Reciprocal modulation of calcium dynamics at rod and cone photoreceptor synapses by nitric oxide

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Abstract

The abundance of nitric oxide (NO) synthesizing enzymes identified in the vertebrate retina highlight the importance of NO as a signaling molecule in this tissue. Here we describe opposing actions of NO on the rod and cone photoreceptor synapse. Depolarization-induced increases of calcium concentration in rods and cones were enhanced and inhibited, respectively, by the NO donor S-nitrosocysteine. NO suppressed calcium current in cones by decreasing the maximum conductance, whereas NO facilitated rod Ca channel activation. NO also activated a non-selective voltage-independent conductance in both rods and cones. Suppression of NO production in the intact retina with N^G-nitro-L-arginine favored cone over rod driven postsynaptic signals, as would be expected if NO enhanced rod and suppressed cone synaptic activity. These findings may imply involvement of NO in regulating the strength of rod and cone pathways in the retina during different states of adaptation.

Nitric oxide (NO) has emerged as an important signaling molecule in the vertebrate retina. Immunocytochemical, immunohistochemical and histochemical (NADPH-diaphorase) methods have revealed that the isoforms of synthesizing enzymes for nitric oxide (NO), nitric oxide synthase (NOS), are abundant and found in almost all layers of the retina (Kelly and Barnes 1997; Haverkamp et al. 1999; Shin et al. 2000). NO has also been shown to affect a large and increasing number of different types of ion channels in retinal neurons (for review see Kelly and Barnes 1997). These include voltage-gated ion channels, ionotropic receptors and gap junction channels. A significant locus of neuronal NOS (nNOS) in the retina is within and around photoreceptors (Weiler and Kewitz 1993; Osborne et al. 1993; Liepe et al. 1994; Kurenny et al.
1994; Kurenni et al. 1995; Kelly and Barnes 1997) suggesting a role of NO in outer retinal function.

Synaptic transmission from photoreceptors to the second order, horizontal and bipolar, cells is triggered by voltage-dependent calcium entry through L-type Ca channels in rod and cone synaptic terminals (Maricq and Korenbrot 1988; Barnes and Hille 1989; Copenhagen and Jahr 1989; Lasater and Witkovsky 1991; Barnes et al. 1993). Calcium entry in turn induces calcium release from intracellular stores via processes that differ between rods and cones (Krizaj et al., 2003). Synaptic cGMP-gated channels, the same type of ion channels which normally are responsible for generating the light-modulated current in photoreceptor outer segments (Fesenko et al. 1985; Haynes et al. 1986; Yau and Baylor 1989), may participate in some aspects of synaptic transmission from cones (Rieke and Schwartz 1994). We showed previously that the NO donor S-nitrosocysteine (SNC) caused a facilitation of Ca channels in salamander rod photoreceptors (Kurenny et al. 1994), an effect due mainly to a shift of the activation curve towards negative potentials.

In the present work, we compared the effects of NO on Ca channels in rod and cone photoreceptors using calcium imaging, patch clamp, and intracellular recording methods. A computer model of the rod and cone responses was constructed to test the potential of the modulatory effects of NO on post-synaptic signals. Our findings show that NO exerts differential modulatory effects on the rod and cone signaling pathways in the vertebrate retina, which suggests that NO could play roles in light/dark adaptation and signal processing at this level of the visual system.

**Materials and methods**
Isolated cell preparation. In accordance with the CWRU IACUC, retinas were removed from larval tiger salamanders (*Ambystoma tigrinum*: Kons Scientific, Germantown, WI). Cells, isolated by trituration following treatment with papain (Sigma, St. Louis, MO), were constantly perfused with a saline solution (in mM: 90 NaCl, 2.5 KCl, 3 CaCl$_2$, 10 HEPES, 10 D-glucose (pH 7.6, NaOH; room temperature). SNC was prepared as described (Kurenny et al. 1994). All experiments were performed at room temperature (21-24°C).

