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Convergence and Divergence of Cones onto Bipolar Cells in the Central Area of Cat Retina

Ethan Cohen and Peter Sterling

Phil. Trans. R. Soc. Lond. B 1990 **330**, 323-328
doi: 10.1098/rstb.1990.0202

Convergence and divergence of cones onto bipolar cells in the central area of cat retina

ETHAN COHEN† AND PETER STERLING

Department of Anatomy, University of Pennsylvania, Philadelphia PA 19104, U.S.A.

SUMMARY

In the central area of cat retina the cone bipolar cells that innervate sublamina *b* of the inner plexiform layer comprise five types, four with narrow dendritic fields and one with a wide dendritic field. This was shown in the preceding paper (Cohen & Sterling 1990*a*) by reconstruction from electron micrographs of serial sections. Here we show by further analysis of the same material that the coverage factor (dendritic spread × cell density) is about one for each of the narrow-field types (b_1, b_2, and b_4). The same is probably true for the other narrow-field type (b_3), but this could not be proved because its dendrites were too fine to trace. The dendrites of types b_1, b_2, and b_4 collect from all the cone pedicles within their reach and do not bypass local pedicles in favour of more distant ones. The dendrites of type b_5, the wide-field cell, bypass many pedicles. On average 5.1 ± 1.0 pedicles converge on a b_1 bipolar cell; 6.0 ± 1.2 converge on a b_2 cell and 5.7 ± 1.5 converge on a b_4 cell. Divergence *within* a type is minimal: one pedicle contacts only 1.2 b_1 cells, 1.0 b_2 cells, and 1.0 b_4 cells. Divergence *across* types is broad: each pedicle apparently contacts all four types of the narrow-field bipolar cells that innervate sublamina *b*. Each pedicle probably also contacts an additional 4–5 types of narrow-field bipolar cell that innervate sublamina *a*. There are several possible advantages to encoding the cone signal into multiple, parallel, narrow-field pathways. These include: tuning of pathways to transmit different temporal frequencies, use of ion channels with widely separated equilibrium potentials (to increase gain), and formation of different regulatory circuits in the inner plexiform layer. The latter possibility would permit different operations (e.g linear or nonlinear) to be performed on the visual signal on its way towards different types of ganglion cell.

INTRODUCTION

In the central area of cat retina, the distribution of cone bipolar cells (38000 mm^{-2}) is denser than the distribution of cones (about 24000 mm^{-2}) (Cohen & Sterling 1990*a*; Steinberg *et al.* 1973). Half of the bipolar cells innervate sublamina *a* of the inner plexiform layer, and the other half innervate sublamina *b* (Cohen & Sterling 1986). Among the bipolar cells of sublamina *b* there are five types, each forming its own independent array (Cohen & Sterling, 1990*a*). We wished to determine the convergence and divergence of cones onto each of these arrays, as this might provide clues to the reason for such an apparent plethora of types.

The convergence and divergence cannot be determined simply from the relative densities of cones and bipolar cells. The ratio of densities merely gives the ratio of the convergence and divergence and not the absolute values (Sterling *et al.* 1988). Therefore, it was necessary to determine the convergence and divergence directly by tracing dendrites from bipolar cells identified as to type through electron micrographs of serial sections back to the array of cone pedicles that synapse upon them. This effort was directed at the cone bipolar cells that had been identified in our previous study (Cohen & Sterling 1990*a*).

† Present address: Department of Physiology, University of Minnesota, Minnesota, MN 55455, U.S.A.

METHODS

The tissue studied here was a patch of retina (25×90 μm) from the area centralis of an adult cat at about 1° eccentricity. It was the same tissue, studied in the same way, as in the preceding paper (Cohen & Sterling 1990*a*).

RESULTS

Forty two cone bipolar cells in this patch of retina sent axons to sublamina *b*. Each cell had already been classified according to type (figure 1) and the dendritic arbors of three members each of type b_1, b_2, and b_4 had been reconstructed. The dendrites of type b_3 were so fine that in no case could they be traced or reconstructed. Only one member of type b_5 was present in the patch; its dendrites were reconstructed over a fairly wide region and found not to collect from any cones within this retinal patch (figure 3, Cohen & Sterling 1990*a*).

