The oscillatory potentials of the mudpuppy retina

Lillemor Wachtmeister* and John E. Dowling

The properties of the oscillatory potentials (OPs) of the mudpuppy electroretinogram (ERG) were studied and compared with the properties of the b-wave of the ERG and the proximal negative response (PNR). Both transretinal and intraretinal ERGs were recorded in response to full-field as well as 200 and 500 μm spot illumination. The OPs differed in behavior from the b-wave in terms of voltage-response relations and the effects of repetitive stimuli. Thus the OPs appear to have a different origin from that of the b-wave. The laminar profile of the OPs was also compared with both the PNR and the b-wave. The OPs reverse in polarity as a function of retinal depth and therefore appear to reflect radial flows of currents within the retina. Thus the origin of the OPs seems different also from that of the PNR, which appears to represent tangential current flows around the amacrine cells. The earlier OPs arise more proximally within the retina than the later ones, suggesting that the individual oscillatory peaks are likely to have different origins. We propose that the OPs may represent feedback loops within retina.

In support of this notion, it was found that the OPs were selectively depressed by GABA, glycine, glutamate, and dopamine. Acetylcholine and carbacholine did not affect the oscillatory responses, suggesting perhaps that the OPs are generated by inhibitory feedback synaptic circuits.

Key words: electroretinogram, oscillatory potentials, proximal negative response, intraretinal potentials, neurotransmitters, mudpuppy retina

The vertebrate electroretinogram (ERG) evoked with high-intensity light stimuli exhibits a series of rhythmic, low-amplitude potentials superimposed on the b-wave. These rhythmic waves are called the oscillatory potentials (OPs). Studies of the human ERG have shown that the threshold and spectral sensitivity of the OPs as well as the temporal and adaptational characteristics of these waves differ from those of the b-wave. The oscillations are most easily elicited when the retina is in the mesopic state of adaptation, and it makes little difference whether this level of adaptation is induced by slow-flickering lights, intermittent high-intensity flashes, or steady background illumination. The origin of the OPs is unknown, although both bipolar cells and amacrine cells have been implicated in their generation. Brindley and Yonemura and Hatta were the first to study the depth profile of the OPs within the retina, and they found the maximum amplitude of the oscillations in the frog retina to be in the inner nuclear layer (INL).
Later, Ogden and Wylie\textsuperscript{24} and Ogden\textsuperscript{25} found the maximum amplitude of the first three OPs in the pigeon, chicken, and monkey to be at the level of the inner plexiform layer, and they suggested that the elements in this neuropil layer (i.e., the axon terminals of the bipolar cells, the processes of the amacrine cells, and the dendrites of the ganglion cells) could all be involved in the generation of these potentials. Recently, Korol et al.\textsuperscript{19} showed that the intravitreal injection of glycine caused morphological changes in many of the amacrine cells in the rabbit retina and the disappearance of the OPs from the ERG. These authors suggested that certain of the amacrine cells may be involved in the generation of the potentials.

The present study was initiated to characterize more fully the properties of the OPs and to determine more precisely their origin. The mudpuppy was chosen as the experimental animal because the characteristics of its retinal cells are well known and both intraretinal and intracellular recordings can readily be made from its eye.

The following three kinds of experiments were undertaken:

1. It is known from human studies that the threshold of the OPs is higher than that of the b-wave and about the same as that of the a-wave.\textsuperscript{28} It is also known that the amplitude of the OPs depends on not only intensity but also interstimulus interval. Thus, in the first part of the study, we investigated whether the OPs in the mudpuppy exhibit similar properties.

2. The amacrine cells extend processes laterally, and when activated, they generate tangential flows of currents within the retina which can be locally recorded as an extracellular field potential, the proximal negative response (PNR). It has been shown that the PNR does not reverse its polarity upon electrode penetration of the retina.\textsuperscript{5} The b-wave, on the other hand, reverses its polarity upon electrode penetration of the retina, indicating that it is generated by structures extending radially through the retina. The second part of the study dealt with depth profile of the intraretinal OPs and a comparison of the behavior of the OPs as a function of retinal depth with that of the b-wave and the PNR.

