Control of Retinal Information Coding by GABA_B Receptors

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The directional selectivity of amacrine and ganglion cells was studied using conventional intracellular recording techniques and drug application in the superfused retina-eyecup preparation of the tiger salamander. Baclofen, a GABA_B receptor agonist, enhanced normal directional responses in some directionally selective third-order neurons. In about 30% of the cells that were not normally directional, baclofen induced direction-selective responses. This effect was particularly marked when 2-amino-4-phosphonobutyrate (APB) was used to isolate the OFF pathway. Comparisons of the effects of APB and baclofen on induced directional cells indicate that directional information in the ON and OFF channels is often handled separately and frequently is not aligned. This tends to obscure the observation of directionality as seen from the soma. Application of picrotoxin blocked both normal directional selectivity and baclofen-induced directional selectivity in some cells. Superfusion of picrotoxin and strychnine together blocked directionality in almost all cells. In both normal and induced directionality, the null direction response varied from cell to cell between a small depolarization, no voltage response, or a hyperpolarization. Injection of positive current often revealed "silent" inhibition. Some induced direction-selective cells did not show any evidence of inhibition in the null direction. The similarities in the response to baclofen, the influence of GABA and glycine antagonists, and the characteristics of the null-direction responses suggest that both normal and induced directionality originate from the same sources or mechanisms. Baclofen also induced orientation selectivity, but this was rarely observed.

We recently found that activation of GABA_B receptors altered the responses of amacrine and ganglion cells in the amphibian retina. Normally, the responses of third-order cells can be categorized as either sustained or transient. Baclofen, a GABA_B receptor agonist (Bowerly et al., 1980), suppressed sustained responses but augmented transient responses. In many sustained cells, baclofen not only suppressed sustained responses, but also brought out transient responses (Slaughter and Bai, 1989). Ikeda et al. (1990) have reported a similar action in sustained OFF, but not on ON, ganglion cells in the cat retina. These results indicate that the transient/sustained dichotomy of retinal ganglion cells can be altered by one type of GABA receptor. The

implications of these results for information processing in the retina are the topic of this study.

It has often been proposed that transient and sustained responses carry different information. Sustained signals relay information on steady-state conditions, while transient signals carry information on rates of change. In the cat retina, there is a correlation between brisk transient and sustained light responses and the α - and β -ganglion cell types (Cleland et al., 1971). Because β -cells have small dendritic fields, while α -cells have large dendritic fields (Boycott and Wässle, 1974), this has led to the hypothesis that sustained signals may carry spatial information while transient signals may carry information on temporal properties, such as motion information (Stone, 1983). When cell responses switch from sustained to transient wave forms, the information contained in their responses may also change. We therefore examined the information content of cells before and after baclofen application. Although the response wave form changed with baclofen treatment, we had no way of knowing what type of information the new signal might be carrying. We arbitrarily investigated directional selectivity and orientation sensitivity because these properties have been well described in vertebrate retinas. Serendipitously, we found that approximately 30% of the nondirectional cells studied showed an "induced" directional response after baclofen treatment. This article presents evidence on this point, compares this induced directional selectivity to conventional directional responses, and suggests implications based on these data.

Materials and Methods

Experiments were performed in the superfused retina-eyecup preparation of the tiger salamander, *Ambystoma tigrinum*. The animal was decapitated and pithed, and then the eye was removed. The eye was dissected to remove the cornea, iris, and lens. The vitreous was dabbed away using a Kimwipe tissue so that the retina was exposed in a hemi-eyecup. Another Kimwipe was positioned around the perimeter of the eyecup to assist in fluid exchange. A perfusion pipette was placed at the rim of the retina, allowing the eyecup to be filled with a Ringer's solution. Through a manifold system and a series of valves, the solution could be changed from a control Ringer's solution to one containing one or more drugs. A detailed description of these methods has been presented previously (Miller and Dacheux, 1976).