(*a*) *Coverage factor*

The first question was whether the dendritic array of

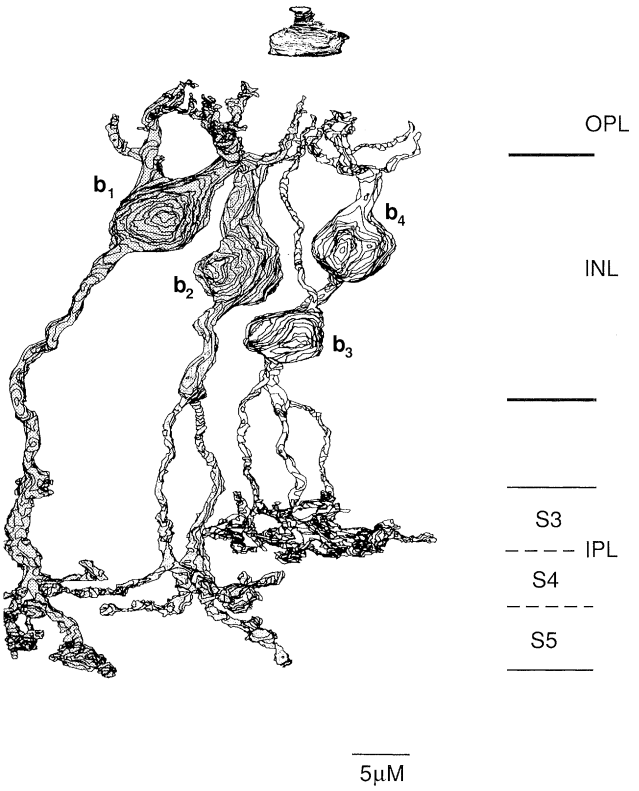

Figure 1. Summary diagram showing four types of bipolar cell that innervate sublamina *b* reaching toward the same cone. (OPL, outer plexiform layer; IPL, inner plexiform layer.)

a given type of bipolar cell has access, at least potentially, to all the cones. In other words, does each array 'cover' all points on the retinal surface? The standard approach to this question is to calculate the 'coverage factor'. This is the density of a cell type times the area covered by its dendrites, assuming they form a circular field (Wässle *et al.* 1978). Figure 2 shows that for types b_1, b_2, and b_4 the coverage factor calculated in this manner was about one. Thus each separate array seems to have at least the potential to contact all the cone pedicles.

The figure also makes clear that the bipolar dendritic fields are not circular. Rather, they vary in shape from oval, to elongate, to cruciate. These variable branching patterns at the level of the individual call are probably not simply 'noisy' growth. Rather, as will be seen, they belong to a larger pattern in which adjacent members of the same cell type branch in complementary fashion so as to completely 'tile' the array of cone pedicles.

(*b*) *Convergence*

We determined the convergence of cones onto specific types of bipolar cell by first reconstructing an array of 35 cone pedicles and then tracing dendrites from the bipolar cells to their terminations beneath particular pedicles (figure 3). In some cases the fine structure of the synaptic contact could be determined (Cohen & Sterling 1990*a*). For the narrow-field types b_1, b_2, and b_4 convergence was modest, with means plus standard deviations of 5.1 ± 1.0, 6.0 ± 1.2 and 5.7 ± 1.5, respectively. The dendrites almost invariably appeared to connect with all the pedicles within their reach and, with one exception, were never observed to snake past a nearby pedicle in favour of a more distant one (figure 3). This exception was in regard to a cone pedicle near the centre of the array, which was smaller than the others. It also lacked the aggregate of large mitochondria characteristic of cone pedicles and contained instead mitochondria such as characteristic

