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the ionotropic glutamate receptors GluR1 and GluR2 to flat contacts between rods and putative OFF CB cells in the normal rodent retina (Hack et al. 1999), as well as from the recent demonstration of such contacts between a specific type of OFF cone bipolar cell, the CB2, and rod spherules in the mouse retina (Tsukamoto et al. 2001). In addition, GluR2 receptors were recently shown to be localized to dendrites of OFF CB cells contacting rod spherules in a large percentage of cells in the rabbit retina (Li et al. 2004).

To investigate whether these alternative pathways transmit rod signals through the mammalian retina, we examined the light responses of GCs in the rabbit, rat, and mouse retina under different light conditions and with specific pharmacological tools. We observed APB-resistant signals frequently in the rabbit, rarely in the mouse, and never in the rat retina. The high proportion of APB-resistant OFF signals in rabbit retina is supported by the high number of rod–OFF CB cell contacts observed anatomically. Our results favor the alternative circuit in which rod photoreceptors directly contact OFF CB cells, and they support the existence of this alternative pathway in rabbit as well as rodent retina.

METHODS
Animals and tissue preparation

Electrophysiological recordings were performed from retinal GCs in albino and pigmented rabbits (2–3.5 kg), mice from the strain Animals and tissue preparation, and rats (5–7 wk old). Light responses in the rabbit retina were recorded from both the whole mount preparation and slices, whereas in the case of mouse and rat retinas, only slices were used. For whole mount experiments, rabbits were dark-adapted for ≥5 h before they were killed by an overdose of pentobarbital sodium. The right eye was removed, and the retina was isolated under infrared illumination (>900 nm). A small piece (~3×4 mm) of the central part of the retina was placed photoreceptor side down in the recording chamber. Retinal vertical slices were prepared from rabbit, mouse, and rat retinas following previously described procedures (Protti et al. 1997). Rabbits were prepared as described above, whereas mice and rats were dark-adapted for 6–7 h and subsequently anesthetized with halothane and decapitated. All procedures were performed under infrared light and in accordance with the guidelines for animal experiments issued by the Federal Republic of Germany (Tierschutzgesetz). The eyes were rapidly enucleated and transferred to a dish containing Ames medium. The cornea was cut along the ora serrata, the lens and vitreous were removed, and the retina was separated from the sclera. The retina was cut into four pieces, one of which was embedded in 2% agar dissolved in Ames medium (kept at 38°C), and the resulting block was rapidly cooled. The block was transferred to a microslicer (Dosaka EM), and 200 μm-thick slices were cut. Slices were kept in Ames equilibrated with a mixture of 95% O2-5% CO2 for 30 min before electrophysiological recording. The tissue could be maintained for ≥8 h in oxygenated Ames medium.

Electrophysiological recordings

During recordings, the tissue was mounted in a recording chamber and continuously perfused with oxygenated Ames medium (35°C, pH = 7.4). Cells were viewed on a video monitor coupled to a CCD camera mounted on a Zeiss microscope (ACM, Zeiss, Oberkochen, Germany) equipped with a water immersion objective (×40/0.75 W). The Nomarski optics of the microscope were modified for infrared illumination (900 nm). GCs were identified by their electrophysiological properties and their morphology, namely large sodium currents (>1 nA) and a large soma. Patch-clamp recordings of cells in the GC layer were performed using an EPC-9 patch-clamp amplifier (Heka Electronic, Landau, Germany). The pipette solution contained Cs+ as the main cation and gluconate as the main anion. The intracellular solution consisted of (in mM) 100 CsGlu, 0.5 MgCl2, 10 Na-HEPES, 5 EGTA, 0.5 CaCl2, 3 Mg-ATP, 0.5 Na GTP, 5 tetrabutylammonium chloride (TBA), and 5 QX-314. TBA and QX-314 blocked voltage-gated potassium and sodium conductances, respectively, thus allowing the recording of light-evoked synaptic currents. The chloride concentration yielded an ECl = −55 mV. Series resistances, which ranged from 10 to 30 MΩ, were left uncompensated. Drugs were diluted in Ames medium and perfused at ~3 ml/min. Ames medium, strychnine, and all other chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany). To block scotopic OFF responses in GCs, a racemic mixture of both D and L forms of APB (Tocris, Cookson, Bristol, UK) at a concentration of 100 μM was used (see also Jin and Brunken 1996; Soucy et al. 1998).
Confocal sections were imaged in 0.5- and observed with an Olympus Vanox photomicroscope (Olympus) mounted with Vectashield (Vector Laboratories, Burlingame, CA) background (ON stimulus) and GCs in whole mount preparations and slices. The GCs were logically identified by Neurobiotin staining. Another parameter used to identify GCs was the presence of robust voltage-dependent Na⁺ currents (>1 nA), which could only be observed immediately after breaking into the cells, before they were blocked by intracellular dialysis with QX-314. Recordings were obtained from ON, OFF, and ON-OFF GCs. Our observations regarding the pharmacological profile of the light responses proved to be equally valid for transient as well as for sustained responses; therefore no further classification has been done. GCs were held at a voltage of −75 mV. Since light-evoked currents consist of a mixture of cationic (Na⁺/K⁺) and anionic (Cl⁻) currents, at this holding potential, the light-driven synaptic currents are inward. Light-evoked currents were elicited by using spots of different light intensities. Under very low light conditions (scotopic), the lowest intensities we tested occasionally failed to evoke light responses. Above a threshold of 6.5 × 10⁻⁷ to 3.25 × 10⁻⁶ cd/m², light-evoked currents were consistently elicited, and intensity-response functions were measured. Under these conditions, light-evoked currents elicited by small spot diameters (50–200 μm) consisted of inward currents and represented responses of the receptive field center. The reversal potential of light-evoked currents was measured by setting the membrane potential to different values between −105 and 45 mV and presenting a spot of optimal size. We found that stimulus-evoked currents reversed at approximately −20 mV (data not shown), suggesting a predominance of excitatory input (as shown in detail in Flores-Herr et al. 2001).