3. Pharmacological studies have suggested that the OPs may be dependent on the integrity of certain synaptic interactions within the retina. For example, Hempel\textsuperscript{18} and Gutierrez and Spiguel\textsuperscript{16} showed that reserpinized rabbits display no OPs but that the OPs reappear when L-dopa is injected intravitreally. If this suggestion is correct, it might be possible to disrupt selectively the OPs by application of particular neurotransmitters (or their blocking agents) to the retina (see also Korol et al.\textsuperscript{19}). Therefore, in the third part of this study, we investigated the effect of some putative neurotransmitters on the OPs.

**Methods**

**Preparation.** Mudpuppies (Necturus maculosus), 20 to 25 cm long, were obtained from a supplier in Wisconsin (Mogul Ed, Inc.). The animals were kept in aerated tanks filled with spring water of 10 to 15 cm depth in a moderately darkened cold room at 5° to 7° C. The water was changed weekly, and commercial fungicide was added to the water to reduce infection from watermold.

At the time of an experiment, a specimen was decapitated and pithed under dim red light illumination provided by a microscope illuminator covered by Wratten No. 29 filter (Eastman Kodak Inc.) (<0.01% < 600 nm). The eye was removed from the head, and the cornea, iris, and lens were excised under a dissecting microscope. Some of the vitreous humor was removed with pieces of tissue paper. The eye was mounted on a cotton pad soaked with amphibian Ringer’s solution and recorded from in a light-shielded Faraday cage.

**Electrodes.** The transretinal ERG was recorded between an indifferent electrode positioned behind the eyecup and an active electrode in the vitreous. The active electrode was a silver–silver chloride wire insulated up to its tip with nail polish, and the reference electrode was a bare piece of chlorided silver wire. The intraretinal potentials were recorded via micropipettes made from omega dot glass tubing (Hilgenberg Glass, West Germany) having an outer diameter of 1.2 mm and an inner diameter of 0.6 mm. The pi-
pettes were pulled on a DK1 type puller (David Kopf Instruments, Inc.) and beveled on fine lapping paper at an angle of approximately 30 degrees. The final pipette opening was approximately 15 μm, but its tip size was only about 0.5 μm. The pipettes were filled with amphibian Ringer's and inserted into a plastic holder containing a silver-silver chloride electrode. The micropipettes were advanced through the retina by means of a stepping hydraulic microdrive (David Kopf Instruments, Inc.). The resistance of the microelectrodes varied between 2 and 10 MΩ.

Recording system. The transretinal and intraretinal potentials were fed to an AC-coupled preamplifier (Tektronix 122 with input impedance of 50 pF and 10 MΩ). Coupling time constants of either 1 sec or 20 msec were used. The high-frequency cut-off response was set at 1000 Hz. The amplifier outputs were monitored on the upper two traces of a four-trace storage oscilloscope (Tektronix type 564-3A74-2B67). The DC-level was monitored continually on a dual-beam oscilloscope (Tektronix type RM 502). Occasionally responses were recorded on a Gould brush recorder (Model 289).

A triggering circuit (Grass SD-6 stimulator plus a 2.5 V relay) was used to synchronize the sweep of the storage oscilloscope with the opening of the shutter of the light stimulator. Amplified signals from a photoresistor were displayed on a third oscilloscope trace. The stored records of the oscilloscope were photographed with an oscilloscope camera (Tektronix C 13).

The amplitudes and the peak times of the on-transient of the PNR, a- and b-waves, and the individual OPs were measured with calipers from photographic or chart records. The amplitudes of the OPs were measured from the baseline drawn between successive troughs of the wavelets and expressed in arbitrary units (a.u.). The peak times were measured from stimulus onset to the peak of the positive (nonreversed) or the negative (reversed) peak of the OPs. The amplitude of the b-wave in all figures was measured from the baseline, but virtually identical results were obtained when the b-wave was measured from the a-wave trough.