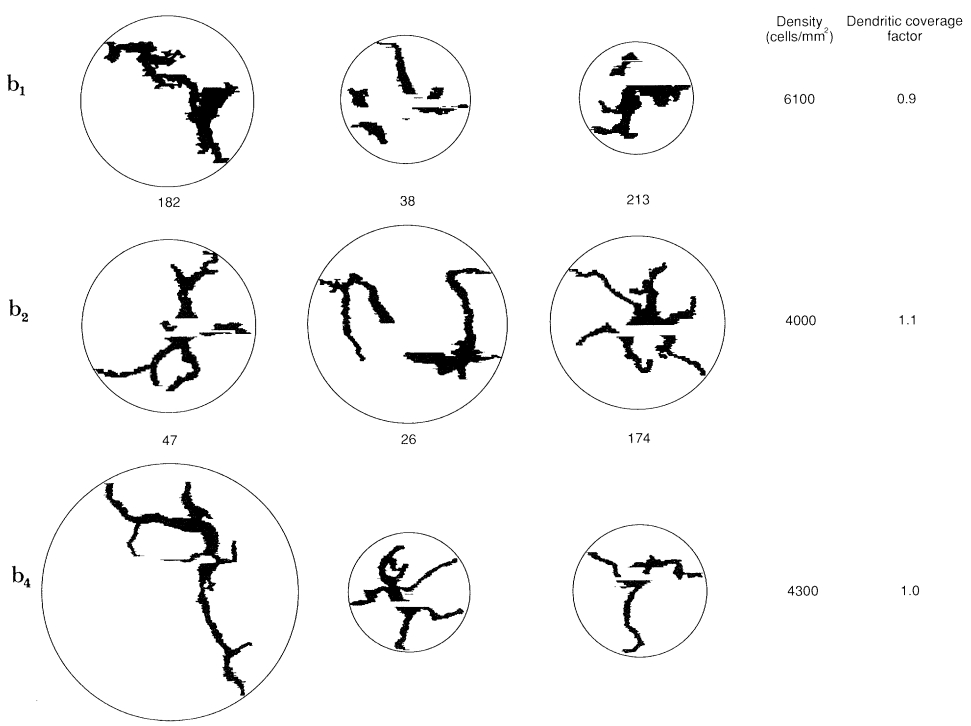

Figure 2. Dendritic arbors of types b_1, b_2, and b_4 bipolar cells in tangential view. Circles show areas used to compute coverage factors.

