Responses of cells in monkey visual cortex during perceptual filling-in of an artificial scotoma

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WHEN we view a scene through one eye, we typically do not see the scotomas created by the optic disc and the blood vessels overlying the retinal surface. Similarly, when a texture field containing a hole is steadily viewed in peripheral vision (artificial scotoma), the hole appears to fill in with the surrounding texture in a matter of seconds, demonstrating that the visual system fills in information across regions where no information is available[1–5]. Here we show that, in monkeys viewing a similar texture field with a hole, the responses of extrastriate visual neurons with receptive fields covering the hole increase gradually to a level comparable to that elicited by the same texture without a hole. The time course of these dynamic changes in activity parallels the time course of perceived filling-in of the hole by human observers, suggesting that this process mediates perceptual filling-in.

The receptive fields of visual area V1 neurons in anaesthetized cats have been shown to expand after several minutes of stimulation with a dynamic texture surrounding the receptive field, suggesting that expansion of the receptive field might underlie filling-in[6]. However, psychophysical studies in humans have shown that perceptual filling-in of a hole in a texture (artificial scotoma) occurs within seconds rather than minutes[3–5]. We re-investigated the neural basis of perceptual filling-in by comparing the time course of filling-in in human subjects with the time course of neuronal responses recorded from monkeys, both observing the same stimuli.

First, we determined the time course of perceptual filling-in in four human subjects. We used a large (16° × 16°) texture with an equiluminant hole in its middle, located 8° from a fixation spot (Fig. 1). While maintaining fixation, subjects indicated when they saw the hole fill in. As the hole size was increased from 1.0° to 12.8°, the time required to see the hole fill in increased steadily. We next recorded from neurons in two rhesus monkeys that were rewarded for maintaining fixation, while the same stimuli as were used with human subjects were presented. For each cell, we centred the hole over the receptive field (average eccentricity of 8°). Responses to the texture with a hole (hole condition) were compared with responses to the same texture without a hole (no-hole condition). The monkeys were not required to signal the filling-in of the hole, but there is behavioural evidence for filling-in across the blind spot in this species[7].

Physiological recordings in visual areas V2 and V3 indicated cells whose firing rate in the hole condition was initially lower than in the no-hole condition but gradually increased to a comparable level; that is, after a few seconds of fixation they responded to the texture with the hole as if it were a texture without a hole. An example of such a cell in V3 is shown in Fig. 2*a*; it responded with a sustained high firing rate to a continuous texture. By contrast, when the receptive field was covered by a hole of 4° in the texture, the cell gave a transient response to stimulus onset followed by a low firing rate, which then 'climbed' to a level comparable to that in the no-hole condition. We term this phenomenon 'climbing activity'. The climbing activity occurred within a time span that was comparable to the time course of perceptual filling-in; that is, the time required by human subjects to perceive the hole fill in (range 6.2–9.8 s) corresponded well with the time period during which the climbing activity approached the level in the no-hole condition (Fig. 2*a*). Significant climbing activity was observed for 10 of 35 cells in V2, and 68 of 127 cells in V3 (see Fig. 3*a* legend). Similarly, the distribution of response changes during filling-in across the entire population showed a shift towards response increases, which was larger in V3 than in V2 (Fig. 2*b*). These results indicate that the perception of filling-in resulted from a minimization of response differences in the hole and no-hole conditions.

The time during which the neuronal responses in the hole and no-hole conditions converged matched the time course of perceived filling-in at other hole sizes as well. The average response of cells with climbing activity in areas V2 and V3 is shown in Fig. 3*a*, for 93 cells tested with the full range of hole sizes. As the hole size increased to 5.6°, the initial transient response to stimulus onset decreased, subsequent activity was reduced, and more time was required for the climbing activity to reach the activity level in the no-hole condition. For hole sizes up to 5.6°, the time at which neuronal responses in V2 and V3 reached a minimal difference between hole and no-hole conditions fell within the range of times at which human subjects reported filling-in (Fig. 3*b*). For a hole size of 12.8°, partial filling-in limited to the hole's corners was occasionally reported. Similarly, climbing activity was strongly reduced and did not reach the firing rate in the no-hole condition (Fig. 3*a*).