Stimulator. White light, restricted to the visible part of the spectrum by infrared and ultraviolet filters, was derived from a tungsten-ribbon filament lamp (General Electric 6.5 V, 2.75 A, and 2950° K). The light was regulated by an optical system which consisted of a series of lenses and apertures mounted on positioning devices, an electromagnetic shutter (Uniblitz 300, Vincent Lab., Inc.), a neutral-density filter wedge, and a series of individual neutral-density filters. The optical stimulator provided variable sized spots of light (100 to 1000 μm) as well as full-field illumination which covered the entire eyecup.

Radiometric calibrations were made with a photodiode at the position normally occupied by the eyecup (PIN-5 United Detector Technology). With all the elements of the optical system in place, maximum output of the system was found to be $6.9 \times 10^{17}$ photons/cm$^2 \cdot$ sec. Light is specified in the text and figures in arbitrary logarithmic notation (base 10). Log 1 = 0 corresponds to maximum output of the optical system.

Baird Atomic interference filters, having a half-bandwidth of 10 nm were used for the spectral sensitivity measurements. The light source was calibrated for difference in quantum flux across the spectrum. At the brightest intensity, 525 nm, the stimulator delivered $2.9 \times 10^{14}$ photons/cm$^2 \cdot$ sec.

Procedure. After dissection of the eyecup the transretinal ERG electrode and the micropipette were lowered into the vitreous humor under dim red light illumination. The transretinal electrode was positioned at the edge of the eyecup while the micropipette was placed in the center. For retinal depth and/or PNR recordings, a 200 or 500 μm spot of dim red light was sharply focused on the retina and centered over the micropipette tip. For transretinal ERG studies, full-field illumination was used.

The retina was allowed to dark-adapt for about 10 to 15 min before an experiment was started. Flashes of 200 msec duration were given at constant intervals of 15 sec, 30 sec, or 1 min. The threshold of the b-wave was monitored continuously to ensure that the retinal sensitivity remained stable during the course of an experiment. A few spectral sensitivity measurements (using a 40 μV criterion b-wave) indicated that the experiments were performed under mesopic conditions. That is, the spectral sensitivity function was found to be broad, flat, and single-peaked, and it approximated a summation of the absorption spectrum of the rod pigment ($\lambda_{\text{max}}$, 525 nm) and the cone pigment ($\lambda_{\text{max}}$, 572 nm) of the mudpuppy retina.3

Micropipette contact with retina was signaled by the appearance of a sudden DC-shift. The penetration of the electrode through the retina (or its withdrawal) was done in steps of 5 to 30 μm, usually with steps of 20 μm. Since the electrode did not penetrate the retina perpendicularly, the micrometer measurements of retinal thickness varied. Therefore percentage retinal depth was
used, and the results showed good agreement. The 0% retinal depth is the retinal surface, and 100% retinal depth is at the distal margin of retina proper (where the negative b-wave showed a dramatic drop in amplitude). The PNR was recorded by carefully adjusting the microelectrode depth and position of the 200 μm stimulus spot to yield a PNR of maximum amplitude.

Observations were made from 49 retinas, and 98 depth penetrations were carried out. All experiments were done at room temperature (65° F), and the responses usually were essentially unchanged for at least 2 to 3 hr and sometimes up to 6 to 7 hr.

Solutions. All drugs were dissolved in an amphibian Ringer's solution (6.5 gm of NaCl, 0.2 gm of NaHCO₃, 0.14 gm of KCl, 0.16 gm of CaCl₂, 0.7 gm of HEPES per liter) whose pH was adjusted to about 7.3. The control Ringer's and the Ringer's containing a dissolved drug were held in plastic tubing. One end of each tube was connected to a micropipette, the other to a micrometer syringe. The micropipettes used for drug addition were recording pipettes whose tips had been broken to a size of 50 to 100 μm. The pipettes were mounted on micromanipulators (Narishige, Inc., C-1), which facilitated positioning their tips close to the surface of the vitreous humor. A small drop of about 0.004 ml was delivered to the retinal surface by twisting the barrel of the micrometer. (The concentrations stated are the concentrations applied and not the effective concentration at recording site.)