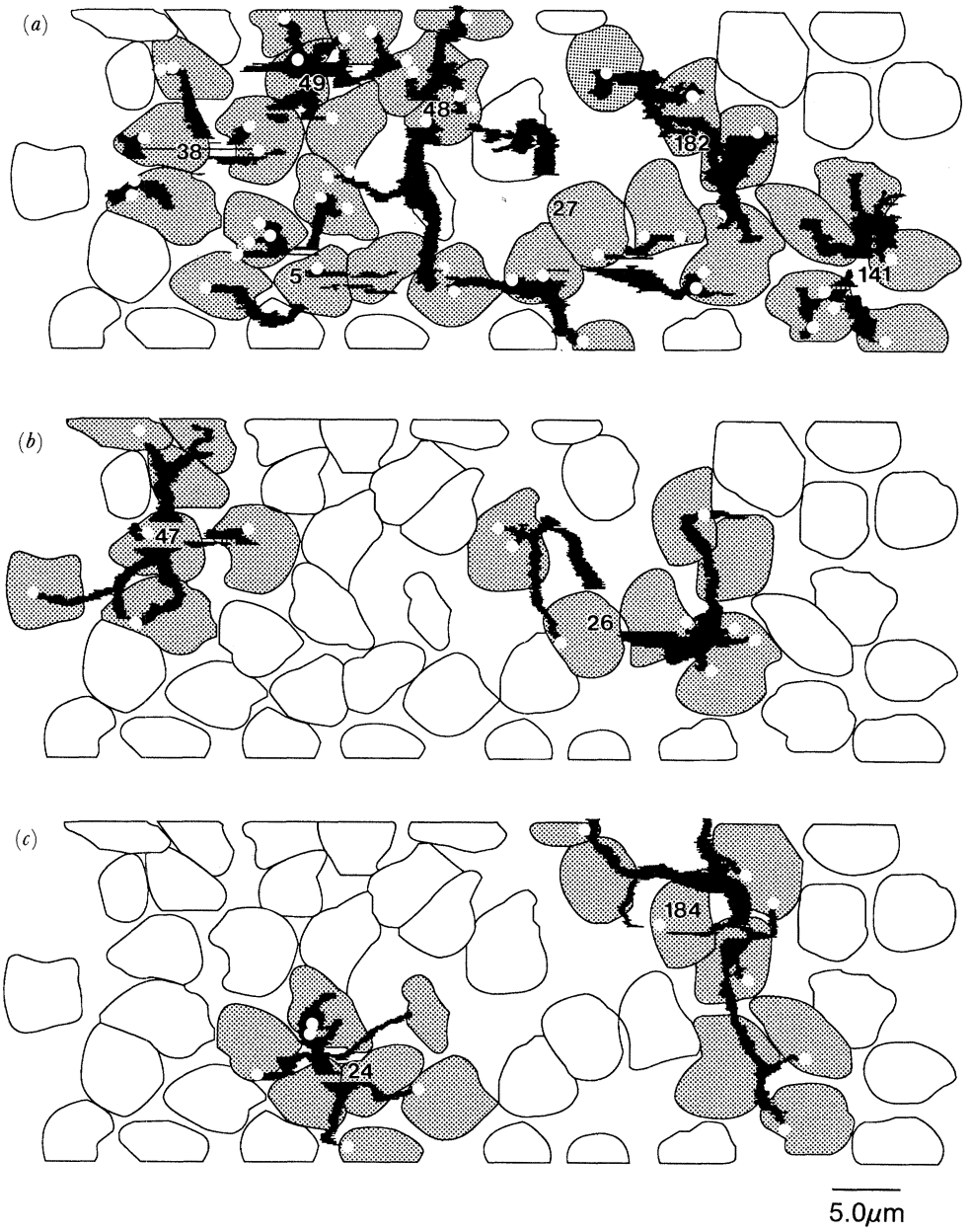

Figure 3. (*a*) Array of 7 b_1 dendritic arbors in tangential view with cone pedicles (shaded) superimposed. Note that b_1 dendritic array completely 'tiles' the pedicle array. White dots mark identified sites of synaptic contact. For b_1 no. 141 five sites of contact were identified for the pedicle at 12 o'clock, two sites for the pedicle at 10 o'clock and one additional site for the pedicle at 7 o'clock whose exact locations on the pedicles were not recorded. (*b*) Two b_2 dendritic arbors in tangential view with same array of cone pedicles as in a superimposed. Note that dendrites connect to the immediately overlying pedicles and collect from cones that also contact b_1 cells. (*c*) Two b_4 dendritic arbors in tangential view with the same array of cone pedicles as in A and B superimposed. Here, too, the dendrites connect to the immediately overlying pedicles and collect from cones that also contact b_1 (and almost certainly) b_2 cells.

of rod spherules. This pedicle contained only six synaptic ribbons while other pedicles at this eccentricity contain 10–13 (Harkins *et al.* 1989). None of the narrow-field bipolar cells were contacted by this pedicle, though at least eight of them had access to it without reaching past any other members of their own types.

(*c*) *Divergence*

To observe directly the full pattern of divergence from one cone to all its post-synaptic cells is technically difficult. The reason is that the post-synaptic processes, being fine, are often lost because their membranes become electron-lucent when they twist parallel to the plane of section. However, once the convergence has been determined accurately, and the density of cones and bipolar cells is known, the divergence is given by the relation:

$$\frac{\text{density of A}}{\text{density of B}} = \frac{\text{conv A}\rightarrow\text{B}}{\text{div A}\rightarrow\text{B}}. \quad \text{(from Freed } \textit{et al.}\text{ 1987).} \tag{1}$$

The divergence calculated in this way for the type b_1 bipolar cell was 1.2. We observed directly five pedicles that diverged to two (but never three) neighbouring b_1 cells. Divergence calculated for types b_2 and b_4 was 1.0.

