level of the plus (+) and asterisk (*) symbols in Figure 3A and are shown here in the horizontal plane at the bases of the terminals. See text for more details. D–F: Schematic versions of A–C. See text for details. G,H: Models of how Müller cells coat cone terminals and tile the space between them. The terminals are squarely packed in G, triangularly packed in H. The actual packing in the retina is a mixture of these geometries.
Figure 3B,C shows that outside the region of the ascending trunk, the neuropil is continuous beneath adjacent terminals, as marked by the arrows. We reconstructed two Müller cells through the depth of the OPL (the green one in Fig. 6A coating terminals #25, #26, #46, and #60 and the cyan one in Fig. 6A coating terminal #24). As the word "trunk" suggests, the Müller process resembles a tree trunk. In the region we studied, the trunk is longer in the Z-direction, \( \text{H} \times 1.5 \text{H} \), than the X-direction, from 0.25 \( \text{H} \times 1.5 \text{H} \) to 1 \( \text{H} \times 1.5 \text{H} \). Thus, Figure 8A shows (solid) trunks in the OPL, below the space between the "footprints" of the terminals (dashed outlines). Within the OPL, below the cone terminal, the surface area of these trunks is \( \sim 30 \text{H}^2 \).

Thus, Müller cell processes (1) ascend between "cylinders" of neuropil that underlie each terminal without substantially penetrating those cylinders, (2) completely coat the entire perimeter of the terminal’s secretory face with at least two layers of glial membrane of one Müller cell, and (3) overlap over half of the perimeter, giving a quadruple layer of glial membrane (Fig. 8B, which is redrawn from the model in Fig. 6H to correspond with the perspective of Fig. 8A). As a result, glutamate released at the secretory face can diffuse inward into a considerable volume of neuropil (roughly \(7.5 \times 0.5 \times 5 \text{H} \), \( \sim 5 \text{H}^3 \), approximately \(200 \text{H}^3 \)) without encountering membranes of Müller cell trunks. Moreover, deep in the OPL, glutamate can diffuse laterally (arrows in Fig. 3B,C) from this volume into the deep neuropil underneath neighboring terminals.
DISCUSSION

The anatomic relationship between Müller cells and cone terminals that we find in macaque is similar to that reported in the only other case studied, the tiger salamander (Sarantis and Mobbs, 1992). Specifically, on their way from the inner nuclear layer to the photoreceptor terminals, Müller cell processes in the tiger salamander do not extend laterally into the OPL beneath each terminal and thus do not interfere with diffusion of glutamate within the OPL underneath each terminal. However, each of their two serially sectioned tiger salamander Müller cells completely surrounded at least six photoreceptors and partially coated several others, many more than what we found in the macaque fovea. The smaller number of photoreceptors coated by the Müller cells in our study may be because we studied a primate, or it may be because we studied the fovea. Finally, the tiger salamander Müller cell processes appear to fill the OPL underneath the regions where terminals abut (cf. Fig. 4 of Sarantis and Mobbs, 1992), but in the macaque, the OPL processes occupy only a small portion of that volume (ovals in Fig. 8A).

One Müller cell per cone terminal in the fovea

The present work resolves uncertainty regarding the numerical relationship of Müller cells to cone terminals in the fovea. Krebs and Krebs (1989) assumed that the arrays of cone terminals and Müller cell nuclei were both monolayers, made counts from tangential sections, and found roughly half the density of Müller cells (17,000 mm\(^{-2}\)) as cone terminals (31,000 mm\(^{-2}\) to 36,000 mm\(^{-2}\)) in macaque fovea. Figure 1, however, shows that Müller cell nuclei reside in roughly two layers, which would double the 17,000 mm\(^{-2}\) value. Indeed, Distler and Dreher (1996) found 30,000 and 37,000 Müller cells/mm\(^2\) in the foveae of two macaque monkeys, which led Chao et al. (1997) to speculate that in the fovea, Müller cell density could be related to cone terminal density. Here, we go further and establish, by three different methods of analysis (Fig. 4), that the fovea contains precisely equal densities of Müller cells and cone terminals.