We first tested the ability of 100 μM APB to abolish ON light responses in scotopic and mesopic conditions. APB is an agonist at the metabotropic glutamate receptor type 6, which blocks the light-evoked responses of all ON-depolarizing bipolar cells, including RB cells. As expected, ON responses of ON and ON-OFF cells were completely blocked in all cases (data not shown). This blockade persisted over a wide range of light intensities. After removal of APB from the bath, light responses were fully recovered (data not shown).

The effect of abolishing synaptic transmission between rods and RB cells using APB was also studied in OFF responses originating from OFF and ON-OFF GCs. OFF responses were elicited either at the cessation of a square light pulse (ON stimulus) or at the presentation of a dark spot on an illuminated background (OFF stimulus). Scotopic OFF stimuli are shown by a black rectangle on a dark gray background, and mesopic/photopic stimuli by a black rectangle on a light gray background. The top trace in Fig. 2A shows a response elicited by an OFF stimulus recorded from an ON-OFF GC under scotopic conditions. Inward currents followed both transitions in light intensity. Due to increased latency and slow response kinetics in scotopic conditions, the ON and OFF part of the light response cannot always be clearly separated when using 400-ms stimuli. APB (100 μM) reversibly abolished the OFF component in 7 (7 OFF and 2 ON-OFF) of 21 cells that presented OFF responses. When using an intensity of 1.625 × 10⁻³ cd/m², stimulus-evoked currents were −51.4 ± 15 (SD) pA (n = 7 cells). In four of these cells, currents in control conditions were −51 ± 17 pA and were reduced by 96% in the presence of 100 μM APB, as shown in Fig. 2A (middle). However, in the presence of 100 μM APB, stimulus-evoked OFF currents were restored by increasing light intensity toward the cone activation levels, either by illuminating with brighter spots or by presenting a...
dark spot on a brighter background (Fig. 2A, bottom). This response under bright conventional conditions indicates direct cone→OFF CB→OFF GC cell signaling, which is expected to be APB-resistant.

In the same set of cells, in agreement with the classical signaling pathway for rod vision, 2 μM strychnine also effectively blocked OFF responses, without affecting the ON component of OFF cells (3 of 3 cells). A representative light-evoked response from an OFF GC elicited with an OFF stimulus is shown in Fig. 2B (control, top). Under scotopic conditions, the OFF component of the response was blocked on perfusion with strychnine (2 μM) without substantially affecting the ON component (Fig. 2B, middle). After adapting the retina to 10,000-fold higher background intensity, strychnine failed to block OFF light responses (Fig. 2B, bottom). Again, this bright stimulus produces a conventional response via OFF CB cells.