Calcium imaging. Cells were loaded with fluo-3 by incubation in 5-10 µM fluo-3 AM ester and 0.01% Pluronic F-127 (Molecular Probes, Eugene, OR). To stimulate calcium entry, the superfusate was switched from 1 mM to 8-10 mM K$^+$ solution, which produces mild depolarization (~ –40 mV) and a small (5-10%) steady-state increase in fluorescence. Solutions with different [K$^+$] were based on the Ringer solution with adjustments in [Na$^+$] to maintain osmolarity. Measurement of fluorescence was made from the entire inner segment of both rods and cones, as synaptic terminals were not visible. Data are presented as mean ± SEM and compared using Student’s *t*-test.

Patch clamp recording. Ca channel currents were recorded in (mM) 70 NaCl, 2.5 KCl, 5 CsCl, 3 CaCl$_2$, 10 TEA-Cl, 20 HEPES, 10 D-glucose (pH 7.6, NaOH). Barium (5 mM) was also used in some experiments. Ruptured and perforated patch configurations were used. The patch pipette solution contained (mM): 100 CsCl, 3 MgCl$_2$, 1 EGTA, 1 ATP-Na$_2$, 10 HEPES (pH 7.2, CsOH). For perforated patch clamp recordings, 150 µg/ml nystatin was added to the pipette solution. Currents were recorded in response to a series of voltage steps between –80 and +40 mV in 5 mV increments. The amplitude of the current at the end of each step was plotted against the corresponding voltage (corrected for the series resistance error) to yield a current-voltage relationship. Leak subtraction of the current voltage relations was perfomed by subtracting a line.
fit to the relation between the voltages of ~80 and ~-45 mV. In Figure 2, both control and test I-Vs had the same leak subtracted in order to show that the leak conductance increased during SNC application. For analysis of modulation of Ca channel kinetic parameters, activation curves were constructed by dividing the leak-subtracted current-voltage data by a line representing the maximum conductance (linear fit to the data in the +10 to +20 mV range), and were fitted by the Boltzmann function $f=1/(1+\exp\{- (V-V_{1/2})/S\})$, where $V_{1/2}$ is the half activation potential and S is the slope factor. Non-selective NO-activated conductance measurements were made in a bath solution containing (mM): 62 NaCl, 2.5 KCl, 3 CaCl$_2$, 0.2 CdCl$_2$, 10 CsCl, 30 TEA-Cl, 10 HEPES, 8 D-glucose (pH 7.6, NaOH).

**Intracellular microelectrode recording in intact retina.** Retinal eyecups were prepared by dissecting away the cornea, lens and iris, and secured in a holder with suction. Continuous superfusion was established at a rate of 1 ml/min with a solution containing (in mM): 95 NaCl, 2.5 KCl, 3 CaCl$_2$, 1.5 MgCl$_2$, 30 NaHCO$_3$, 6 D-glucose, and bubbled with 95% O$_2$ and 5% CO$_2$ (pH 7.6). Following 1 hr of dark-adaptation, 150-200 MΩ microelectrodes filled with 3M potassium acetate plus 200 mM KCl recorded horizontal cell (HC) light responses. Voltage responses were recorded with a WPI M707 amplifier and digitized with Indec Systems hardware and Basic-Fastlab software. Light flashes were of 500 ms duration with un-attenuated intensity of $2 \cdot 10^{-7}$ µW/µm$^2$. Recordings were all obtained from individual HCs that were incubated in control or drug solutions for one hour prior to recording. N$^G$-nitro-L-arginine (NNA) was obtained from Calbiochem (San Diego, CA).

**Computer simulation of the light response.** The rod photoreceptor model was created using NEURON software (M. Hines and J.W. Moore, www.neuron.duke.edu or www.neuron.yale.edu) with a single-compartment design with leak conductance of 0.01 nS and
capacitance 16 pF. Conductances associated with voltage gated ion channels were: 1) for rods, Ca channels (Kurennyi et al. 1994; Kurennyi and Barnes 2000), non-inactivating potassium Kx channels (Kurennyi and Barnes 1994; Kurennyi and Barnes 1997), non-selective cation h channels (Malcolm et al. 2003), and delayed rectifier-like K channels (Kamiyama et al. 1996) were included, and 2) for cones, Ca channels (this work), h channels (Barnes and Hille 1989), delayed rectifier-like K channels, and calcium activated K and Cl channels (Kamiyama et al. 1996) were included.

