Nitric Oxide Differentially Modulates On and Off Responses of Retinal Ganglion Cells

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Running Title: NO modulates visual responses of ganglion cells

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ABSTRACT
Several lines of evidence suggest that nitric oxide (NO) can regulate diverse retinal functions, but whether this gas is capable of modulating the visual responses of retinal output neurons has not been established. In the present study, the effects of NO on rod-driven responses of retinal ganglion cells were tested by making whole-cell patch-clamp recordings from morphologically identified ganglion cells in the isolated ferret retina. Bath application of L-arginine, the substrate of nitric oxide synthase, and SNAP, the NO donor, was found to differentially affect On and Off discharge patterns. The introduction of these drugs significantly decreased visual responses of retinal ganglion cells, but the effects were more pronounced on Off than on On discharges. The peak discharge rates of On responses were usually reduced by about 40%, but not completely blocked. In contrast, Off responses were completely blocked in most cells. These differential effects were observed in On-Off cells as well as in cells that yielded just On or Off discharges. The Off responses that were blocked by NO were also blocked by APB and strychnine, suggesting the involvement of the APB-sensitive rod pathway.
INTRODUCTION
Nitric oxide (NO), a gas with a half life of a few seconds (Snyder & Bredt, 1992), has been implicated in diverse biological functions, including control of information processing in the visual system (Cudeiro and Rivadulla, 1999; Goldstein et al., 1996). In the retina, nitric oxide synthase (NOS), the enzyme synthesizing this messenger molecule (Marletta, 1994), has been localized to several different cell types, including pigment epithelium cells (Bredt et al., 1990; Goureau et al., 1993), Müller cells (Liepe et al., 1994), amacrine and ganglion cells (Dawson et al., 1991; Koistinaho et al., 1993; Yamamoto et al., 1993a) as well as photoreceptors (Yamamoto et al., 1993b).

There is also evidence that NO may play a role in the physiological regulation of diverse retinal functions, ranging from transduction to the gating of the output signal. For instance, NO has been reported to modulate voltage-gated ion channels in rods and cyclic-nucleotide-gated channels in both rods and cones (Kurenny et al., 1994; Rieke & Schwartz, 1994; Savchenko et al., 1997). This is in line with the finding that light stimulation induces NO release in the retina, but the cell types involved in such light-induced release are yet to be determined (Neal et al., 1998). NO has also been found to decrease electrical coupling in horizontal cells (DeVries & Schwartz, 1989; Miyachi et al., 1990), and to modulate cGMP levels in bipolar cells (Nawy & Jahr, 1990, 1991; Shiells & Falk, 1990), and to reduce coupling between AII amacrine cells and On-cone bipolar cells (Mills and Massey, 1995). In ganglion cells, NO donors have been shown to modulate cGMP-gated conductances (Ahmad et al., 1994; Kawa and Sterling, 2002), and to increase the amplitude of N-type calcium currents (Hirooka et al., 2000).
In view of the rather extensive literature documenting the myriad effects of NO on membrane properties of retinal cells, it is surprising that little is known yet about the effects of this gas on the light-evoked responses of ganglion cells, the output neurons of the retina. Studies that have relied on electroretinogram (ERG) and compound action potential recordings from the optic nerve during application of NO-related compounds reveal that this gas can modulate retinal activity (Goldstein et al., 1996; Maynard et al., 1995), but it remains to be established how NO affects the responses of individual ganglion cells.

In the present study we sought to answer three main questions. First, does NO affect the visual responses of retinal ganglion cells in the dark adapted retina? Second, can the effects of NO be related to a specific class of retinal ganglion cells defined on the basis of salient morphological properties? And third, is the influence of NO on light responses of ganglion cells mediated by specific rod driven pathways? To address these issues we relied on whole-cell patch-clamp recordings from morphologically identified retinal ganglion cells in the isolated retinal preparation. Our results indicate that NO acts to dampen the responses of all three major classes of ganglion cells in the ferret retina. Moreover, we show that Off responses are more sensitive to NO modulation than On responses. We also demonstrate that the blockade of responses by NO to light offset is mediated by the APB-sensitive Off retinal pathway, suggesting that inhibition of glycinergic synapses may be involved in this phenomenon.
MATERIALS AND METHODS

All procedures were in compliance with National Institutes of Health guidelines and were approved by the campus animal use committee. Animals were dark-adapted overnight prior to the experiments and all procedures, including animal surgery, dissection of retinas, and recordings from cells were made in complete darkness. Infrared goggles were used to visualize the tissue on the dissecting and recording microscopes and to maneuver in the recording room. LEDs (850 nm) were used to provide light to the dissecting microscope while the illumination from the recording microscope was passed through an ≥850 nm cut-filter.