A light bench equipped with a 100-W tungsten-halogen lamp was used to project diffuse light or a light slit (which was as narrow as 100 μ m in width) onto the retina. Driven by a motor, the slit could sweep across the entire eyecup with variable speeds. The length of the slit and the range of slit movement were controlled by an aperture installed in front of the slit. The direction of slit movement was controlled by rotating the slit holder. The light beam was interrupted by an electro-mechanical shutter controlled by an ATARI computer, and the intensity of the light stimulus was attenuated by neutral density filters.

An alternative light stimulus consisted of images generated by an ATARI computer onto a black-and-white Ikegami monitor (using a slightly modified version of a program developed by Dr. Stephen Massey). These images were focused onto the retina through a light bench

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Figure 1. Responses of a sustained ON cell to a 100- μ m-wide light slit moving across the retina in the directions indicated by the arrows. The long axis of the slit was orthogonal to the direction of motion. *a*, The responses of the cell under control conditions, when the neuron gives no indication of directional selectivity. *b*, The responses to the same stimuli, but after the retina was treated with APB and baclofen. Under these conditions, the cell showed a preference for motion of the slit in the -135° direction and a null response to the opposite direction (45°). There was a hyperpolarizing response to movements in the null direction.

system. Diffuse light, small spots, bars, and edges could be focused onto the retina and moved in different directions at various speeds. The stimulus contrast could also be modified. Similar results were obtained with both optical systems. Optimal slit speed was generally between 0.5 and 2 mm/sec.

Electrophysiological recordings were made using standard intracellular electrodes, with resistances of 200–400 M Ω when filled with 2 M potassium acetate and 1 mM EGTA. The electrical signal was recorded with a WPI 707A amplifier, viewed on a Hitachi V-134 storage oscilloscope, and stored on a Brush 440 penwriter and a Vetter video cassette recorder. The control pulses for the mechanical slit or the computer-generated stimuli were recorded in conjunction with the voltage records. A function generator was used to produce sine- or square-wave current pulses while DC currents were applied using a current source in the World Precision Instruments amplifier. Current injection was used to change the cell's membrane potential, measure changes in cell input resistance, and inject Lucifer yellow for morphological identification.

Although some cells were identified morphologically, this was not done routinely, and therefore we do not attempt to distinguish between amacrine and ganglion cell responses.

Results

Directional selectivity could be induced in third-order neurons of the tiger salamander retina by activating GABA_B receptors. The effect was particularly marked when 2-amino-4-phosphonobutyrate (APB) was used to isolate the OFF pathway (Slaughter and Miller, 1981). This phenomenon is illustrated in Figure 1. Figure 1*a* shows the responses of a sustained ON cell to a bar moving across the retina. In each case, the long axis of the slit was orthogonal to the direction of motion. Although the responses were not identical, there was no indication of an en-

Figure 2. Comparison of the synaptic responses to a steady light and a 100- μ m moving slit before and after drug application. This figure shows the variety of directional responses that are evoked. *Preferred* and *null* refer to the resultant induced directionality. *a*, Responses of an ON-sustained third-order neuron. Under control conditions, there was no apparent directional preference. Application of APB and baclofen blocked the ON response and evoked a transient OFF EPSP to a stationary light stimulus; the response of the cell to a moving slit showed a distinct directional selectivity, which disappeared upon return to control solution. *b*, Responses of a nondirectional, transient, ON-OFF inner retinal neuron. Application of APB blocked the ON response to a stationary light stimulus, leaving an OFF EPSP, but the cell remained nondirectional. When baclofen was added in the continued presence of APB, the cell exhibited directional selectivity to a moving slit. The *bottom row* shows a partial recovery. *c*, In this cell, APB alone suppressed responses to all directions of movement (*second row*), but with the addition of baclofen the cell became directional. Positive current revealed a small hyperpolarization in the null direction (*bottom row, right*). *d*, Frequently, baclofen alone did not induce directionality, though the combination of APB and baclofen resulted in directional responses.