Is there a reason that the densities of Müller cells and cone terminals are strictly equal? Equality of numbers is required for the tiling relationship between foveal Müller cells and cone terminals shown in the model in Figure 8B, and this arrangement may be important for optimal functioning of midget cell circuits in macaque fovea. Consider the midget ganglion cell whose receptive-field center is driven by the one (white) cone terminal in this figure and whose receptive-field surround is driven by the six (light gray) adjacent cone terminals. Correspondingly, the three (black) Müller cells that coat the (white) cone terminal also coat the six (light gray) adjacent terminals. The three Müller cells coating each cone terminal thus may constitute a functional unit of transmitter uptake, providing a common chemical environment for the cones responsible for a midget ganglion cell’s center and surround. The fovea would be special in this regard because, in this region, midget ganglion cell centers are driven by one cone.

The situation in peripheral retina is different. Indeed, Krebs and Krebs (1987) found twice as many Müller cells (16,000 mm\(^{-2}\)) as cone terminals (8,000 mm\(^{-2}\)) at an eccentricity of approximately 30 degrees from the fovea, and Distler and Dreher (1996) found a similar density of Müller cells at that eccentricity. The number of Müller cells per cone terminal thus appears to increase above one outside the fovea, where Müller cells serve numerous rods as well as cone terminals.

Müller cells and macular disease

Because Müller cells affect retinal metabolic and synaptic function, they could be a primary cause of some retinal diseases. For example, Müller cells have been implicated in juvenile X-linked retinoschisis (Yanoff et al., 1968; Deutman, 1977; Condon et al., 1986; Reid et al., 1999). In light of its 1:1 relationship to cone terminals, the macula may be particularly vulnerable to Müller cell pathology. Indeed, Müller cells have been proposed as the initiator of cystoid macular degeneration (Loeffler et al., 1992), idiopathic macular holes, and foveomacular schisis (Gass, 1999). In addition, DiLoreto et al. (1995) reported that changes in Müller cells that are normally regarded as “reactive”, namely, production of glial fibrillary acidic protein, preceded degenerative changes in photoreceptors in age-related retinal degeneration in rat, raising the possibility that Müller cell disease may underlie some forms of age-related macular degeneration in humans.

The geometric relationship between Müller cells and foveal cone terminals suggests that death of one (blue) Müller cell (in Fig. 6H) could partially denude the three (white) cone terminals that it served and greatly expand those terminals’ immediate extracellular space. Without a narrow synaptic cleft, vesicular release might not cause the large peaks in glutamate concentration needed by autoreceptors or glutamate transporters on the cone terminal to control release (Picaud et al., 1995; Scanziani et al., 1997; Min et al., 1998; Roska et al., 1998; Koulen et al., 1999). The absence of this negative feedback mechanism might increase the tonic release rate, leading to exhaustion of the synaptic terminal and perhaps death of the white cones. Conceivably, loss of the one (blue) Müller cell and the three (white) cones would create large extracellular spaces for the six surrounding (red and green) Müller cell, causing their death by the death of their surrounding cones, and so on. This mechanism is obviously highly speculative, but it suggests a novel approach to thinking about macular disease.

Müller cell framework permits transmitter diffusion to many receptor sites

The glial cell framework for cone terminals differs strikingly from that of other central synapses. For example, Bergman glia completely coat all synapses on Purkinje cell spines, thus segregating every active zone from its neighbors (Lehre and Danbolt, 1998). By contrast, astrocytes in CA1 of hippocampus coat approximately half of the axospinous synapses, with coatings that are often incomplete (Ventura and Harris, 1999). These glial frameworks are of fine mesh, and glutamate can diffuse within a two-dimensional cleft for at most 100 nm before encountering glial membrane and their associated transporters. Spillover of glutamate from one synapse to a neighboring one may occur, particularly where astrocytes fail to separate two active zones and because synapses wrapped in the same glial package will experience more mutual spill-over than those separated by glial membranes (Lehre and Danbolt, 1998; Rusakov and Kullmann, 1998). During intense release, transmitter may even spill over to affect...
low affinity receptors at adjacent synapses (Luján et al., 1997; Kullmann and Asztely, 1998; Rusakov et al., 1999).