The experimental protocol used was the following. Responses were first recorded after applying control Ringer's of the same volume to the retina. Then Ringer's solution containing the drug was applied, and responses were recorded again. Occasionally the recovery of the response after the drug application was followed. Recovery sometimes took up to 1 hr.

Results

Properties of the mudpuppy OPs. Fig. 1 shows the transretinal ERG evoked with full-field flashes of increasing intensity of 200 msec duration and an interstimulus interval of 30 sec. Threshold responses of the mudpuppy b-wave were recorded with intensities of about Log I = —3.5. Tiny OPs could be detected superimposed on the b-wave at intensities as low as Log I = —3.0. Both the OPs and b-wave increased in amplitude with increasing stimulus intensities (Fig. 1). How-
Fig. 2. a, Summed amplitude of the OPs and the amplitude of the b-wave of the transretinal ERG in relation to stimulus intensity. The same recording conditions were used as in Fig. 1. The OPs are expressed in arbitrary units (a.u.). The b-wave increased in amplitude for about 2 log units and then leveled off. The summed amplitude of the OPs showed a linear increase over a range of at least 3 log units. b, Amplitude of the PNR in relation to stimulus intensity. The stimulus light was a 200 μm spot of 200 msec duration delivered at an interval of 30 sec. The voltage-intensity function of the PNR was steeper than that of the b-wave or the OPs.

interval with the summed amplitude of O₁ to O₅. The maximum amplitude for the OPs occurred with an interval of 1 min between flashes. The b-wave amplitude, on the other hand, showed no such maximum; rather its amplitude increased gradually over the range of stimulus intervals used. The PNR, like the OPs, showed a maximum amplitude with an interstimulus interval of about 1 min (Fig. 3, b). Thus the amplitudes of both the OPs and the PNR were enhanced by prior stimulation; the b-wave, on the other hand, showed no such effect.

Not all OPs were equally changed in amplitude with altered stimulus interval. Fig. 4 shows that the second and third oscillatory peaks were considerably enhanced by decreasing the interstimulus interval from 3 min to 30 sec but that oscillatory peaks 1, 4, and 5 were altered little if at all. The recordings of Fig. 4 were made with a shortened time-constant setting of the amplifier (20 msec). Such recordings enhance the relative amplitudes of the OPs and facilitate their measurement. In Fig. 5, recordings are compared from the same preparations made with the same stimulus intensity and interstimulus interval but with different time-constant settings (1 sec vs. 20 msec). The OPs were much more obvious when the shortened time constant was used; however, the peak times of the potentials were not significantly affected by altering amplifier time constant. For most recordings shown in this paper, a 20 msec time constant was used.

The timing of the OPs varied depending on both stimulus intensity and interstimulus interval. The variation with intensity can be seen in Fig. 1, and the variation with interstimulus interval is shown for all seven OPs in Fig. 6. In Fig. 6 the time to peak for each potential was measured from the start of the stimulus flash. The first OP (O₁) slightly increased its peak latency with increasing in-
terstimulus interval. The third and following OPs (O3 to O7), on the other hand, decreased in peak latency with increase of interstimulus interval. Thus the OP peaks were farther apart the closer the interstimulus interval. The PNR, on the other hand, did not change its peak time with variations in interstimulus interval.

**Depth profile of the OPs.** To compare the depth profile of the OPs, b-wave, and the PNR, a 500 μm spot stimulus was used. Responses evoked with a spot of this size and a 30 sec interstimulus interval showed both an identifiable PNR and prominent OPs superimposed upon the b-wave (Fig. 7). If a 200 μm spot was used, a larger PNR was elicited, but only very low-amplitude OPs were observed. With full-field stimuli, on the other hand, large OPs were evoked, but only a small PNR. In the depth recordings the OPs were identified by time from stimulus onset. In Fig. 7 they are labelled O1 to O5. Occasionally one or two extra positive waves appeared in intraretinal recordings (Fig. 7, asterisk), but these potentials were not investigated systematically. We have reason to believe that these potentials may be related to the PNR (see below).