Retinal preparation. Retinas were obtained from ferrets (Marshall Farm USA, North Rose, NY) ranging in age from postnatal day 35 (P35) to P55, with the day of birth denoted as P0. Following a lethal dose of barbiturate (Nembutal 200 mg/kg i.p.), the eyes were removed and placed in oxygenated L15 at 37°C for 12 min. The retinas were then carefully peeled from the eyecup and stored at room temperature in Eagle’s minimal essential medium (EMEM), continuously bubbled with 95% O₂ and 5% CO₂. A small piece of retina was placed ganglion cell layer up in the recording chamber and stabilized with an overlying piece of filter paper. A 2 mm hole in the filter paper provided access for the recording electrode. Cells were visualized through a 40x objective mounted on a upright epifluorescence microscope (Nikon).

During recordings the retina was perfused continuously with EMEM (1.5 ml/min) through a gravity fed line, heated with a Peltier device, and
continuously bubbled with 95% O₂ and 5% CO₂. A calibrated thermocouple monitored the temperature in the recording chamber, maintained at 35°C. Recordings from individual cells usually lasted 30-120 minutes, and retinal segments from which recording were made typically remained viable for 8-12h. Patch electrodes were filled with a solution containing (in mM): KCl, 140; HEPES, 10; EGTA, 0.5; 0.5 mg/ml Nystatin; 2.5 mg/ml Pluronic F-68; 0.5 % Lucifer Yellow; pH 7.4. By the end of the experiment the soma and the dendritic arborizations were usually completely filled, suggesting that recordings were made in the whole-cell configuration. Once complete filling was achieved, the retina was removed and fixed in 4% paraformaldehyde for 6-8 hours at 4°C.

*Electrophysiology.* Patch pipettes with a tip resistance between 3 and 7 MΩ were pulled from thick-walled 1.5 mm-OD boroscillate glass on a Sutter Instruments puller (P-97). Current-clamp recordings were made with an Axopatch 200B patch-clamp amplifier. The data were low-pass filtered at rates between 1 and 2 kHz and digitized at rates between 1 and 4 kHz before storage on a computer for subsequent off-line analysis. To attain whole cell access, vitreous and the outer limiting membrane overlying the recording area were removed by gently brushing the retinal surface with the tip of a glass pipette. Recordings were obtained by patching onto cells with clear, non-granular cytoplasm. High-resistance seals were obtained by moving the patch electrode onto the cell membrane and applying gentle suction. After formation of a high-resistance seal between the electrode and the cell membrane, transient currents caused by pipette capacitance were electronically compensated by the circuit of the Axopatch 200B amplifier. If the seal resistance dropped below 1 GΩ during the recording the
experiment was terminated. The series resistance was 7-16 MΩ. After attaining whole-cell configuration the resting membrane potential was read off the amplifier. The value of the resting potential was monitored regularly throughout the recording, and if significant changes were observed, the recording was terminated.

*Light stimulus.* Light evoked responses were obtained by delivering spots of light from three computer controlled LEDs (having $\lambda_d = 463, 569, 651\text{nm}$) through the camera port of the microscope. Spectral power was measured with a silicon photodiode and linear readout system (United Detector Technology, 81 Optometer) and a spectroradiometer/photometer (Photo Research, PR703-A) scanning from 390 to 720 nm in 2 nm steps. These instruments were calibrated relative to standards of the National Institute of Standards and Technology. The number of quanta delivered to the retina was specified per receptor per second, assuming that the inner segments form the optical aperture, based on a diameter of 0.315 $\mu\text{m}$ (Weidman and Greiner, 1984). The photoreceptors were stimulated at very low intensities (9-174 quanta per receptor per second), in the scotopic range based upon reasonable assumptions available from other species (Soucy et al., 1998).