A two-state kinetic scheme was used for Ca channels in rods and cones, and Kx and h channels in rods. In this scheme, the rate constants for activation (α_i) and deactivation (β_i) of the channel of type i were described by the equations: 

\[ \alpha_i = \alpha_0,i \exp\left\{ \frac{(V-V_{1/2,i})}{S_i} \right\} \] 

\[ \beta_i = \beta_0,i \exp\left\{ -\frac{(V-V_{1/2,i})}{S_i} \right\} \]

The activation (n_i) of the channels was numerically calculated from the equation \( \frac{dn_i}{dt} = \alpha_i (1-n_i) - \beta_i n_i \), and the ionic current was obtained as \( I_i = G_{max,i} n_i (V-E_i) \), where \( G_{max} \) is the maximum conductance and \( E \) is the reversal potential.

The model parameters for rods were: \( \alpha_{0,Ca} = 3.1 \), \( V_{1/2,Ca} = -20 \), \( S_{Ca} = 6 \), \( G_{max,Ca} = 1 \), \( E_{Ca} = 40 \); \( \alpha_{0,Kx} = 0.66 \), \( V_{1/2,Kx} = -49.9 \), \( S_{Kx} = 5.7 \), \( G_{max,Kx} = 0.85 \), \( E_{Kx} = -74 \); \( \alpha_{0,h} = 1.64 \), \( V_{1/2,h} = -82 \), \( S_{h} = -5.33 \), \( G_{max,h} = 1.41 \), \( E_{h} = -32 \). The parameters for cones were: \( \alpha_{0,Ca} = 3.1 \), \( V_{1/2,Ca} = -16.6 \), \( S_{Ca} = 5.7 \), \( G_{max,Ca} = 4.92 \), \( E_{Ca} = 40 \).

The cone h channel was modeled using the five state model (Barnes and Hille 1989):

\[ C_1 \leftrightarrow C_2 \leftrightarrow O_1 \leftrightarrow O_2 \leftrightarrow O_3 \]

where \( \alpha = 18/(1+\exp\{(V+88)/12\}) \) and \( \beta = 18/(1+\exp\{-(V+18)/19\}) \).

Because the four gating particles in this model are identical, the numerical calculations can be based on a first order equation for a single gating particle: \( dr/dt = \alpha_{h}(1-r) - \beta_{h} r \). The model assumes that any two particles in the open state would render the channel open. Thus, open probability
can be calculated as $n_h = 1 - (1 + 3r)(1 - r)^3$, and the h current as $I_h = G_{\text{max},h} n_h (V - E_h)$, where $G_{\text{max},h} = 3.5$, $E_h = -32.5$.

The delayed rectifier-like $K_V$ channel (Usui et al. 1999) was implemented in rods and cones using standard Hodgkin-Huxley formalism. The $K_V$ current was expressed as $I_{K_V} = G_{\text{max},K_V} (m_{K_V})^3 h_{K_V} (V - E_{K_V})$, where $dm_{K_V}/dt = \alpha_{m_{K_V}} (1 - m_{K_V}) - \beta_{m_{K_V}} m_{K_V}$ and $dh_{K_V}/dt = \alpha_{h_{K_V}} (1 - h_{K_V}) - \beta_{h_{K_V}} m_{K_V}$. The constants were: $\alpha_{m_{K_V}} = 5(V - 100)/(1 - \exp(-(V - 100)/42))$, $\beta_{m_{K_V}} = 9\exp((20 - V)/40)$, $\alpha_{h_{K_V}} = 0.15\exp(-V/22)$, $\beta_{h_{K_V}} = 0.4125/(1 + \exp((10 - V)/7))$, $G_{\text{max},K_V} = 2$, and $E_{K_V} = -80$.