For the depth studies, the transretinal and intraretinal ERGs were recorded simultaneously (Fig. 7). The top response in each figure shows the transretinal ERG. This served as control to ensure that conditions remained stable during the experiment, and it also provided a measure of the peak times of the various OPs. In this and other transretinal recordings, positivity is up; in the intraretinal recordings, positivity is down. In the transretinal recording, the b-wave was always positive as were the OPs. The deflection in the transretinal record that appeared to correspond to the PNR was also positive in these recordings. With a micropipette on the surface of the retina, the PNR was always recorded as negative, whereas the b-wave and OPs were positive, as in the transretinal recordings. The difference in PNR polarity between the two types of recordings probably relates to the fact that the 500 μm stimulating spot was centered with respect to the micropipette but eccentrically positioned with regard to the transretinal ERG electrode (see Methods and ref. 5).

Fig. 7 shows that when the electrode was advanced to the midpoint of the retina, the b-wave reversed to a negative potential; the PNR did not reverse, however, and re-
Fig. 4. OPs of the transretinal ERG recorded with a short time constant (T = 20 msec) in response to full-field stimulus of maximal intensity delivered at an interval of 3 min (top) and 30 sec (bottom). The second and third OPs are enhanced as the interstimulus interval is decreased.

Fig. 5. Transretinal ERGs in response to full-field stimulus of maximal intensity and 200 msec duration delivered at an interval of 30 sec. The top trace was recorded with a long time constant (T = 1 sec), and the lower trace with a short time constant (T = 20 msec). The OPs are comparatively enhanced in amplitude when the shortened time constant is used. The peak times of the OPs are not significantly affected by altering amplifier time constant.

All the OPs, (with the possible exception of O₅) had reversed their polarity by this level of the retina; that is, all were now negative (up). Fig. 8 shows a number of records obtained during a penetration of the retina. The PNR was the initial negative potential which was maximal in amplitude at a retinal depth of about 30%. Note that its polarity was always negative. The OPs all reversed, but at various depths within the retina. By casual inspection, this often is not easy to see, but if the records are carefully aligned, the reversal points are obvious. Compare for example O₁ and O₄. In this experiment O₁ has reversed by a depth of 25%, but O₄ did not reverse until the retina had been penetrated to a depth of 55%. Note that the wavelets that occurred earlier than O₄ did not reverse polarity with retinal depth. This suggests that they may be related to the PNR. That these early wavelets are also not obvious in the transretinal recordings provides further evidence that they are different from the OPs and may be more closely related to the PNR.

Fig. 9 shows in graphic form the behavior of the b-wave, PNR, and OPs during another retinal penetration. As the electrode penetrated the retina from the vitreous side, the b-wave reversed its polarity at a depth of about 35% (i.e., its isopotential point). The PNR, on the other hand, did not reverse its polarity over the entire depth it could be recorded, i.e., up to about 50% of retinal depth. The OPs all reversed their polarity but at different levels. The open symbols represent nonreversed (positive) OPs. The closed symbols represent reversed (negative) oscillatory peaks. The first oscillatory peak reversed to a corresponding negative one at the retinal depth of about 20% to 25%. At the retinal depth where the b-wave was isopotential (ca. 35%), the first three OPs had reversed their polarity. The later OPs, on the other hand, did not reverse their polarity until the electrode was at a retinal depth of about 40% or more.

The relative sequence of changes in intraretinal activity was quite constant from one penetration to another in any one retina as well as between different preparations. There also was no significant difference in the sequence of reversal observed when the indifferent electrode was placed in the vitreous.