Figure 3. Baclofen often induces directionality in the OFF pathway. *a*, Comparison of responses to stationary and moving stimuli as in Figure 2. The cell showed a rather sustained response to the moving slit, but no directionality. After baclofen was applied, the responses to a moving slit were enhanced and showed double peaks in both directions. The second peak showed a weak directional preference (arrows), though the overall response did not show much difference between preferred and null directions. APB blocked the ON responses and enhanced the directionality. *b*, Responses to a 100- μ m moving slit. Under control conditions, there was a slight separation between ON and OFF responses, but no evidence of directionality. Baclofen enhanced the ON and OFF responses, and a directional response appeared in the OFF pathway (arrows), which was augmented with the addition of APB.

hanced response to a particular direction of motion. The polar plot in the center of the circle maps the peak EPSP amplitude in each direction. The control plot was fairly symmetrical. After applying 100 μ M APB and 100 μ M baclofen (Fig. 1*b*), the same stimulus produced a directional response, with a maximal EPSP produced by movement in the -135° direction, a null response in the opposite direction, and intermediate responses in other directions. The solid-line polar plot maps this response, while the dotted line shows the control responses from Figure 1*a*. Of 154 third-order neurons tested with this protocol, 55 cells manifested an induced directional selectivity after treatment with baclofen plus APB. In Figure 1*b*, the null direction produced a small hyperpolarization of the membrane potential. Note, however, that this hyperpolarization was present before drug treatment and may not have played a role in the formation of direction selectivity. Of the 55 induced directional cells, 21 showed no voltage response or a small hyperpolarization to movement in the null direction. The remaining 34 cells showed depolarizations in all directions, though the amplitudes were much smaller in the null directions.

Figure 2 shows a comparison of the responses of third-order neurons to steps of light and moving slits during the application of APB and baclofen. Previous reports, using stepped-light stimulation, demonstrated that APB blocked ON responses but not OFF responses (Slaughter and Miller, 1981), while baclofen suppressed sustained responses but enhanced transient responses (Slaughter and Bai, 1989). Figure 2*a* shows a cell that produced

a relatively sustained response to a stepped-light stimulus (left column) but did not show any directional preference to a moving bar of light (middle and right columns, labeled "preferred" and "null" based on the drug-induced directionality). After treatment with APB and baclofen, the cell responded to a stepped-light stimulus with a transient OFF response and showed directional selectivity. In this cell, the null direction produced a hyperpolarizing response. The bottom trace shows a partial recovery. Figure 2*b* shows a transient, ON-OFF, third-order neuron (left column). When a moving slit was used, the cell responded with brief EPSPs to all directions of motion, with no directional preference. After APB was applied, the ON response was suppressed, leaving a transient, OFF EPSP, but the cell was still nondirectional. When baclofen was added in the presence of APB, the cell became direction selective. In this cell, there was no voltage response to movement in the null direction. The bottom row shows a partial recovery, after the drugs were removed, with a return of the ON response and a loss of directionality. The lack of a directional effect with APB alone suggests that induced directional selectivity was due to baclofen and not APB. Figure 2*c* shows the responses of another transient, ON-OFF, third-order neuron. In this case, APB blocked the ON response to a step of light, leaving a sustained ON hyperpolarization and reducing the voltage response to all directions of movement. There were no directional differences. Addition of baclofen made the OFF response more transient and induced a directional response. Baclofen most often produces a 10–15-mV

Figure 4. Responses of a third-order neuron to movement of a wide bar. This cell shows opposing directional preferences in the ON and OFF pathways. In *a*, the ON response had a directional preference to the 180° direction, while in *b* the OFF pathway showed a preference for the -45° direction.

hyperpolarization of third-order neurons, as it did in this cell. We therefore tested whether the directional properties induced by baclofen were due solely to this hyperpolarization. As shown in the bottom row of Figure 2*c*, when positive current was used

Figure 5. An example of a cell, stimulated by a wide bar as in Figure 4, in which the induced directional selectivity of the ON and OFF channels appears to be aligned. APB still enhances the directionality in the OFF pathway.

to depolarize the cell back to near the original resting membrane potential, the directionality was still present. The EPSP in the preferred direction was reduced, presumably due to the reduction in driving force. Also, a hyperpolarizing potential was revealed for null direction stimuli, indicating the presence of “silent” inhibition. However, in most experiments, neither baclofen nor APB alone induced directional selectivity, which was only seen when both of these drugs were used in combination, as illustrated in Figure 2*d*. Of 55 induced direction-selective cells, baclofen alone induced directional responses in 10 of them. Even when baclofen alone induced directional selectivity, the addition of APB usually enhanced this directionality. We also found two cells in which APB alone produced a weak directionality. In these two cells, the addition of baclofen enhanced this directionality. The remaining 43 cells only became directional in the presence of both baclofen and APB.