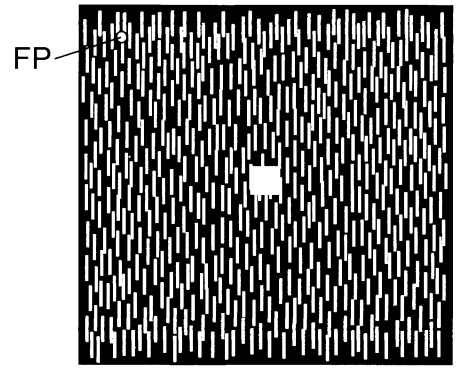

FIG. 1 Schematic representation of the stimulus, which consisted of three frames played at 20 Hz for 12 s. Each frame consisted of small bars (0.2° × 1.4°), randomized in position, and spaced 0.9° apart on average. The hole was equiluminant to both the texture and the background (23 cd m^{-2}). From a short distance, the reader might experience filling-in of the small hole while steadily fixating the fixation point (FP), even though, unlike the experimental stimulus, the hole is more luminant than the texture and the texture is static, conditions which delay filling-in[3–5].

METHODS. Human observers fixated the fixation point at the centre of the display and signalled when they saw the hole fill in by releasing a button. Trials per subject for each hole size ranged from 10 to 40. In both monkeys, areas V2 and V3 were localized by previous physiological mapping[19,20], and in one monkey the recording sites were confirmed with magnetic resonance imaging (MRI) scans taken with tungsten electrodes (clearly visible in the scans) located in typical recording sites. Standard physiological techniques were used, and eye movements were monitored with the scleral coil technique[21]. The monkeys were rewarded with fruit juice for maintaining fixation. To allow for occasional movements caused by eye blinks, the fixation window was set at 2.5° for one monkey and 3.5° for the other. Trials with eye movements outside those ranges were aborted and discarded during data analysis. The standard deviation of gaze from the fixation point was 0.39° in one monkey and 0.79° in the other, with no change in average eye position during the trial. The eye was located within 1° of the fixation point for 97% of the total trial time in one monkey and 92% of the time in the other.

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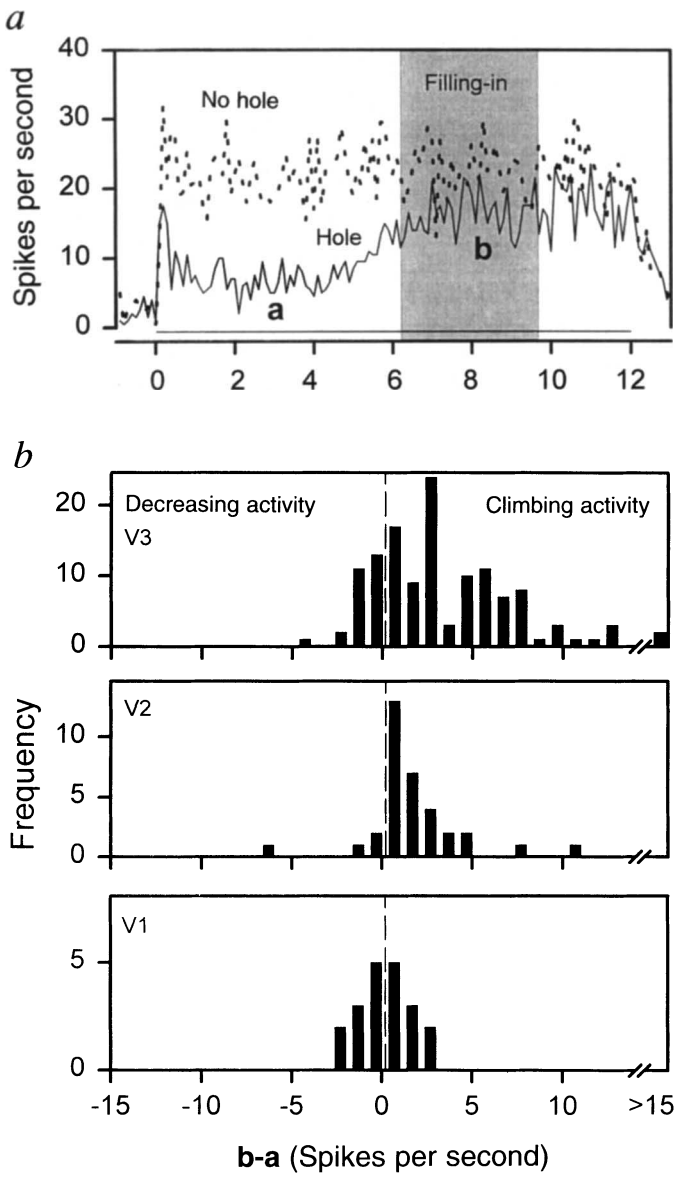