Pharmacological studies. Fig. 10 illustrates that when drops of 0.1 to 10 mM gamma-aminobutyric acid (GABA) were applied to the open eyecup, a quite selective decrease in the amplitude of the OPs was observed.
within 1 min. The b-wave, on the other hand, was hardly affected by any of these concentrations of GABA. Thus, a few minutes after the application of 10 mM GABA, the ERG evoked was very smooth and showed only the hint of a single oscillatory potential (Fig. 10). The same effect could also be induced by the application of similar amounts of glycine, glutamate, and in most experiments, dopamine. On the other hand, acetylcholine (ACh) and carbacholine (CCh) in corresponding concentrations did not alter the response; that is, neither the oscillatory potentials nor the b-wave was affected by these drugs.

The action of various concentrations of GABA upon the stimulus-response-function of the oscillatory potentials and b-wave is shown in Fig. 11. A relatively low concentration of GABA (0.1 mM) reduced selectively the summed amplitude of the OPs (Fig. 11, a), whereas the V-log I function of the b-wave was virtually unaltered (Fig. 11, b). With the higher concentrations of GABA (10 mM), no OPs could be recorded at all. The b-wave with such doses was nearly as sensitive as the control b-wave; however, its maximum amplitude was reduced somewhat.

Although all the oscillatory potentials were affected by GABA, glycine, and glutamate, the earlier OPs (O₁ and O₂) appeared relatively more sensitive to the drugs than the later OPs (O₃ to O₅). This effect is shown in Fig. 10. Note that with a dose of 1.0 mM GABA, O₁ and O₂ were not distinguishable, but O₃ to O₅ were still present.

Discussion

The ERG of the mudpuppy displays a series of OPs whose properties resemble in many respects those of OPs observed in the human and other vertebrate ERGs. In both the human and mudpuppy, intensity and interstimulus interval are important factors in the generation of large-amplitude OPs. As is to be expected, the interstimulus interval that produces the maximum-amplitude OPs is shorter in the human (30 sec) than in the mudpuppy (60 sec). However, in both species, up to seven OPs are seen very consistently from prepara-
Fig. 7. Simultaneous transretinal and intraretinal ERGs recorded with a long time constant in response to a 500 μm spot of maximal intensity delivered at an interval of 30 sec. In the transretinal recording, positivity is up; in the intraretinal recording, positivity is down. When the microelectrode has advanced to the midpoint of retina, the b-wave has reversed to a negative potential. The PNR has not reversed and is still negative (up). The OPs, on the other hand, have reversed (with the possible exception of O5) by this level of the retina.

Fig. 8. Intraretinal ERGs recorded with a short time constant at different retinal depths. The same stimulus conditions prevailed as in Fig. 7. The OPs reverse at various depths within retina. For example, O1 is reversed at a depth of 25%, but O4 does not reverse until the retina is penetrated to a depth of 55%.

One of the major findings of this study was our observation that the intraretinal OPs reverse their polarity with retinal depth. This has not been reported previously. The PNR, on the other hand, does not reverse during electrode penetration of the retina. Thus the OPs appear to reflect radial flows of current within the retina, indicating that the origin of the OPs must be different from that of the PNR, which appears to represent tangential current flow around amacrine cells. This conclusion disagrees with previous notions that the amacrine cell potentials could be the source of the OPs.16,19

Another finding of considerable interest is that the individual oscillatory peaks reverse at different retinal levels and in sequence, which suggests a different origin for the first OP as compared with the second and third OPs and later ones. This is shown in Fig. 12 where the reversal point and maximum negative amplitude of the OPs are compared with the anatomical structures of the mud-puppy retina and some of the characteristics...
Fig. 9. Peak times of the individual OPs in relation to retinal depth. Depth is expressed in percent. Crosses represent the peak time of the PNR. Open symbols represent peak times of the nonreversed positive OPs. Closed symbols represent peak times of the reversed negative OPs. Full-field stimulus of 200 msec duration delivered at an interval of 30 sec was used. As the electrode penetrates retina from the vitreous side, the b-wave reverses its polarity at about 35% of depth. However, PNR remains negative over the entire depth it can be recorded (from about 10% to 50%). The OPs reverse their polarity at different levels (O1 at about 20%, O2 and O3 at about 30%, and O4 and O5 at about 40% of retinal depth).