However, in the remaining 12 cells (4 OFF and 8 ON-OFF), OFF responses, although partly reduced in amplitude, always persisted in the presence of 100 μM APB. Figure 2C represents a typical APB-resistant light-evoked current recorded in scotopic conditions and shows that, although the amplitude of the light-evoked currents was reduced by application of APB, a substantial inward current still persisted. Slower kinetics of the light response after application of 100 μM APB (as in Fig. 2C) was not seen in all cells tested. In the remaining 3 of 7 cells for which currents were measured in response to the same stimulus intensity, the mean amplitude of light-evoked currents in control conditions was −52 ± 16 pA (n = 3) and it was only reduced by 7% by 100 μM APB. This strengthens the idea that 100 μM APB blocks the light responses of ON CB and RB cells without substantially affecting OFF CB cell signaling. Consistent with this result, in this group of cells, 2 μM strychnine exhibited only partial blocking effects in scotopic conditions with stimulus-evoked currents being reduced to 58% of their control amplitude (3 cells), implying that these responses might be independent of the RB cell-AII amacrine cell circuit. A representative recording of the strychnine effect on OFF responses is shown in Fig. 2D, in which presentation of an OFF stimulus elicited an inward current (top), and application of strychnine partly, but not fully, blocked this response (bottom). In summary, these experiments indicate the presence of rod input to OFF GCS, independent of RB cells.

**Rod contacts of OFF cone bipolar cells**

We set out to investigate the anatomical substrate of the alternative rod pathway in the rabbit retina, as previously described in the rodent retina (Hack et al. 1999; Tsukamoto et al. 2001). This was accomplished by dye-injecting single OFF CB cells with Neurobiotin and Lucifer yellow and double labeling the tissue with an antibody against mGluR6. Each whole mount retina was examined with a confocal microscope. The mGluR6 antibody labeled the dendritic tips of RB cells and ON CB cells, as previously reported (Vardi et al. 2000). Each rod spherule was identified as two bright dots, one for each of the two rod bipolar dendrites forming the central pair...
in a tetrad of processes that invaginate the rod terminal (Rao-Miroznik et al. 1995). Cones were identified by multiple ON CB cells contacting one cone pedicle forming a lightly labeled cluster of fine dendritic tips. This pattern of mGluR6 labeling provides a map for the locations of rods and cones.

When viewed in whole mount, the dendritic tree of each OFF CB cell formed a typical pattern of branching dendrites with terminal clusters. As shown in Fig. 3, most of the dendritic tips contact cone pedicles, and almost every cone within the dendritic field is contacted (Fig. 3). Occasionally, the terminals of two separate dendrites meet at a single cone pedicle. As might be expected for OFF CB cells, the dendritic tips were not double labeled for mGluR6.

While most OFF CB cell dendrites terminate at cone pedicles, we also found examples that clearly terminate at rod spherules, identified by the presence of two brightly stained RB cell dendritic tips. Two examples are highlighted by arrows in Fig. 3B. We have intentionally been very conservative in identifying rod contacts from OFF CB cells. The dendrites of OFF CB cells certainly pass close to many rod spherules as they traverse the outer plexiform layer to make contact with the relatively sparse cone array. However, we cannot assign these as rod contacts, as opposed to passing coincidence, without the presence of a third synaptic marker. Instead, we have chosen to look for the terminal contacts of OFF CB dendrites. If an OFF CB dendrite terminates in an area where there are no cones but an obvious rod spherule is present, we have counted this as a rod contact. At such contacts, the OFF CB dendrites are never double labeled for mGluR6 but always occur basolateral to the rod bipolar dendritic tips at the base of the rod spherule (Fig. 3B). We are aware that this approach may lead to a substantial underestimate of rod contacts, but these examples are very likely to represent rod input to OFF CB cells. We have observed this connectivity pattern in several OFF CB cells, showing the existence of a putative locus for signal transmission from rods directly to OFF CB cells.

**Light responses in the mouse retina**

Light-evoked currents were recorded from cells in the GC layer in mouse retinal slices. Cells were identified as GCs using the same criteria as described for rabbit GCs. The light stimulus consisted of a full-field pulse (ganzfeld). Typical light responses at a holding potential of $-75$ mV consisted of inward currents that reversed between $-55$ and $-35$ mV, suggesting that there was also a strong inhibitory input presumably originating from the surround illumination.