One explanation for the importance of APB may be that the ON and OFF channels interact to interfere with the formation of directional selectivity. This possibility was suggested by data such as that illustrated in Figure 3. Although induced directionality was most pronounced when baclofen and APB were used in combination, baclofen alone often revealed a directional preference in the OFF pathway, which was enhanced by APB. Under control conditions, the cell in Figure 3*a* produced small, transient responses to a step of light and small but maintained responses to a moving slit. There was no evidence of directional selectivity. After baclofen was applied, the responses to stationary and moving slit stimuli were enhanced. In response to movement, there were prominent paired EPSPs. The double peak represents ON and OFF response components because the first peak was blocked by APB. In the presence of baclofen, the overall voltage response was still not directional, but the amplitude of the OFF EPSP was larger in the preferred direction than in the null direction (Fig. 3*a*, arrows). That is, baclofen

Figure 6. A third-order neuron in which baclofen alone induced directional selectivity. The addition of APB reduced this directionality (from a preferred: null ratio of 3.7 in baclofen to 2.5 in APB plus baclofen), suggesting that in this cell the selectivity was primarily in the ON channel.

alone induced directional selectivity in the OFF pathway. After APB was added to block ON responses, the directional selectivity in the OFF pathway became more pronounced. Figure 3*b* illustrates another cell in which baclofen alone induced directional selectivity in the OFF pathway. This cell showed a large, transient EPSP as the slit entered the receptive field and a smaller EPSP as the slit passed through the receptive field. There was no evidence of directionality. Baclofen made the OFF response (Fig. 3*b*, arrows), but not the ON response, directionally selective. Again, APB not only blocked the ON responses but also accentuated the directional preference established by baclofen in the OFF pathway.

Based on these findings, we attempted to separate systematically the ON and OFF pathways to analyze the baclofen-induced directional properties of each pathway. An ideal method to accomplish this would be to isolate the ON and OFF pathways separately while using baclofen. Unfortunately, there is no pharmacological agent available that can selectively block the OFF pathway. We attempted to use low doses of kynurenic acid, which is more effective at blocking OFF responses, compared to ON responses, in the inner retina (Slaughter and Miller, 1983; Coleman et al., 1986). However, the relative block was inconsistent, and for these long-term experiments, there was a progressive block of the ON channel. An alternative approach is to use a wide bar to separate temporally the responses to the leading and trailing edges of the stimulus. Although this stimulus paradigm did not totally separate ON and OFF, it did provide evidence that the ON and OFF pathways may be misaligned, as illustrated in Figure 4. The cell in this figure responded to a step of light with ON and OFF transient EPSPs. We used a light

Figure 7. Baclofen enhances the directionality in some normally directional cells.

bar wider than the diameter of the eyecup, so that the leading edge swept completely across the retina before the trailing edge began. Figure 4*a* shows the response to a bar moving in the 0°/180° directions; in Figure 4*b*, the bar is moving in the 45°/–135° directions. In each case, the cell responded to the moving bar with distinctly separate EPSPs, representing the cell's responses to the leading and trailing edges of the moving bar. This cell did not show directional selectivity under control conditions. After baclofen application, the responses were enhanced, but there was only slight evidence of directionality in the leading- and trailing-edge responses. In Figure 4*a*, as APB was added to the baclofen-treated retina, the leading-edge responses were slowly blocked. In the third and bottom rows of Figure 4*a*,

Figure 8. Different types of normal directional responses encountered in tiger salamander retinas. Null-direction responses consisted of no voltage change (*top row*), a small depolarization (*middle row*), or a hyperpolarization (*bottom row*).