However, this spillover is confined to two dimensions and small spatial scale (Danbolt, 2001).

For giant central synapses, such as the calyceal synapse in the auditory brainstem, multiple active zones are unseparated by glial fingers (Otis et al., 1996). The calyx appears to be topologically one-dimensional, because each active zone directly abuts a postsynaptic process across a 20-nm cleft. However, spillover can occur on a larger scale into the second dimension. In the glomerular synapse in the thalamus, a spheroidal cluster of glutamatergic and GABAergic processes are wrapped in a sheet of astrocytes (Majorossy et al., 1965; Peters and Palay, 1966; Guillery, 1969); each active zone abuts a postsynaptic process, forming an inherently one-dimensional synapse. However, transmitter also spills into the higher dimensional extra-cellular space of the cluster before reaching the astrocytic shell. In these examples, primary transmission occurs on the scale of 20 nm.

By contrast, as can be appreciated in the photomicrographs in Figure 3, the Müller cell framework permits diffusion of glutamate from its (−20) release sites associated with synaptic ribbons on the secretory face of one cone terminal to the large number (500) of receptor clusters on dendrites associated with a terminal (Chun et al., 1996). The secretory face of a foveal cone terminal has an area of ~50 μm², giving a domain of ~2.5 μm² per release site. If this domain were configured as an area 0.8 μm high by 3 μm wide, no receptor cluster would be more than 1,500 nm from a release site alongside a synaptic ribbon (Schein et al., 1996).

In more detail, these receptor clusters are distributed over a broad scale of distance and dimension (Schein et al., 1996; Vardi et al., 1998, 2000; Morigiwa and Vardi, 1999; Haverkamp et al., 2000, 2001): A pulse of glutamate released at the active zone encounters in one dimension, 10 nm away, iGluR receptor clusters directly across the synaptic cleft (on horizontal cell spines). It encounters along the two-dimensional surface of the terminal, ~600 nm away, receptors near the mouth of the invagination (on the invaginating dendrites of inner midget and diffuse bipolar cells), ~1,000 nm away, iGluR receptors on triad-associated contacts (on OFF midget bipolar cell dendrites), and ~1,500 nm away, iGluR and mGluR6 receptors on basal contacts (on outer and inner diffuse bipolar cell dendrites, respectively). In three dimensions, it encounters iGluR receptors (on horizontal cell dendrites) 1,500–1,800 nm away. Glutamate should be able to diffuse these distances in a time scale on the order of a millisecond (Sarantasis et al., 1993; Clements, 1996; Ribble et al., 1997; Overstreet et al., 1999). That a pulse of transmitter could diffuse 1,000 nm or more to cause an excitatory postsynaptic current may seem surprising, but recent electrophysiological recordings from bipolar-cone pairs provide ample confirmation (DeVries and Schwartz, 1999; DeVries, 2000).

Indeed, glutamate could even diffuse from the center of the secretory face of the cone terminal laterally along the secretory face to receptors at the perimeter of the terminal and Müller cell membrane, a distance of 2–3 μm, in less than 10 msec. Glutamate transporters on Müller cell processes (see below) bind glutamate quickly in this time frame (Bergles and Jahr, 1998). Therefore, for this diffusion to occur, it is critical that Müller glial processes are absent from the neuropil immediately underlying the cone terminals, which is exactly what we show here.