As in the rabbit retina, APB reversibly abolished responses at all intensities tested. We studied light responses from 18 ganglion cells (10 OFF and 8 ON-OFF) that presented OFF responses in scotopic conditions. In 17 of them, 100 µM APB completely abolished OFF responses at low light intensities. Representative responses are shown in Fig. 4. In ON-OFF GCs, inward currents were elicited at both onset and offset of the stimulus (Fig. 4A, top). These responses were completely blocked after bath application of 100 µM APB (Fig. 4A, middle). An increase of stimulus intensity (2–3 log units brighter) rescued OFF responses still in the presence of APB, whereas ON responses remained hindered due to blockade of signal transfer at the photoreceptor–ON-depolarizing bipolar cell synapse. OFF GCs (Fig. 4B) exhibited a similar behavior: responses to an OFF stimulus were abolished by 100 µM APB (middle) but were recovered by using a brighter background (bottom). The effect of 2 µM strychnine on OFF responses recorded from ON-OFF and OFF GCs was also tested. In ON-OFF cells, strychnine abolished the OFF component of light-evoked currents in response to scotopic stimulation without affecting the ON responses (Fig. 4C, top and middle). Using a brighter uniform illumination as background in the presence of strychnine, application of an OFF stimulus resulted in recovery of the OFF response and an increase in the amplitude of the ON response (bottom). Figure 4D shows the effect of strychnine on light-evoked responses of OFF GCs. OFF responses were evoked by presentation of an OFF stimulus (top) and were abolished by 2 µM strychnine (middle). Using brighter background intensity during perfusion with strychnine rescued OFF responses (bottom). The ON response is most likely inhibitory chloride input to the cell that was not present or masked by spontaneous membrane fluctuations at lower light intensities. Strychnine fully abolished the OFF responses in seven of seven cells (5 ON-OFF and 2 OFF).

**FIG. 3.** Synaptic contacts established by OFF-cone bipolar cells with rod and cone terminals. A: neurobiotin-injected Ba3 bipolar cell (green) was double labeled with antibody to mGluR6 (red), which labels rod bipolar dendritic tips in the form of bright doublets within the rod spherules. ON cone bipolar dendritic tips form dimmer, larger clusters beneath each cone pedicle. B: in the zoomed image of the area indicated by the square in A, the cone pedicles are outlined by circles. In this region, most of the dendritic tips from this Ba3 cell contact cones. However, several branches terminate at the rod spherules as indicated by arrows. Scale bar in both A and B is 5 µm.
The peak amplitude of OFF responses originating from OFF and ON-OFF GCs was measured, normalized, and plotted as a function of stimulus intensity. Intensity-response curves for OFF responses in control conditions and in the presence of pharmacological agents are shown in Fig. 5. In control conditions, OFF responses were elicited by using minimal intensities in the scotopic range. Application of both APB and strychnine abolished responses at low light levels. However, when the light intensity was raised to about three log units above the scotopic threshold, in the presence of either APB or strychnine, OFF responses could be evoked. Further increases of the light intensity resulted in increasing responses. Changes in the shape of the intensity-response curve observed on drug application could be attributed to higher light intensities needed to elicit responses and, in the case of strychnine, to removal of glycinergic inhibition.

The one cell where the OFF responses were not fully blocked by APB was an ON-OFF GC. Figure 6A shows the effect of 100 μM APB on this cell. Inward currents evoked at light onset were completely abolished on application of APB (left). Under these conditions, however, an inward current was still elicited at light offset, indicating the presence of an APB-resistant OFF component. When OFF stimuli were used (Fig. 6B), responses were partly reduced by APB, but not abolished. Note that the ON component of the response is fully blocked (right). Normalized peak current amplitude is plotted as a function of stimulus strength for the OFF responses of this cell in Fig. 6C; the sensitivity profile in the presence of APB matches almost perfectly with the control responses, providing evidence for an APB-resistant OFF component in scotopic conditions. In summary, APB-resistant responses were found in a very small proportion of GCs in the mouse retina.
Light responses in the rat retina

Light-evoked currents were also recorded from cells in the GC layer of rat retinal slices. Cells were identified according to the criteria used for rabbit and mouse GCs. As in the previous sections, light responses were inward currents and reversed between $-55$ and $-35$ mV.

As previously described for the rabbit and mouse retinas, 100 \( \mu \)M APB completely and reversibly abolished light-evoked ON responses recorded from all GCs at all intensity levels, and this blockade was reversible. Light-evoked OFF responses were recorded from a total of 20 (11 OFF and 9 ON-OFF) GCs in scotopic conditions. In all cases, stimulus-evoked OFF currents were fully blocked by 100 \( \mu \)M APB, an effect that was reversible. Figure 7A shows the response of an OFF GC in scotopic conditions (top) that is completely blocked by 100 \( \mu \)M APB (middle). However, using brighter background illumination in the presence of APB allowed OFF responses to recover (bottom). This indicates input mediated by OFF CB cells in the photopic range.