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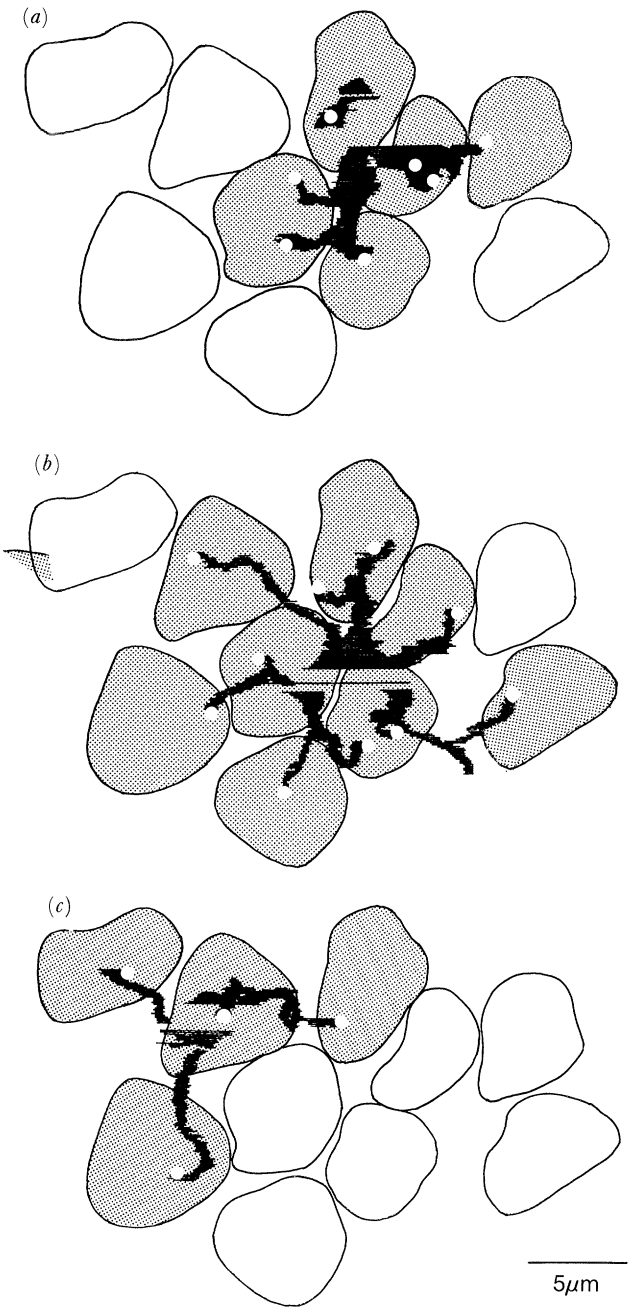

Figure 4. Co-spatial dendritic arbors of types (*a*) b_1, (*b*) b_2, and (*c*) b_4 bipolar cells, (cell numbers 213, 174 and 192, respectively in Cohen & Sterling (1990*a*)) with overlying cone pedicles (shaded). Dots represent sites of identified synaptic contact. Note that b_1, b_2, b_4 branch toward and collect synapses from all pedicles above them without skipping. Certain cones are seen directly to contact all three bipolar types.

The divergence calculated separately for each of these types (b_1, b_2, b_4) implies that every cone (except the small dark one) diverges to all three types. Evidence on a local scale that a pedicle which contacts a b_1 bipolar cell also contacts a b_2 or a b_4 cell, or both, is shown in figure 3: 35 pedicles in the upper panel innervate an array of 7 b_1 bipolars. The middle and lower panels show groups of pedicles in the same array to which dendrites were traced from individual b_2 and b_4 bipolar cells. Of 35 pedicles that contact b_1 cells, at least 12 and 9, respectively also contact b_2 and b_4 cells. Almost certainly the pedicles in the lower panel to which dendrites of cells b_2 and b_4 could not be traced nevertheless probably do contact types b_2 and b_4. This seems inescapable because the dendritic fields of particular b_2 and b_4 cells are situated directly beneath these pedicles, and we show in figures 3 & 4 that such bipolars collect from the pedicles directly above them.