Figure 9. An example of null-direction inhibition, revealed by injection of positive current (bottom row), in an induced directionally selective cell.

during the early stages of APB application (before the ON responses were completely blocked), there was a distinct 180° directional preference for the leading edge (arrows). Figure 4*b* shows responses of the same cell to movement along the $-45^\circ/135^\circ$ axis. After the ON pathway was fully blocked by APB (bottom row), the trailing edge showed a -45° directional preference, almost diametrically opposite to the leading edge.

In a few cases, we found that both the leading- and trailing-edge responses to a wide bar stimulus showed the same directional preference after baclofen application. An example is shown in Figure 5, which reproduces recordings from a third-order neuron that responded to a step of light with ON and OFF transient IPSPs (not shown). There was no directional preference under control conditions, but baclofen produced a similar directional preference in both the leading- and trailing-edge responses. Addition of the APB to the baclofen still enhanced directionality in the OFF pathway.

We also found some cells in which baclofen induced directional selectivity, but the addition of APB suppressed this directionality, as illustrated in Figure 6. Based on the studies described above, this probably represents a baclofen-induced directionality in the ON pathway.

Attempts were made to correlate normal and induced directional selectivity for the purpose of determining if induced directionality arose *de novo* or derived from sources or mechanisms related to normal directionality. Baclofen enhanced normal directional responses, as illustrated in Figure 7. We found that baclofen enhanced directionality in three normally directional cells and had no effect on directionality in three others. Therefore, baclofen would be expected to enhance the overall signaling of directional information in the inner retina. As mentioned previously (Fig. 2), the null direction response in induced directionality consisted of one of three response types. Of the 55

Figure 10. Null-direction inhibition in a normally directional cell. Positive current produced a hyperpolarization that was converted to a depolarization by negative current. This is the same cell shown in Figure 7.

baclofen-induced directional cells, seven showed hyperpolarizations in the null direction; the remainder showed either a small depolarization or no voltage response. The same variety of null direction responses was seen in normally direction-selective cells, as illustrated in Figure 8. In addition, current injection would often reveal inhibition in the null direction, as illustrated in Figure 9 for induced directional cells and in Figure 10 for normally directional cells. In Figure 9, APB and baclofen induced directionality, but a clear inhibition was not evident. When the cell was depolarized (bottom row), the inhibitory input in the null direction became apparent. In the normally directional cell shown in Figure 10, positive current revealed a hyperpolarization in the null direction (middle row), while negative current inverted the polarity (bottom row), indicating the presence of null-direction inhibition. Recordings were obtained from 23 normally directional cells. In response to movement in the null direction, 13 showed small depolarizations, six produced hyperpolarizations, and four cells showed no potential change. All of these data are similar to observations of induced directionality and suggest that induced and normal directionality develop through the same mechanisms.

The pharmacology of normal directionality has been extensively studied. In rabbit retina, both GABAergic inhibition (Caldwell et al., 1978) and cholinergic excitation (Ariel and Daw, 1982) have been linked to the formation of directional properties. These studies have been used to lend support to Barlow and Levick's (1965) original model, which suggests that directional selectivity is generated by asymmetrical inhibitory inputs. Similar pharmacological results were reported in turtle retina (Ariel and Adolph, 1985), suggesting that these two transmitter systems are involved in directional encoding in all vertebrate retinas. Therefore, we examined the role of inhibitory amino acid neurotransmitters on directional properties in the tiger salamander. Picrotoxin, a GABA_A receptor blocker, reduced directional selectivity in some but not all cases. Picrotoxin suppressed normal directionality in three out of six cells tested (e.g.,

Figure 11. Picrotoxin can block normal direction selectivity in some cells.

Fig. 11) and suppressed induced directional selectivity in three out of five cells (e.g., Fig. 12). In these examples, picrotoxin blocked directionality principally by enhancing the null-direction responses. This is similar to its mechanism of action in rabbit and turtle (Caldwell et al., 1978; Ariel and Adolph, 1985).