FIG. 2 Responses of cortical neurons during perceptual filling-in. *a*, Typical responses (binwidth, 100 ms) in the hole condition (solid line) and no-hole condition (dotted line) from a single V3 cell. Conditions were randomly interleaved, with up to 20 trials in each. The horizontal line indicates the 12-s stimulus presentation time, preceded and followed by a 1-s period in which activity was recorded in the absence of a stimulus. The hole in the texture was 4°, centred over the receptive field (measured by minimum response field method) which was 3.5° in size at an eccentricity of 7.2°. The orientation of the bars and square hole were typically chosen to match the preferred orientation of the cell. Preliminary data indicate that climbing activity also occurs for orthogonal orientations. The shaded zone indicates the range of average times required by human observers to report filling-in. *b*, Distribution of response changes in V3, V2 and V1 during perceptual filling-in (4° hole). Response changes were calculated for each cell by subtracting the firing rate in a period extending from 1.5 to 4.0 s after stimulus onset (labelled **a** in Fig. 2*a*) from the firing rate during perceptual filling-in (labelled **b** in Fig. 2*a*). According to a Wilcoxon paired-comparison test, there was a significant increase in activity (**b**-**a**) across the population in V3 ($P<0.001$) and in V2 ($P<0.001$), but not in V1 ($P=0.82$). At the time filling-in occurred (shaded zone), there was no significant difference between the activity in hole and no-hole conditions in the V3 population ($P=0.31$) although a significant difference remained in V2 ($P<0.001$) and in V1 ($P<0.001$). Data shown for V1 are from cells with an average eccentricity of 24.5°, where the human subjects experienced strong filling-in. The average receptive field size (2.5°) at this eccentricity was comparable to that of cells in V2 (2°) and V3 (3.7°) at the smaller eccentricities (9.1° and 7.6°, respectively) studied in these areas. Results from 13 V1 cells recorded at an eccentricity of 4.5° (not shown) were similar to those obtained at a 24.5° eccentricity. The distribution and statistical tests in V2 do not include one cell which exhibited inhibitory responses, its behaviour being the mirror image of the cells with climbing activity.

Other correlations between the behaviour of the human observers and the behaviour of the cells were then explored. With a hole of 4°, perceptual filling-in could be prevented by shrinking the overall size of the texture to a 4.4° × 4.4° field, that is, by creating a narrow frame of texture around the hole. We tested this stimulus on seven V3 cells that showed climbing activity with the standard large texture, but none of them exhibited climbing activity with the reduced field (Fig. 4*a*). Therefore, the hole's boundaries *per se* do not cause either climbing activity or filling-in; rather, both require a sufficiently large field of texture in the receptive field surround. We could also prevent perceptual filling-in and climbing activity by flickering the texture field surrounding the hole at a rate of 1.9 Hz (Fig. 4*b*).

Limited data in V1 indicated only weak climbing activity. Significant climbing activity was observed for only 4 of 20 cells recorded at an eccentricity of 24.5°, and 0 of 13 cells recorded at an eccentricity of 4.5°. Furthermore, there was no overall increase in response across the V1 population during filling-in at either eccentricity, unlike the case in V2 and V3 (Fig. 2*b*). The data show that climbing activity is more pronounced and more prevalent in extrastriate cortex than in V1, and within extrastriate cortex it appears to be stronger and more prevalent in V3 than in V2 (see Fig. 2*b*).

To test whether climbing activity is due to expansion of the receptive field[6], we quantitatively mapped the receptive field and surround with moving or flashing light bars presented before, during, and after stimulation with the texture display. In some experiments we used two bar contrasts (Michelson indices of 23% and 67%) to test for changes in contrast gain[8]. In the 16 V3 cells with climbing activity that were tested, there were no changes in the size or profile of the receptive field, which indicates that increases in neither receptive field size nor contrast gain mediate climbing activity or filling-in (Fig. 4*c*, *d*). We suggest that climbing activity is caused by a dynamic change in the balance between excitation and inhibition in the receptive field and surround[9–13]. The excitatory onset responses to the texture with the hole, even with large hole sizes, indicate that the cells receive excitatory input from surrounding regions extending well beyond the classical receptive field. The subsequent sharp fall-off of activity suggests that these excitatory inputs are followed by strong inhibition (Fig. 3*d*). We propose that the inhibition adapts over time, unmasking previously ineffective excitation from the texture, causing climbing activity. The closer the texture is to the centre of the receptive field, the stronger are the excitatory inputs, which explains why climbing activity occurs faster with smaller hole sizes. When the neuronal response to the surround texture becomes comparable to the response to a continuous texture, the subject experiences filling-in. Both long-range horizontal connections between pyramidal cells[14–16] and feedback connections[17] between areas may underlie the interaction