(*d*) *Divergence at a single triad*

According to previous studies (Nelson & Kolb 1983; McGuire *et al.* 1984; Cohen & Sterling 1990*a*), dendrites of bipolar types b_2, b_4, and a_2 are 'fully invaginating'; that is, they penetrate the pedicle deeply to terminate just beneath the arciform density in intimate association with the ribbon. Dendrites of b_1 are 'semi-invaginating'; they penetrate less deeply into the pedicle and form their post-synaptic densities at some distance from the arciform density and the ribbon. Since each cone contacts types b_1, b_2, b_4, and possibly also the a_2 bipolar, the question arises, can all this occur at the same triad? We have observed that one triad can accommodate both a fully invaginating and a semi-invaginating process (figure 10*a*, in Cohen & Sterling (1990*a*)), but how often this occurs, or whether more than one fully invaginating process plus one semi-invaginating process can be accommodated at the same triad is unknown. Thus whether each of the 17 triads present in a cone pedicle of the area centralis (Harkins *et al.* 1989) diverges to all four bipolar types or whether the triads are divided among them, remains to be determined.

DISCUSSION

(*a*) *Wide-field bipolar cell*

Famiglietti (1981) suggested that the wide-field bipolar cell, which we call b_5 (figure 3 and 9 in Cohen & Sterling (1990*a*)), collects selectively from short wavelength (blue) cones. There is now a web of circumstantial evidence to support this idea. Dendrites of the b_5 cell appeared to bypass all the cones in the region that we studied. Conceivably the b_5 cell does connect to these cones via filaments too fine to be traced, but this seems improbable because the cell is in every other respect robust. Furthermore, this type has been observed in Golgi impregnations to emit clusters of postsynaptic terminals from robust dendrites, but only at a few intervals over its wide field (Famiglietti 1981; Kolb *et al.* 1981; Pourcho & Goebel 1987). Finally, the b_5 axon is pre-synaptic to certain *on* ganglion cells, but not to alpha or beta cells (Cohen & Sterling 1990*b*). This is consistent with evidence that certain *on* ganglion cells, but not alpha or beta cells, respond selectively to short wavelength stimuli (Cleland & Levick 1974). Thus the b_5 bipolar cell may correspond to the 'blue cone' bipolar cell identified in primate retina by Mariani (1984), which also skips nearby cones in favour of more distant ones and which also terminates in sublamina *b*.

(*b*) *Narrow-field bipolar cells*

At least three out of the five types of cone bipolar cell

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than innervate sublamina *b* (b_1, b_2, b_4) have narrow dendritic fields that contact all the overlying pedicles without skipping. They also have relatively dense distributions (4000–6100 cells mm^{-2}), which tile the cone array completely. Consequently each cone (with one exception) diverges to all three types. Although the dendrites of type b_3 were too fine to trace, the fact that the b_3 is a small cell and is densely distributed (4000 mm^{-2}) suggests that it, too, has a narrow field. Therefore, it probably collects from the same overlying cones as the other narrow-field cells. Moreover, this pattern is probably duplicated for the 4–5 types of narrow-field bipolar cell that innervate sublamina *a* (Famiglietti 1981; Kolb *et al.* 1981; McGuire *et al.* 1984; Pourcho & Goebel 1987) since they must also be densely distributed (Cohen & Sterling 1990*a*). If so, then every cone in the area centralis, except for the rare blue one (de Monasterio *et al.* 1981), must diverge to 9–10 different types of bipolar cell!

What reasons could there be for this striking divergence of the signal from each cone into multiple, parallel pathways? The various bipolar types might represent different filters, each tuned to transmit a particular region of the spatial or temporal frequency spectrum. Bipolar dendrites might bear different post-synaptic receptors coupled to ion channels with widely separated equilibrium potentials (Saito 1979). This could increase the gain and improve linearity for transmitting the cone signal across the outer plexiform layer (Attwell 1986; Miller & Slaughter 1986). Bipolar axons might release different transmitters onto the ganglion cell or else release the same transmitter onto different post-synaptic receptors. In either case different ion channels would be modulated for transmitting the cone signal at the ganglion cell. This could provide a mechanism to increase linearity and gain across the inner plexiform layer. Finally, bipolar axons might form different types of regulatory circuit in the inner plexiform layer.