The effect of strychnine and picrotoxin on a normally direction-selective cell is shown in Figure 13. In this cell, baclofen enhanced the directional preference (second row), and positive current revealed a silent inhibition in the null direction (third row, arrow). Addition of picrotoxin and strychnine, after recovery from baclofen, increased the size of the EPSPs in all directions and eliminated directionality in this cell. A similar suppression was observed in induced direction-selective cells, as illustrated in Figure 14. Initially, this cell was not directional. When baclofen was applied, a weak directional preference was revealed (second row), and this was enhanced by the addition of APB (third row). Applying picrotoxin and strychnine in the continued presence of baclofen and APB caused a suppression of this directional preference. In induced directionality, picrotoxin and strychnine in combination suppressed directional responses in 11 of 13 cells. However, we were only able to test picrotoxin and strychnine together on two normally directional cells. In one cell, directional encoding was blocked, but in the other it was not. Unfortunately, this sample is too small to permit any conclusions to be drawn.

Another trigger feature that may be induced by baclofen is orientation sensitivity. Using moving slits, we found very few examples of orientation-sensitive cells, and even in these cells the orientation preference was generally weak. However, we found one very dramatic example of induced orientation sensitivity. This was in a transient, ON-OFF, third-order neuron illustrated in Figure 15. Under control conditions (Fig. 15*a*), this cell showed no evidence of orientation sensitivity to a narrow bar (100 μ m) moving in the directions indicated by the arrows. After application of 100 μ M baclofen, the cell responded with enhanced EPSPs to a horizontally oriented (0–180°) bar, but with reduced responses to other orientations of the moving bar. When 100 μ M APB was added to baclofen, the orientation became more finely tuned (Fig. 15*c*). The horizontally oriented bar was the only stimulus that produced an EPSP. The EPSP was smaller than that in baclofen alone, suggesting that the ON and OFF EPSPs were somewhat additive, though not tuned to the same orientation.

Figure 12. An example of an induced directionally selective cell in which picrotoxin suppressed this directionality.

Discussion

The overall conclusion from these studies is that the information content of retinal neurons is a dynamic property that is under neurotransmitter control. In our experiments, activation of GABA_B receptors resulted in a prominent encoding for directional information in approximately one-third of the nondirectional cells studied. Directionality was the trigger feature that we decided to evaluate a priori, and in this sense the study is biased. The information coding induced by baclofen may relate to other features as well as, or perhaps better than, directionality. Nevertheless, there was a dramatic increase in the number of third-order neurons carrying directional information after activation of GABA_B receptors.

One question we attempted to address is how this induced directional information arose and what this implies about the functioning of the retinal network. There are two alternative explanations for the induction of directional responses. One is that the original responses of the network were modified in a manner that resulted in de novo generation of directional responses. The other possibility is that the directional signals were always present, but went unnoticed because they were not a dominant component of the cell's response until baclofen was applied. Several observations suggest the latter. As noted previously (Bai and Slaughter, 1989), transient responses can sometimes be seen in sustained cells, especially after they have been hyperpolarized by negative current injection. In addition, when APB blocks ON responses in some sustained ON cells, transient OFF responses appear (Arkin and Miller, 1988; McReynolds and Lukasiewicz, 1989). These data indicate that information carried by transient signals is contained, though not evident, in the sustained responses of third-order neurons. Similarities between the properties of induced and normal directionality also suggest they originate from similar mechanisms. The null-direction responses are similar in both cases, as is the inhibitory amino acid pharmacology. In addition, baclofen often enhanced normal directional responses, which might contribute to the appearance of directionality in nondirectional neurons. Taken together, the most parsimonious explanation is that directional information is contained in cells that show no apparent direc-

Figure 13. A normally directional cell in which baclofen enhanced this directionality. Under control conditions, positive current revealed null-direction inhibition. The combination of picrotoxin and strychnine (after baclofen washout) blocked directionality.

tionality, and that this directional information derives from the same sources found in conventional directional cells.