*Drug application.* L-arginine (Sigma, 1 or 5 mM), D-arginine (Sigma, 1 or 5 mM), S-nitroso-N-acetylpenicillamine (SNAP, Sigma, 1 mM, Hirooka et al., 2000), strychnine (Sigma, 50 $\mu\text{M}$), and DL-2-amino-phosphonobutyric acid (APB, Calbiochem, 50 $\mu\text{M}$) were freshly dissolved in EMEM on the day of the experiment and administered through a gravity fed line. The solutions were heated with a Peltier device and continuously bubbled with 95% $\text{O}_2$ and 5% $\text{CO}_2$. A six-position rotary valve (Western Analytical
Products) was used to switch between bath and drugs solutions. The washout time for chemicals usually took 6 to 20 minutes.

*Morphological analysis.* Recorded cells were visualized and identified as ganglion cells before the electrode was withdrawn, and only neurons unequivocally identified as retinal ganglion cells (Wingate et al., 1992) were included in this study. Images of the labeled cells were taken in the wholemount configuration with an upright Nikon Eclipse E600 microscope equipped with epifluorescence or a BioRad MRC-1024ES confocal microscope using a computerized imaging system (Bio Rad CoMOS, version 7.0), and cell class was determined from these images.
RESULTS
The results are based on successful recordings from a total of 73 retinal ganglion cells that were studied under different conditions described in the sections below.

Effects of NO on light-evoked responses of retinal ganglion cells
Recordings were made from 55 cells to flashing spots of light, before, during and after addition of either D-arginine or L-arginine to the bath solution. In every instance, when D-arginine, the biologically inactive stereo-isomer of arginine was applied to the bath solution, there was no appreciable change in the visual responses. By contrast, the visual responses of all but a few ganglion cells (47 of 55) were affected by L-arginine application. In all cases, the introduction of L-arginine, the precursor of NO, produced a decreased responsiveness in the light evoked responses, but the magnitude of this effect differed for On and Off discharges.

In the majority of neurons that responded only to light onset (n = 37), L-arginine was found to significantly decrease On discharges (29 of 37). Two examples of this effect are shown in Figure 1. One of these cells responded to light with a transient discharge (Fig 1A), while the other yielded a more sustained response (Fig 1B). Note that in both cases, there was a significant decrease in responsiveness after application of the L-arginine and that the light-evoked responses remained stable after D-arginine application.

Figure 2 (left side of panel) shows the magnitude of this effect expressed in terms of the average peak firing rate at onset of light stimulation. The average peak firing rate was calculated by counting the number of spikes within a window that encompassed the highest firing rate
and dividing the spike number by the duration of the window. As may be seen, L-arginine decreased the peak firing rate by about 40% from 206.3 ±19.3 to 120 ±23.8 spikes/sec (mean ±SE, n=29, p<0.05, two-tailed t-test).

Most cells manifested spontaneous activity (i.e., action potentials not evoked by the light), and L-arginine usually reduced the frequency of these discharges (Fig. 2, right side of the panel). For the overall sample, this effect was significant (28.2 ±3.3 to 18.6 ±1.1 spikes/sec; mean ±SE, n=29, p<0.05, two tailed t-test). The reduced discharges observed to light onset, however, were not always associated with a decrease in spontaneous activity levels since in some cells (n=5) L-arginine caused a decrease in light-evoked responses without affecting spontaneous activity.

Application of L-arginine affected the responses of all cells that yielded only Off discharges. In most cases (5 of 8) the introduction of this drug completely blocked Off responses as shown in Figure 3A. In the three remaining cells, Off responses were reduced in peak firing frequency by 22.5%, 36.7%, and 43.1 % of their controls (the peak firing frequency in bath solution).

The differential effect of L-arginine on On and Off discharges was also apparent in the 10 ganglion cells that responded to both light onset and offset (Fig. 3B). In this sample, Off responses were completely blocked by L-arginine in 6 cases and decreased in 4 others (by 27.7%, 33.2%, 48.2, and 52.6% of control values). By contrast, responses to light onset were decreased in 8 cases (ranging from 22.3% to 54.2%) and unaffected in 2 other cells (<5% of the control values). In no case were On responses completely blocked by NO.