There is actually some evidence for most of these possibilities. The published recordings from b_1 and b_2 bipolar cells suggest that the two types differ in their temporal responses: the b_1 gives a strong transient-plus-sustained response, but the b_2 appears to give pure sustained response (figure 5; Nelson & Kolb 1983). Thus the b_1 cell may be tuned to higher temporal frequencies that the b_2. On the other hand, the narrow-field bipolar cells probably differ very little in the *spatial* filtering properties because the receptive field of the cone is relatively large compared to the cone spacing (Nelson 1977; Smith & Sterling 1990). Consequently the receptive field centre of a bipolar cell collecting from eight cones (e.g. b_2) will hardly be different from one that collects from only four (b_1) (Smith & Sterling 1990).

The b_1 and b_2 dendrites may well bear different glutamate receptors since their responses to light are apparently of opposite polarity (figure 5; Nelson & Kolb 1983; Miller & Slaughter 1986). Therefore, these types might represent mechanisms to increase the gain of transmission across the outer plexiform layer to sublamina *b*. Furthermore, these cells converge on the same type of ganglion cell (on-beta) where they may

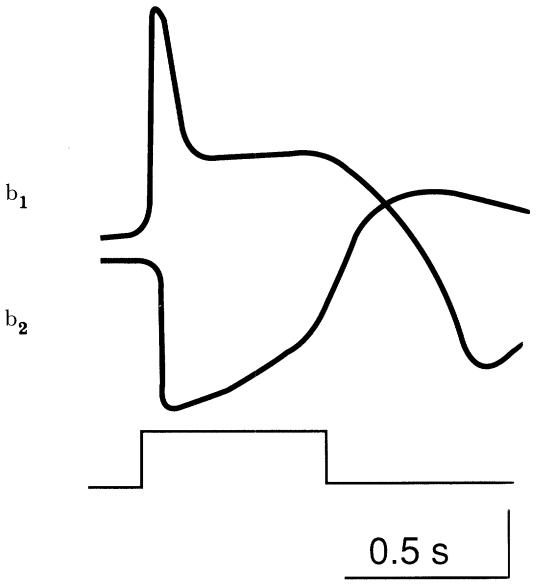

Figure 5. Intracellular recordings (Nelson & Kolb 1983) from b_1 and b_2 bipolar cells (termed CB5 and CB6 by Nelson & Kolb) showing a different time course of response. The b_2 recording has been rescaled from the original for purposes of comparison.

either release different transmitters (or the same transmitter onto different receptors) and thus be responsible for a 'push-pull' amplification (McGuire *et al.* 1986; Bolz *et al.* 1985). Finally, the b_4 axon displayed quite a different circuitry than the other narrow-field types. Whereas b_1, b_2, and b_3 axons all connect strongly to the on-beta cell, the b_4 axon does not (Cohen & Sterling 1990*b*). Furthermore, whereas the b_1, b_2, and b_3 axons all connect only moderately to amacrine cells (50% or less of their output), the b_4 axon connects mainly to amacrines (70% of its output), and its feedback circuits from amacrines may be different as well (Cohen & Sterling 1990*a*). In short, quite a few of the possible advantages to diverging the cone's signal to multiple types of bipolar cell may actually be exploited.

We thank Dave Carter, Sam Hong, and Ellen Magyarits for technical assistance, Suzanne Leahy for artwork, and especially Maria Koebert for extensive contributions to preparing the manuscript. We are grateful to Professors Ed Pugh, R. W. Rodieck, and B. Boycott for many suggestions. Supported by NIH grants EY00828 and EY07035.

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(*Submitted by Professor B. B. Boycott, F.R.S.; received 22 March 1990; Revised 2 August 1990*).

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