We compared the pharmacology of directional responses in the amphibian retina with published reports in other vertebrates. In rabbit and turtle retinas, drugs that interfere with GABAergic and cholinergic systems eliminate directional selectivity. We found a similar GABA sensitivity in some of our normal and induced direction-selective cells. For example, picrotoxin suppressed directionality in about half of both the normal and induced directional cells. A combination of picrotoxin and strychnine almost always suppressed directionality, indicating a role for glycine in the formation of amphibian directional responses. This result differs from observations in rabbit and turtle (Caldwell et al., 1978; Ariel and Adolph, 1985), where picrotoxin alone always suppressed directionality. However, Werblin et al. (1988) have proposed that glycine may play a role in directionality in the tiger salamander retina. They have suggested that wide-field glycinergic amacrine cells produce asymmetric inhibition that generates directional preferences. Unfortunately, we did not test strychnine alone to evaluate this hypothesis. In two induced and one normally directional cell, strychnine and bicuculline in combination failed to block directionality. This may represent an artifactually weak antagonism by these drugs in these cases. Alternatively, it may indicate that another transmitter provides the asymmetric inhibition or that inhibition is not always required to generate directionality. Recently, the importance of inhibition in the formation of directionality in the cortex has been questioned (Douglas et al., 1988).

The retina may generate directional information through several different mechanisms. Early studies by Barlow and Levick (1965) suggested that a mixture of excitation and asymmetric,

Figure 14. A nondirectional cell in which baclofen alone produced a weak directionality, which became more pronounced with the addition of APB (third row). Treatment with picrotoxin and strychnine, while still in the presence of baclofen and APB, suppressed this induced directionality.

hyperpolarizing inhibition produces directional responses. In amphibia, similar observations were made by Werblin (1970). There has been extensive modeling of this mechanism (e.g., Torre and Poggio, 1978; Koch et al., 1983). Marchiafava (1979), experimenting in turtle retina, found that null-direction movement evoked small depolarizations, at least part of which was an inhibitory potential, suggesting the importance of silent inhibition. Watanabe and Murakami (1984) found that directional cells in the frog retina arose from at least two different synaptic mechanisms. They reported that ON-OFF cells showed a hyperpolarizing response in the null direction, but ON cells and OFF cells had no inhibitory responses to null-direction movement. They suggested that the timing of excitatory potentials might regulate direction coding, somewhat analogous to the low-pass-filter model in the fly (Reichardt, 1969; Poggio and Reichardt, 1973). In our studies, the spectrum of null-direction responses and the differences in inhibitory amino acid pharmacology also suggest that several mechanisms may contribute to formation of directional signals.

Our results indicate that many different inputs impinge on third-order neurons and that baclofen can change the weighting of these various synaptic inputs. The concept that cells may receive inputs from multiple modalities and use a filtering process to enhance one particular modality has analogies with the distributed network systems of parallel processors and models of neural networks such as one proposed by Hopfield and Tank (1986). These models suggest that changes in synaptic weighting of the input modalities can be used to alter the form of the output signal. A similar effect appears to occur in the retina,

Figure 15. Induced orientation responses of a third-order neuron to moving 100- μ m-wide slits, similar to the paradigm in Figure 1. When baclofen was applied (*b*), the cell responded much more selectively to bars moving along the vertical axis compared to control (*a*). The solid line in the center of *b* is a polar plot of peak response in the presence of baclofen; dotted lines represent control responses. When APB was added to the baclofen (*c*), the responses became even more narrowly tuned.

where baclofen increases the synaptic weighting of transient signals and decreases the synaptic weighting of sustained signals. One consequence of this altered synaptic weighting is the relatively enhanced transmission of directional information. DeVoe et al. (1985, 1989) have found directional properties in bipolar, horizontal, and amacrine cells, indicating that this information may be distributed broadly to include the early layers of the retina as well as our contention that it is diffusely distributed in the inner retina.

This distributed system model contrasts with the well-established model of neuronal specialization and signal decomposition that states that particular groups of cells have dedicated

functions and are hard-wired to carry one part of the overall signal. Many examples of this principle can be found in the visual system, including the color system, the rod-cone system, the ON-OFF system, and the linear/nonlinear ganglion cell system. In cat retina, the linear/nonlinear physiological separation (X- vs. Y-cells; Enroth-Cugell and Robson, 1966) corresponds to a morphological separation (β vs. α ; Boycott and Wässle, 1974), indicating that this is a fundamental dichotomy in the retina. In the monkey retina, a similar division is found between P- and M-ganglion cells (Shapley and Perry, 1986). It is unclear whether the amphibian retina establishes a strict linear/nonlinear system similar to that found in the mammals, but it is clear

that even in the cat the temporal response characteristics of X- and Y-cells are variable and depend on ambient conditions, such as light adaption. Our observation that baclofen makes cell responses more transient suggests that there is some flexibility between sustained and transient responses. The implication is that there is a basic hard-wired anatomical system upon which neurotransmitter regulation is superimposed. The actions of baclofen suggest that neurotransmitters can alter the influence of this wiring diagram not only by eliminating a pathway through inhibition, but also by modifying the relative influence of the synaptic inputs.