We typically used a concentration of L-arginine of 1 mM, although in a number of instances we also tested the effects of a 5 mM concentration.
There was no indication that the differential effects described above on On and Off discharges were dependent on the concentration of L-arginine. This may be seen in Figure 4A which shows the percentage of On and Off responses that were either not changed appreciably (i.e., within 5% of control values), reduced in peak firing frequency (ranging from 11% to 70% of control values) or completely blocked (responses inhibited 100%) following either 1 or 5 mM application of L-arginine. Note that none of the On responses were blocked when the higher concentration of L-arginine was applied (depicted with hashed bars in Fig. 4A) and that 44.4% of the Off responses were completely blocked at the lower concentration. Thus, it seems unlikely that the differential effects of L-arginine on On and Off responses of retinal ganglion cells reflect a drug dosage effect. Figure 4B illustrates the distributions of On and Off responses with respect to the degree of response inhibition observed after L-arginine application. The On and Off responses presented in Figure 4A&B include all responses from On cells, Off cells, and On-Off cells that were tested with L-arginine.

We also employed an alternate means for increasing NO production by introducing into the bath solution the NO donor SNAP. A total of 7 cells were tested (4 On and 3 Off). SNAP caused a decrease in the responses of 4 On cells (ranging from 19.2% to 53.4%) and the complete block of Off responses in 2 cells, with 1 Off cell showing a response decrease (38.5%). Figure 5 illustrates the effect of SNAP application for an On (Figure 5A) and an Off cell (Figure 5B). As was the case with L-arginine, SNAP application had a more pronounced effect on the Off pathway.

The resting membrane potential of the cells we recorded was -57.4±4.6 mV (n=73). In most cells, there was no appreciable change in the membrane potential following application of L-arginine or SNAP (61 of 73).
In some cells (12 of 73), the membrane potential initially become more hyperpolarized (by 2-5 mV at its peak) followed by a period of depolarization. The effects of the light-evoked responses did not appear to be related to these effects on the membrane potential of the recorded cells.

*Morphologically Identified Cell Classes*

The cells from which recordings were made were filled with Lucifer yellow, allowing us to establish their morphological class (Figure 6). This revealed that NO influenced the visual responses of all three major cell classes found in the ferret retina. The percentage of alpha, beta, and gamma cells affected by NO was 75% (6 of 8), 88.5% (23 of 26), and 75% (9 of 12), respectively. (Note that the number of filled cells from which recordings were made is lower than the overall sample because in some cases we could not recover the labeled neurons, although these were identified as ganglion cells by the presence of a labeled axon before the electrode was removed.) The 8 cells insensitive to NO were encountered in experiments in which other neurons from the same retina were found to be sensitive to this gas. We could not distinguish these neurons from the overall sample on the basis of their salient morphological properties.

*Effects of NO on current-evoked responses of retinal ganglion cells*

The effects of NO on light-evoked responses of retinal ganglion cells could be mediated by presynaptic and/or postsynaptic mechanisms. To assess the possibility that NO could influence the visual responses of retinal ganglion cells by affecting the excitable membrane properties of these
neurons, we examined the effects of NO on current-evoked responses. In all but a few cases, responses to direct current injections were not affected (25 out of 30), even though the visual responses of these neurons were altered by NO. In this sample, 19 were On cells, 5 were Off cells, and 6 were On-Off cells. In the majority of On cells (14 of 19) the visual responses were decreased by L-arginine (ranging from 18.4% to 58.5%), and for 2 of the 14 cells firing patterns to current injection changed after L-arginine application from a maintained to a rapidly adapting pattern (Figure 7). For a given cell, several current levels were used (typically in 10 steps, ranging from 10 to 180 pA) and the rapidly adapting feature was evident at several different current levels, indicating the robustness of the effect.

All Off cells were affected by NO (4 had completely blocked responses and 1 was reduced by 36.7% of control value), with the current-evoked firing pattern changing from maintained to rapidly adapting in 2 of the blocked cells. For 6 On-Off cells, the On responses were reduced in 5 cases (ranging from 29.7% to 47.6%), while the Off responses were blocked in 4 cases and reduced in 2 others (by 27.7% and 48.2%, respectively). In one of the On-Off cells the current evoked firing pattern was altered by L-arginine in the manner described above. In this neuron the On response was decreased and the Off response was blocked by application of the drug.