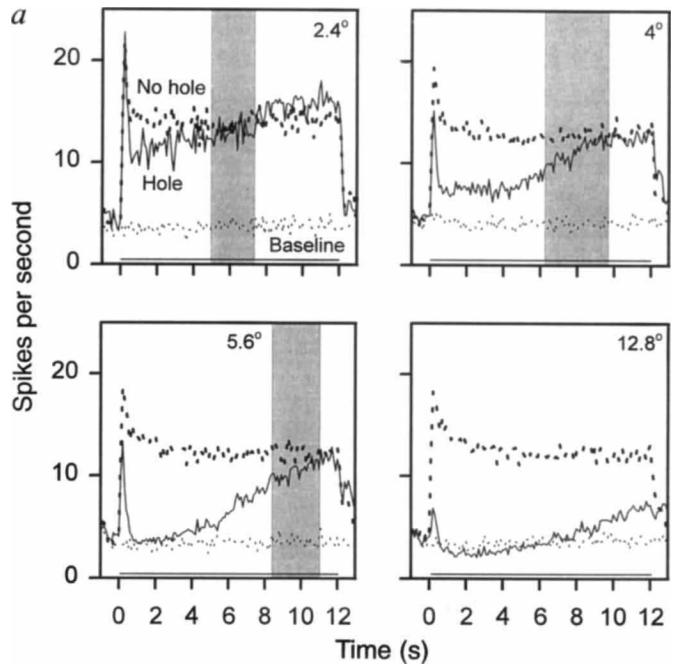

FIG. 3 Correlation between the time course of climbing activity and that of perceptual filling-in. *a*, Average activity in the hole condition (solid line) and no-hole condition (heavy dotted line) of V2 and V3 neurons with significant climbing activity. Fine dots show baseline activity recorded without any stimulus. Hole size is given at the top of the panels. Range of average times required by observers to report filling-in is indicated by the shaded zones. Other conventions as in Fig. 2. Climbing activity was evaluated with paired *t*-tests (two-tailed, $P<0.05$) in which firing rates in a 2.5-s interval starting 1.5 s after stimulus onset were compared with the rates in the last 2.5 s of stimulus presentation. A few cells that showed a response increase in the no-hole condition were excluded from the count of cells with climbing activity. For cells that initially responded similarly in the hole and no-hole conditions there was less opportunity to develop climbing activity. This was particularly true at hole sizes of 3.2° and smaller (which were not as conspicuous to the subjects as the larger holes), for which we found significant climbing activity in only 12% of the cells. *b*, Comparison between the average time required by humans to report filling-in (open symbols) and the average time it took neurons with climbing activity to minimize the response difference in the hole and no-hole conditions (solid symbols). Data from individual observers are shown with thin, solid lines. The slopes of linear regression lines fit through the observers' data ranged from 0.8 to 1.1 ($r^2>0.90$ for three subjects and $r^2=0.73$ for the fourth) compared with 0.9 for the cells ($r^2=0.94$). For the neuronal data, the smallest difference between the firing rates in the hole and no-hole conditions was determined for each 1-s period beginning 1 s after stimulus onset, for each cell and hole size. The time at which the difference was smallest was considered as the 'filling-in time' for that cell at that hole size. Data at the smallest hole size were pooled from hole sizes of 1.0° and 1.6°.