Fig. 10. Effect of different concentrations of GABA on the OPs of the transretinal ERG evoked by full-field stimulus of maximal intensity and 200 msec duration delivered at an interval of 30 sec. The effects appeared within 1 min after the addition of the drug and gradually increased, reaching a maximum after about 3 to 5 min when these recordings were taken. A selective decrease of the OPs occurred. The first (O1 and O2) appeared relatively more sensitive to the drugs than did the later OPs (O4 to O5).

of the other inner retinal potentials. The reversal point of the first oscillatory peak (O1) is located at the border between the inner plexiform layer and INL, distal to the PNR maximum but proximal to the isopotential b-wave. The reversal points of O2 and O3 lie in the middle of the INL, whereas the reversal points of O4 and O5 fall even more distally within the INL. The maximum negative amplitude of O1 to O3 is also located more proximally in the retina than is the locus for the maximal negative amplitude for O4 and O5.

The neuronal events generating the first OP occur earlier in time than the events generating the later OPs, and we have found that the first OP reverses more proximally in the...
Fig. 11. Stimulus-response curves in relation to different concentrations of GABA applied to the eyecup. The same stimulus conditions were used as in Fig. 10. a, Summed OPs of the transretinal ERG. The OPs are expressed in arbitrary units (a.u.). A relatively low concentration of GABA (0.1 mM) reduced selectively the summed amplitude of the OPs. b, The b-wave of the transretinal ERG. The V Log I function of the b-wave was virtually unaltered when a low concentration of GABA (0.1 mM) was used. Higher concentrations reduced the maximum amplitude of the b-wave, but the sensitivity was nearly the same.

retina than do the later ones. This suggests that the chain of events underlying the OPs starts in the proximal part of retina and travels distally. One possible explanation for such a series of radial current loops moving from proximal to distal in the retina would be a feedback system. This notion supports a previous suggestion by Brown, who proposed that the OPs might represent neural feedback circuits.

Since the polarity of the first OP reverses at a retinal depth where the amacrine cells are located, the first OP might be initiated by the activity of the cells in the amacrine cell layer feeding back onto cellular structures located in the more distal part of the INL. Two possible feedback systems have been described within this region of the retina. The first is from the amacrine cell processes back onto bipolar terminals or onto other amacrine cell processes or perikarya. The second one involves the recently discovered interplexiform cell, which is presynaptic to amacrine cell processes in the inner plexiform layer and to bipolar cell dendrites and horizontal cell processes in the outer plexiform layer. If synaptic feedback by the amacrine or interplexiform cells gives rise to the OPs, it might be expected that these interactions could be affected by application of certain putative neurotransmitters to the retina. Indeed, our pharmacological results showed that the OPs could be selectively depressed by GABA, glycine, and usually dopamine. Histochemical and physiological studies have suggested that these substances are likely inhibitory synaptic transmitters in the inner plexiform layer of the retina (see review by Neal). It has also been shown that certain amacrine cells selectively take up these drugs. On the other hand, acetylcholine has also been implicated as playing a role in
the inner plexiform layer, perhaps as an excitatory transmitter of amacrine and bipolar cells.\textsuperscript{20} We found that neither acetylcholine or carbacholine affected the OPs. This may indicate that only inhibitory feedback synapses are involved in the generation of the OPs.

The OPs were also extinguished selectively by the application of glutamate to the retina. No role for this amino acid has been proposed in the inner plexiform layer, although the substance is known to block transmission between photoreceptors and the second-order neurons.\textsuperscript{9, 21} Since the b-wave is not substantially affected by concentrations of glutamate that selectively abolish the OPs, this effect of glutamate would not appear to be related to photoreceptor neurotransmission. Rather, it may suggest a role for glutamate in the inner plexiform layer of the retina.

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REFERENCES

9. Dowling, J. E., and Ripp, H.: Effect of magnesium...