If changes in excitable membrane properties are involved in mediating the effects of NO on the visual responses of retinal ganglion cells, then the discharge patterns evoked by current injection should change after NO application in these neurons. For the vast majority of cells this was not the case; there was no clear relation between the effects of NO on light-
evoked responses and current-evoked responses. The fact that NO changed current-evoked discharge patterns of some ganglion cells suggests that these neurons might be a functionally distinct subgroup with membrane excitability sensitive to NO. This may relate to the fact that some ganglion cells with their dendrites arborizing in On and Off layers of IPL show increased cGMP in response to NO donors (Blute et al., 1998).

NO blocks the APB-sensitive Off retinal pathway

In the dark-adapted retina Off responses in retinal ganglion cells can be mediated by two different pathways. One of these is APB-sensitive and involves rod bipolar cells and glycinergic synapses between AII amacrine cells and Off-cone bipolar cells (Sharpe and Stockman, 1999). In this pathway, APB hyperpolarizes rod bipolar cells, thereby preventing their release of glutamate (Slaughter and Miller, 1981; Bolz et al., 1984; Müller et al., 1988). The other Off retinal pathway is APB-resistant and involves a direct input from rods to Off-cone bipolar cells (Soucy et al., 1998; Hack et al., 1999; Wang et al., 2001). The presynaptic modulation of Off responses in retinal ganglion cells could be mediated by one or both of these pathways. In order to establish the retinal circuitry underlying the blockade of Off responses by NO, we compared the effects of NO, strychnine, a glycinergic receptor blocker, and APB. SNAP, strychnine and APB all were found to block Off responses in seven of eleven cells tested (see Figure 8). In the other four Off cells studied, APB, strychnine and SNAP all failed to abolish Off responses (see Figure 9). Collectively, our data suggest that in the dark-adapted retina total blockade of ganglion cell Off responses by NO is
mediated by the APB-sensitive rod pathway, while the APB-insensitive pathway is involved in the reduction of Off responses.
DISCUSSION

The results of the present study demonstrate that NO can modulate the visual responses of all three major classes of ganglion cells (alpha, beta and gamma) in the ferret retina. In all cases, the effect of NO is to dampen the visual responses to flashed stimuli, with the Off pathway being more sensitive to NO modulation. These findings provide the first direct evidence that NO is capable of influencing the responses of individual ganglion cells, thereby affecting the transfer of information from the retina to higher levels of the visual system.

It should be emphasized that the results cannot be due to non-specific or toxic effects of the drugs we employed. This claim is supported by the fact that D-arginine, the biologically inactive stereo-isomer of arginine, had no effect on visual responses. Both L-arginine and SNAP yielded similar results, and increasing the dosage of L-arginine from 1 to 5 mM did not change the differential sensitivity of On and Off pathways. Perhaps, the most compelling demonstration of the specificity of the effects is evident in those cases where NO differentially affected the On and Off responses within a single On-Off cell.

*Possible sites of action for NO modulation of visual responses*

In studies using dissociated cells NO has been shown to affect channel properties of photoreceptors (Kurenny et al., 1994; Rieke & Schwartz, 1994; Savchenko et al., 1997), horizontal cells (DeVries & Schwartz, 1989; Miyachi et al., 1990), bipolar cells (Nawy & Jahr, 1990, 1991; Shiells & Falk, 1990), and ganglion cells (Ahmad et al., 1994; Kawa and Sterling,
2002; Hirooka et al., 2000). NO donors have also been shown to increase cGMP in a variety of retinal cells including photoreceptors, horizontal cells, different types of amacrine cells, bipolar cells, as well as ganglion cells (Blute et al., 1998; Baldridge and Fischer, 2001). Thus, multiple action sites could contribute to the effects of NO on visual responses of ganglion cells.