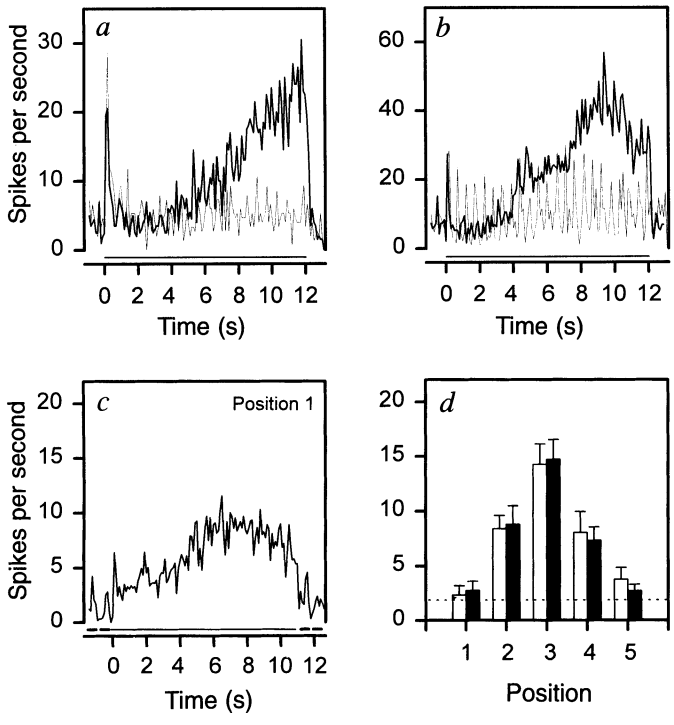

FIG. 4 The role of surround-stimulus characteristics and receptive field expansion in perceptual filling-in and climbing activity. *a*, Single V3 cell showing strong climbing activity when its receptive field was covered by a hole of 4° in a large dynamic texture (thick line), and absence of climbing activity when its receptive field was surrounded by a narrow frame of the same, dynamic texture (thin line). Similar results were obtained in six additional V3 cells with climbing activity; four of the cells tested with the narrow frame showed the same onset transient immediately followed by a low level of activity as they did with the large texture field. *b*, Single V3 cell showing strong climbing activity to a texture with a 4° hole over its receptive field (thick line), and response to the same stimulus flickered at 1.9 Hz (thin line). The flickering strongly suppressed the climbing activity. Instead, the cell gave transient responses timelocked to the flicker. This same flickering stimulus was tested on three other V3 cells that showed climbing activity, and in all cases the flickering suppressed or eliminated the climbing activity. Both the narrow texture frame and the flickering stimulus prevented filling-in in human subjects. *c*, Responses of a single V3 cell to a bar swept just outside the receptive field borders before and after presentation of a texture stimulus (4° hole) that caused climbing activity. One end of the 1.5°-long bar touched the receptive field edge (position 1 in *d*). The two bold line segments on the left of the abscissa indicate the forwards and the backwards motion of the bar before the presentation of the textured stimulus (thin line on abscissa); the two bold line segments on the right indicate the bar's forwards and backwards motion after the presentation of the texture stimulus. *d*, Average receptive field profiles of four cells with climbing activity. Receptive fields were mapped with moving bars just before (open bars) and after (solid bars) presentation of the texture with hole. The bars were swept through areas occupied at a different time by the texture (position 1 and 5) or through the hole and receptive field (positions 2–4). The dotted line indicates average spontaneous activity. Error bars indicate standard errors. There was no evidence for receptive field expansion.

between excitation and inhibition which leads to climbing activity and perceptual filling-in. In permanent scotomas, such as the blind spot, inhibition from the surround may be adapted in a more permanent manner so that filling-in is instantaneous[7,18,22]. □

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Differential properties of two gap junctional pathways made by AII amacrine cells

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THE retina is sensitive to light stimuli varying over more than 12 log units in intensity. It accomplishes this, in part, by switching between rod-dominated circuits designed for maximum utilization of scarce photons and cone circuits designed for greater acuity. Rod signals are integrated into the cone pathways through AII amacrine cells, which are connected by gap junctions both to other AII amacrine cells and to cone bipolar cells. To determine the relative permeabilities of the two junctional pathways, we have measured the distribution of biotinylated tracers across this heterologous cell assembly after injecting a single AII amacrine cell. We found that neurobiotin (relative molecular mass, 286) passed easily through both types of gap junctions, but that biotin-X cadaverine (relative molecular mass, 442) passed through AII/bipolar cell gap junctions poorly compared to AII/AII gap junctions. Thus, the AII/bipolar cell channel has a lower permeability to large molecules than does the AII/AII amacrine cell channel. The two pathways are also regulated differently. Dopamine and cyclic AMP agonists, known to diminish AII–AII coupling[1], did not change the relative labelling intensity of AII to bipolar cells. However, nitric oxide and cGMP agonists selectively reduced labelling in bipolar cells relative to AII amacrine cells, perhaps by acting at the bipolar side of this gap junction. This suggests that increased cGMP controls the network switching between rod and cone pathways associated with light adaptation.