Just beneath the terminal’s secretory face, glutamate reaching the edge of the terminal encounters multilayered Müller cell wrappings that should reduce lateral spillover between terminals (Fig. 8B), but not necessarily to zero (S. DeVries, personal communication). Müller cell membranes at this site thus help preserve independent activity between the midget bipolar cells that provide the private pathways for each foveal cone and are responsible for visual resolution in macaque fovea as fine as the dense array of foveal cones.

Glutamate diffusing more deeply into the neuropil encounters Müller membrane only at the trunks (Fig. 8A, ovals), so lateral spillover deep in the OPL (arrows in Fig. 8B,C) to receptor clusters on horizontal cells underneath neighboring terminals may proceed unimpeded. Unlike glutamate receptors on midget bipolar cells, those on horizontal cells are presumably designed to respond to glutamate from a larger distance, that is, to slower and lower concentration pulses. Because horizontal cells already sum responses over large numbers of cone terminals, this inter-terminal spillover is not disadvantageous.

**Does the glial framework’s transport capacity match the estimated flux of glutamate?**

The Müller cell removes neuroactive substances (e.g., potassium, GABA, and glutamate) released into the extracellular space by synaptic activity (Newman et al., 1984; Ripps and Witkovsky, 1985; Newman and Reichenbach, 1996). Scavenging should be vigorous to prevent disasters like spreading depression and neurotoxicity but should also be precisely tuned to optimize the spatiotemporal concentration of each substance. Although neurons, including cone terminals and bipolar dendrites (Massey et al., 1997; Vandenbranden et al., 2000), also express transporters, the following speculative discussion follows findings that Müller glia dominate glutamate transport in the retina (Kennedy et al., 1974; White and Neal, 1976; Ehinger, 1977; Lehre et al., 1997; Bergles and Jahn, 1998), as glial cells probably do elsewhere as well (Lehre and Danbolt, 1998; Rusakov and Kullmann, 1998; Pow et al., 2000).

The band of Müller cell membrane that coats the perimeter of a cone terminal, ~25 μm in length (Fig. 8B), rises 2 μm from the base of the terminal with a 1.5-fold lapping, to present a membrane surface area of ~75 μm² of Müller cell per cone terminal. Beneath the terminal, within the OPL, the Müller cell trunk has a surface area of ~30 μm² (ovals in Fig. 8A). Although the density and distribution of EAAT1 transporters on Müller cell membrane has not been determined, here we assume a density of 5,000 EAAT1 μm⁻² (Derouiche and Rauen, 1995; Rauen et al., 1995; Lehre and Danbolt, 1998). In that case, these two Müller cell surface areas could represent 375,000 and 150,000 EAAT1 molecules per cone terminal. Müller cells contain three additional excitatory amino acid transporters (Eliaosof et al., 1998a,b), and as noted above, the neurons contain transporters as well, so these numbers of EAAT1 molecules may underestimate the total number of glutamate transporters.

Glutamate transporters require ~100 msec for transport (Wadiche et al., 1995; Otis and Jahn, 1998; Wadiche and Kavanaugh, 1998). The precise number of glutamate...
molecules in a synaptic vesicle is not known, with estimates ranging from 400 to 4,000 (Danbolt, 2001). Assuming a glutamate concentration of 100 mM (Danbolt, 2001), the synaptic vesicles in our material, with an inner diameter of 35 nm, would have 1,500 molecules, in the middle of this range. Assuming a release rate $\sim 1,000$ quanta sec$^{-1}$ from $\sim 20$ active zones each releasing at 50 quanta sec$^{-1}$ (Ashmore and Copenhagen, 1983; Rieke and Schwartz, 1996; Rao-Mirotznik et al., 1998), we estimate that each cone terminal releases $1.5 \times 10^6$ glutamate sec$^{-1}$, or $\sim 150,000$ glutamate molecules over the 100-msec transport time. Thus, despite the impressive tonic flux, the Müller cell framework by itself, with perhaps $\sim 500,000$ glial EAAT1 transporters per cone terminal, seems to create an adequate sink for glutamate, in these estimations several times as much as needed.

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LITERATURE CITED