While the precise site of NO action on the visual responses of ganglion cells remains to be established, our results indicate that this gas affects the intrinsic membrane properties of relatively a small proportion of these neurons. The current-evoked discharges of only a few ganglion cells were influenced by NO. These observations imply that there may be a population of neurons with membrane properties that are NO sensitive within the major three cell classes defined on the basis of their salient morphometric properties. A subgroup of alpha ganglion cells has been previously documented on the basis of somatostatin immunoreactivity (White and Chalupa, 1991). It also seems unlikely that the differential effects of NO on On and Off responses could reflect photoreceptor action. Thus, the primary influence of NO appears to be on retinal circuitry presynaptic to ganglion cells.

One or both of two retinal Off pathways could have been involved in mediating the effects of NO on Off ganglion cell responses: an APB sensitive pathway and an APB resistant pathway. The APB sensitive pathway transmits rod signals via rod bipolar cells to AII amacrine cells, then to Off-cone bipolar cells by glycinergic synapses, which innervate the dendrites of Off ganglion cells (Sharpe and Stockman, 1999; Soucy et al., 1998). APB blocks this pathway by hyperpolarizing rod bipolar cells.
Strychnine, the glycinergic receptor blocker, also blocks this pathway by inhibiting glycinergic synapses (Müller et al., 1988). Recently, an APB resistant Off pathway has been discovered (Soucy et al., 1998; Hack et al., 1999). This involves rod photoreceptors that synapse directly on Off-cone bipolar cells, which in turn connect with Off ganglion cells. We have shown that this pathway is functional in the dark-adapted ferret retina (Wang et al., 2001). There is another APB resistant pathway that involves the gap-junction between rod and cone, then to cone bipolar cells. This pathway has been reported operating at mesopic rather than scotopic intensities (Daw et al., 1990; Smith et al., 1986; Steinberg, 1969). Whether or not this pathway is functional in the ferret retina under scotopic intensities remain to be established. Since NO inhibits glycine release in the retina (Neal et al., 1997), our results can be taken to suggest that the inhibition of glycinergic synapses could be the mechanism by which NO blocks ganglion cell Off responses. Other sites of action could also be involved. For instance, NO could decrease endogenous dopamine release in the retina via a cGMP-independent mechanism (Bugnon et al., 1994). Consistent with this notion, dopamine has been found to enhance the responses of Off cone bipolar cells (Maguire & Werblin, 1994).

We have not attempted to establish the sites of NO action on On ganglion cell responses, but the available literature suggests several possibilities that would be worth pursuing in future studies. For example, NO has been reported to decouple gap junctions in the retina (DeVries & Schwartz, 1989; Miyachi et al., 1990), and in particular, NO has been shown to effectively reduce coupling between AII amacrine cells and On-cone bipolar cells (Mills and Massey, 1995). Another target of NO, the cGMP-
gated channel, has been localized to On bipolar cells (Nawy & Jahr, 1990, 1991; Shiells & Falk, 1990), but this serves to increase light responses in On bipolar cells so it is unlikely to play a role in the reduction we observed in ganglion cell light responses.

Role of retinal NO in visual information processing

The results of the present study demonstrate that NO can provide a powerful effect on the visual responses of retinal output neurons, especially to light offset. In all but a small proportion of ganglion cells (86%) NO dampens visual responses, and in many cases Off discharges were completely blocked by the actions of this gas. By contrast, in the dorsal lateral geniculate nucleus, blocking NO synthesis has been reported to reduce the visual responses of virtually all X and Y cells (Cudeiro et al., 1994). This effect could be reversed by application of L-arginine, but L-arginine administration alone had no appreciable effect on visual responses. In the cortex, manipulations of the NO system have been reported to produce reductions as well as enhancements of visual responses (Cudiero et al., 1997). Thus, the available evidence indicates that NO can modulate visual responses in different ways along the retino-geniculo-cortical pathway. The significance of such diverse modulation by NO to the processing of visual information remains to be established.

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REFERENCES


**Figure legends:**

Figure 1. Whole-cell current-clamp recordings from two retinal ganglion cells in the dark-adapted ferret retina. Light-evoked responses were obtained by delivering spots of light through the camera port of the microscope. Both cells responded only to light onset. L-arginine (5 mM) reduced the On responses of both cells, while D-arginine (5 mM) had no effect on such responses.