The effect of strychnine was also tested in 10 of the 20 cells. Strychnine efficiently blocked OFF responses in scotopic conditions in all 10 cells tested (6 OFF and 4 ON-OFF GCs). Figure 7B shows the action of strychnine on an ON-OFF GC. An inward current was evoked with an OFF stimulus (top), and strychnine fully blocked this response. Note that the ON response is enlarged, presumably by removal of glycinergic inhibition (middle). A further increase of the background illumination during perfusion with strychnine made it possible to obtain OFF responses once more (bottom). Again, these represent direct photopic input from OFF CB cells.

Analysis of the intensity-response relationship confirms the blocking action of APB for OFF responses at low intensities. When higher stimulus intensities were applied during the application of APB, light responses could be generated via direct input from OFF CB cells. Further increases in light intensity produced larger responses (Fig. 8). These results suggest that, in the rat retina, the majority of rod level signals are processed via the RB pathway, which is sensitive to APB. We were unable to detect alternative rod-driven pathways in this sample of GCs.
DISCUSSION

Although a profound knowledge of retinal circuitry has been accumulated over the last 100 yr (Masland 2001; Ramon y Cajal 1972; Wässle and Boycott 1991), recent results have modified our views of signaling in the rod pathway. The simplest rod circuit is via RB cells (Bloomfield and Dacheux 2001; Daw et al. 1990; Famiglietti and Kolb 1975; Kolb and Famiglietti 1974; Müller et al. 1988). In addition to this well-documented circuit, recent evidence suggests the existence of alternative parallel pathways that can also carry rod signals (Deans et al. 2002; DeVries and Baylor 1995; Hack et al. 1999; Soucy et al. 1998; Tsukamoto et al. 2001). We have conducted physiological studies to investigate the presence of these alternative routes in three mammalian species of interest for retinal studies. Additionally, we have explored the morphological basis for one alternative rod pathway in the rabbit retina.

In rabbit and mouse retina, we observed that light signals are able to flow through a circuit independent of the RB and AII amacrine cell interneurons. Our conclusions, which support the existence of an alternative pathway, are based on the observation that some OFF light responses persisted in the presence of APB and strychnine. These data imply that both rod cells, and bars are their corresponding SE (control: n = 12, APB: n = 4). Lines show the fit of the experimental points to the Hill function. For fitting parameters see Methods and Fig. 5.

Sensitivities to APB and strychnine. These data imply that both rod cells, and bars are their corresponding SE (control: n = 12, APB: n = 4). Lines show the fit of the experimental points to the Hill function. For fitting parameters see Methods and Fig. 5.

FIG. 8. Sensitivity curves showing the intensity-response function for OFF responses from ON-OFF and OFF ganglion cells in control conditions and in the presence of 100 μM APB. Peak amplitudes were normalized and plotted against the log of light intensity. Symbols represent the average of different parameters see METHODS and Fig. 5.

Our electrophysiological results in the rabbit retina are very similar to the observations made by DeVries and Baylor (1995). However, while they suggested that APB-resistant signaling could occur via rod→cone gap junctions, we interpret these results in a different manner. Because it is very likely that single cones contact all types of CB cells (Boycott and Wässle 1991, 1999; Grünert et al. 1994), if rod signals were to spread to the cones and were subsequently transmitted to the CB cells, theoretically all OFF and ON-OFF GCs should exhibit APB-resistant OFF responses. However, this was not the case (DeVries and Baylor 1995; this study), and therefore we propose that these signals do not arise from rod signals spreading into cones but from direct rod→OFF CB cell contacts. Such contacts, at least in mouse retina, have been shown to involve only one type of OFF CB cell, and hence, it is highly likely that their signals reach only a subset of GCs. In rabbit retina, all OFF CB cells establish dendritic contacts with rod spherules in a relatively high proportion (Li et al. 2004). These results are in agreement with Deans et al. (2002), who showed that, in scotopic conditions, GC ON responses from connexin 36 knockout mice, but not OFF responses, were suppressed. Because Cx36 KO mice most likely lack functional gap junctions between rods and cones, the persistence of light responses in OFF GCs suggests that rod→cone signaling does not play a major role for the OFF pathway at scotopic intensities.