In the mammalian retina, ganglion cells, the final output of the retina, receive input from both rod and cone pathways. Cones contact ON and OFF cone bipolar cells which synapse directly with ganglion cells. In contrast, rods synapse onto a single type of rod bipolar cell, which does not contact ganglion cells directly, but relays its signal through the AII, or rod amacrine cell[2–4]. Rod input into the ON pathway occurs through gap junctions between AII amacrine cells and ON cone bipolar cells. The AII also makes gap junctions with other AII amacrine cells (Fig. 1). Tracer coupling across both types of junctions is found with neurobiotin, but not Lucifer yellow[1].

Coupling is most commonly found between similar cells of the same type (homologous). Channels may be formed from pairings of a single type of connexon (homotypic)[5,6] or two different connexon types (heterotypic)[7–10]. Total permeability between two cells is the product of the number of open channels times the single-channel permeability. If two gap junctions contain a single connexon type, differences in total permeability to a given tracer can only reflect differences in number of open connexons. Under these conditions, the relative partitioning of dye will be independent of tracer size. Different connexons, on the other hand, can selectively favour passage of one tracer over another. Channels with a small pore diameter will select against larger-diameter tracers[11–13]. Consequently, if the relative distribution of tracer across two pathways changes with different-sized tracers, then their gap junctions must contain channels with fundamentally different permeabilities. Neurobiotin and biotin-X cadaverine both bear a single positive charge and have similar detection efficiency, as seen by dotblots. It is therefore likely that differences in permeability ratio reflect differences in connexon type, although post-translational modifications of a single type cannot be excluded.

Figure 2 shows examples of AII and bipolar cell staining following injection of biotin-X cadaverine (BXC) or neurobiotin (NBT) into a single AII amacrine cell. Although the number of amacrines stained is similar (Fig. 2*a*,*d*), far fewer bipolar cells (Fig. 2*b*, *e*) are labelled with biotin-X cadaverine (M_r 442) than with neurobiotin (M_r 286) (Fig. 2*c*, *f*). Failure of Lucifer yellow (M_r 443) to pass either channel most likely reflects charge selectivity of the channels[14]. A quantitative representation of neurobiotin labelling is depicted in Fig. 3*a*, which shows the relative staining intensity of AII amacrine cells (circles) and cone bipolar

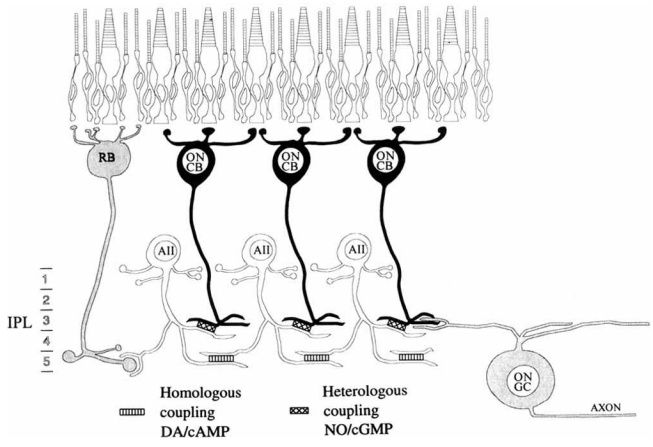

FIG. 1 The ON pathways in mammalian retina. Rods and cones (top) synapse on rod bipolar or cone bipolar cells. ON cone bipolars synapse directly upon ganglion cells (ON GC), rod bipolars (grey, RB) synapse only upon the AII amacrine cell, which transmits rod information to both OFF (not shown) and ON cone bipolar cells (ON CB). AII amacrine cells make gap junctions with ON cone bipolar cells in sublaminae 3 and 4 and also with other AII amacrine cells. The heterologous AII-to-bipolar cell connexons have a lower permeability to large ions than the homologous AII–AII connexons, and are gated by NO and cGMP. The homologous gap junctions are gated by dopamine (DA) and cAMP. IPL, inner plexiform layer.