Figure 2. The average peak firing rates and the spontaneous firing rates of On cells before and after bath application of D-arginine and L-arginine. L-arginine significantly reduced both average peak firing rates of On responses and the spontaneous firing rates for On cells (* p<0.05, two-tailed t test). D-arginine did not change either average peak firing rates or the spontaneous firing rates.

Figure 3. Effects of L-arginine on Off responses of Off cells and On-Off cells. In A: light-evoked responses were recorded from an Off ganglion cell in the dark-adapted retina. Bath application of L-arginine (1 mM) completely abolished the Off response. The Off response recovered after L-arginine was washed out. In B: responses of an On-Off ganglion cell in the dark-adapted retina. Application of L-arginine (1 mM) selectively blocked the Off response. This effect was reversed after the drug was washed out. D-arginine application had no effect.

Figure 4A. The percentage of On and Off responses that with L-arginine application (i) did not change, (ii) had a reduced peak firing rate or (iii) had completely blocked responses. The On and Off responses presented here
include all responses from On cells, Off cells, and On-Off cells. The numbers at the top of the bars indicate the fraction of responses in each group. The proportion of cells exposed to 5 mM L-arginine is indicated by the height of the hatched bar within each group. Note that none of the On responses were blocked by either 1 or 5 mM L-arginine application, while 61% of Off responses were completely blocked by this chemical at both concentrations of this drug (17% tested with 5mM L-arginine and 44% tested with 1mM L-arginine). Figure 4B shows the number of On and Off responses as a function of percent reduction in peak firing rate after L-arginine application. These values are expressed as a percentage of control responses, recorded in bath solution. Thus 100% denotes complete blockade of visual responses.

Figure 5. The NO donor, SNAP, had effects similar to those of L-arginine on light-evoked responses of retinal ganglion cells. A: SNAP (1mM) reduced the On response of an On cell. B: SNAP (1mM) blocked the Off response of an Off cell.

Figure 6. The figure shows examples of Lucifer yellow filled retinal ganglion cells (alpha, top; beta, middle & gamma, bottom) from which recordings were made. The cells were from P38, P41 and P47 ferrets, respectively.

Figure 7. The effects of L-arginine and D-arginine on current-evoked responses of an On retinal ganglion cell. L-arginine (1 mM), the biologically active form of arginine, changed the firing pattern of the current-evoked response from sustained to rapidly adapting. In contrast, D-arginine (1 mM), the biological inactive form of arginine, had no effect on
the firing pattern of the current-evoked response. The holding potential was –70 mV, and the current injected was 100 pA.

Figure 8. Light-evoked responses of an Off cell. The Off response was blocked by bath application of NO donor, SNAP. The response recovered after switching back to normal bath solution. The Off response of the same cell was also blocked by strychnine and APB applications. The Off responses recovered after the drugs were removed from the bath.

Figure 9. Light-evoked responses of an Off cell. The Off response was not blocked by SNAP application. The Off response from the same cell was also insensitive to strychnine or APB applications.
Figure 1 Top

A  On  Off  B  On  Off

bath solution

d-arginine

l-arginine

bath solution

30 mV

2 sec
Figure 2 Top

![Bar Chart]

- **Peak Firing Rate**
  - Bath solution
  - d-arginine
  - l-arginine
  - n=29, n=19

- **Spontaneous Firing Rate**
  - n=24, n=19
Figure 3 Top

A

On

Off

B

On

Off

bath solution

d-arginine

l-arginine

bath solution

30 mV

2 sec
Figure 4  Top

A

On responses (L-arginine 1 mM)
Off responses (L-arginine 1 mM)
L-arginine 5 mM

percentage

no change  reduced  blocked

10/47  37/47  11/18

B

On responses
Off responses

number of responses

magnitude of L-arginine inhibition
Figure 5 Top

A  On  Off  B  On  Off

bath solution

SNAP

bath solution

30 mV

2 sec
Figure 6 Top
Figure 7 Top
Figure 8 Top

On

Off

bath solution

SNAP

bath solution

strychnine

bath solution

APB

bath solution

30 mV

2 sec
Figure 9 Top

On

Off

bath solution

SNAP

strychnine

APB

30 mV

2 sec