Soucy et al. (1998) have also described an alternative pathway for scotopic vision in both normal and coneless mouse retina, which carries signals to all OFF and ON-OFF GCs. The anatomical substrate suggested to transmit these signals are flat contacts established by a particular type of OFF CB2 with rod spherules (Tsukamoto et al. 2001), and where ionotropic glutamate receptors have been localized (Hack et al. 1999). In the mouse retina, however, we only observed APB-resistant OFF responses in 1 of 18 cells. What possible explanations could account for this difference? First, we recorded synaptic currents by doing patch-clamp recordings in the slice preparation, whereas Soucy et al. (1998) recorded spike trains with a multielectrode array in the whole mount retina. If when doing multielectrode recordings, there were a bias toward recording from particular GC types that happened to be those cells receiving input from an APB-resistant pathway, the role of this pathway might have been overestimated. Along a similar line of reasoning, if GCs that receive input from CB2 have a particular morphology that made them unsuitable for our recordings, or if either CB2 or their postsynaptic GCs do not survive the slicing procedure, we may have possibly missed recording some APB-resistant OFF responses. Nevertheless, in the rabbit retina, we have obtained similar results recording from slices and from whole mount retina, suggesting that the functionality of the alternative pathway is independent of the preparation. Second, we dark-adapted the animals for a substantially longer time period than Soucy et al. (6 vs. 1 h), since in preliminary experiments, we observed that after short dark-adaptation periods the retina was unresponsive to low intensity stimulation. Therefore a difference in photoreceptor sensitivity between the two studies cannot be excluded. Finally, according to Soucy et al. (1998), the appearance of APB-resistant OFF
responses was dependent on stimulus contrast, being most prevalent at high contrast. To detect APB-resistant OFF responses at all intensities, we used the maximum possible contrast (100%), thus ruling out a difference between the stimulus employed to evoke responses in their experiments and in this study.

Although no more than 20% of rods were found to make contacts with the CB2, it was proposed that rod→rod gap junctions in the mouse retina may be the route by which all rod signals could be fed into CB2 cells and their corresponding GCs (Tsukamoto et al. 2001). It is well established, however, that GCs stratify at different strata in the inner plexiform layer, where each particular type receives input from only a few bipolar cell types (Boycott and Wässle 1999; DeVries 2000; Roska and Werblin 2001). Consequently, CB2 bipolar cells are expected to synapse onto some but not all GC types. Additionally, only an estimated 5% of rods were shown to possess flat contacts with glutamate receptors (Hack et al. 1999). Taking into account these considerations and consistent with our results, signaling through an APB-resistant pathway is expected to be transferred to only a subset of OFF and ON-OFF GCs. Our data suggest that, in rodents, the percentage of OFF GCs might be considerably lower than in rabbit.

Soucy et al. (1998) also observed APB-resistant light responses in coneless mice, in which cones were genetically ablated. Genetic manipulation of particular cell types in the retina may result in widespread effects. For example, removal of cones from the retina results in degeneration of the rods (Ying et al. 2000). Moreover, in a retinal degeneration model, RB cells form ectopic contacts with cones in the absence of rods (Peng et al. 2000). Taken together, this may hint that, in the coneless mice, wiring changes could take place, such that a larger number of cone bipolar cell types receive rod input than in the normal mouse, thus explaining the results observed in the coneless mice. However, no difference in the APB sensitivity of OFF responses between the normal and coneless retina was observed, making an indirect effect due to cone ablation unlikely.

It remains to be elucidated in which other species the alternative pathways are present and whether they play an important role. In the developing retina of the ferret, APB-resistant OFF responses have been recorded under dark-adapted conditions from the majority of OFF and a small percentage of ON-OFF GCs, although it is not known whether this pathway is also functional in the mature retina (Wang et al. 2001). In the cat retina, both ON and OFF responses recorded under scotopic conditions from brisk sustained (X) and brisk transient (Y) cells were completely blocked by APB, and OFF responses were blocked by strychnine (Müller et al. 1988). These two GC classes comprise only about 50% of all GCs of the cat retina, and responses from other GC types have not been characterized so far. However, expression of a GluR1 glutamate receptor subunit has been observed in dendrites of OFF CB cells contacting rod spherules of the cat retina, suggesting the possibility that this pathway may also be present in the cat retina (Kolodziej et al. 2003). In the dark-adapted monkey all light-mediated behavior is abolished on APB application, whereas in mesopic and photopic conditions, only the ON responses have been shown to be blocked by APB (Dolan and Schiller 1989). In addition, no contacts between rods and OFF cone bipolar cells have been reported in the primate retina (Boycott and Dowling 1969). However, it is possible that in the primate retina, where midget and parasol GCs make up ~90% of the GCs, an alternative rod pathway is only present in some of the remaining 10% of the GCs.

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