The interplay between the ON and OFF systems in the generation of directional selectivity is an intriguing but unresolved factor in this study. In most cells, the baclofen-induced directionality was enhanced when APB blocked the ON system. In some cells, where baclofen clearly induced directionality in the OFF pathway, blocking the ON pathway enhanced this OFF directionality. In some other cells showing baclofen-induced directionality, APB blocked this directionality, suggesting that in these cases the induced directionality was in the ON pathway. Using a wide bar, we could show a few instances where the ON and OFF pathways were not aligned. Presumably this lack of alignment negated directionality unless the ON pathway was eliminated by APB.

The wide bar experiments did not duplicate the effects of APB, indicating that detection of a moving edge is influenced by neuronal activity away from that edge. Sakai and Naka (1988, 1990a,b) have found widespread sign-conserving interactions within the ON or OFF pathways and also sign-inverting interactions between ON and OFF systems in the catfish retina. They reported that OFF cells are connected to other OFF cells, providing a positive-feedback system, while ON cells provide a negative feedback to suppress OFF cells. Therefore, elimination of the ON system would tend to enhance the OFF system. Recent reports that OFF ganglion cell activity is augmented after APB (Massey et al., 1983; Wässle et al., 1986; Arkin and Miller, 1987) are consistent with this mechanism. It appears that APB's effects on directional responses may be the result of a number of factors, two of which may be that (1) the directional preference of the ON and OFF inputs may not be in register, and (2) directional responses due to excitation in the OFF pathway may be suppressed by activity in the ON pathway.

This interference between ON and OFF systems may not create as much interference for the cell as it does for our recordings. The ON and OFF sublamina are anatomically separate, so that in many cells local processing of the ON and OFF inputs will be discrete. On the other hand, our recordings are from the soma, which is looking at the combined ON and OFF inputs. Therefore, there may be local directional processing in each sublamina that is equivalent to what we see when the ON sublamina is eliminated by APB. In fact, we found two cells in which APB alone revealed a directional response in the OFF pathway, suggesting that in these cells the OFF pathway was normally directional, but that this was obscured by ON pathway activity.

The effects we observed were all produced by the exogenous application of pharmacological agents and not by stimulating synaptic mechanisms. Is this effect artifactual or relevant to the functional capabilities of the retina? There is a diffuse GABAergic innervation of the inner plexiform layer, and most amacrine and ganglion cells have GABA_B receptors, indicating that the endogenous activation of the GABA_B pathway in the cells

we studied is feasible. In most of our experiments, APB was required to see induced directionality, and clearly APB is not released in the retina. However, the sublamination in the inner retina results in an anatomical and physiological separation of ON and OFF inputs to third-order neurons (Famiglietti and Kolb, 1976). Therefore, at the level of individual dendrites, the GABA_B-modulated inputs can act independently on the ON and OFF pathways. This ON/OFF separation may also be a significant property of normal directional selectivity. For example, the cholinergic amacrine cells, which are involved in processing directional information (Ariel and Daw, 1982; Ariel and Adolph, 1985), are unistratified and divided into ON and OFF cell types (Masland and Mills, 1979; Masland et al., 1984; Tauchi and Masland, 1984; Massey and Redburn, 1985). This separation of ON and OFF signals may be important in the generation of normal directionality, even though directional ganglion cells are most often ON-OFF cells (Oyster, 1968). Based on the presence of GABA_B receptors, the preponderance of GABA-releasing neurons in the inner plexiform layer, and the morphological separation of ON and OFF synaptic zones, it seems feasible that the normal retina is capable of duplicating our experimental manipulations of synaptic weighting and modifications of information processing.

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