The calcium activated $K$ current (Usui et al. 1999) was expressed with some modifications as $I_{K_C} = G_{\text{max},K_C} (m_{K_C})^3 h_{K_C} (V - E_{K_C})$, where $dm_{K_C}/dt = \alpha_{m_{K_C}} (1 - m_{K_C}) - \beta_{m_{K_C}} m_{K_C}$ and $h_{K_C} = 0.5 I_{Ca}/(0.3 + 0.5 I_{Ca})$. The kinetic constants were: $\alpha_{K_C} = 15(V - 80)/(1 - \exp(-(V - 80)/40))$ and $\beta_{K_C} = 20\exp(-V/35)$, $G_{\text{max},K_C} = 0.5$, and $E_{K_C} = -80$.

The calcium activated $Cl$ current (Usui et al. 1999) was expressed with modifications as $I_{Cl(Ca)} = G_{\text{max},Cl} m_{Cl} (V - E_{Cl})$, where $m_{Cl} = 1/(1 + \exp((0.37 - 0.5 I_{Ca})/0.09))$, $G_{\text{max},Cl} = 6.5$, and $E_{Cl} = -45$. The effects of NO were modelled by changing $V_{1/2}$ for rod $I_{Ca}$ (Kurenny et al., 1994) to $-24.3$ mV and by reducing cone $G_{\text{max},Ca}$ to 3.79 nS (this work).

**Results**

**NO donor SNC inhibits calcium entry into cones and enhances it in rods.** Our goal was to investigate how SNC affected calcium-dependent fluorescence in rods and cones. We used low concentrations of KCl (8 to 10 mM) to slightly depolarize the cells to the level expected in the dark-adapted state (near $-40$ mV) and activate Ca channels. Since Ca channels in photoreceptors
do not exhibit pronounced inactivation (Kurenny et al. 1994; Wilkinson and Barnes 1996; Kurennyi and Barnes 2000), steady levels of increased fluorescence due to calcium entry were seen. Application of SNC reversibly suppressed the fluorescence signal in cones and enhanced it in rods (Fig. 1). On average, 1 mM SNC suppressed fluorescence by $5.6 \pm 0.8\%$ (n=24) in cones, while 2 mM SNC increased the signal by $2.6 \pm 0.3\%$ in rods (n=21).

![Figure 1 near here]

Calcium imaging indicated that SNC suppressed Ca channels in cones, a modulation that stands in contrast to the facilitation seen in rods. Changes in calcium-dependent fluorescence in photoreceptors loaded with fluo-3 can be explained almost entirely by the activity of L-type voltage gated Ca channels (Baldridge et al. 1998). In both rods and cones, calcium-induced calcium release (Krizaj et al., 2003), which could be directly sensitive to NO, as well as modulated extrusion and/or uptake via PMCA and SERCA or other systems, could contribute to the modulated fluo-3 signal. Moreover, under some conditions, unclamped photoreceptors can produce regenerative depolarizations, such that NO regulation of channels other than the voltage-gated Ca channels themselves could lead to changes in these responses and hence in Ca$^{2+}$ influx. In salamander rods, NO modulation of $I_{Kx}$ and $I_h$ was not detected (Kurenny et al., 1994). We patch clamped cone photoreceptors to directly measure ionic currents and compare the actions of SNC in photoreceptors.

**NO suppresses calcium channel activity in cones.** In our previous study (Kurenny et al. 1994), we showed that 2 mM SNC facilitated Ca channels in rods by shifting the activation curve to
negative potentials by about 4 mV with an insignificant increase in the maximum conductance.

Here we show that SNC inhibits currents through Ca channels in cones via a different mechanism. Fig. 2 illustrates an example of a calcium current recording in a cone made with the perforated patch clamp technique. Current amplitude was suppressed at all potentials where Ca channels are typically activated in cones, but at potentials negative to this range, leak conductance was increased. The Ca channel activation curve was slightly shifted (2 mV) to positive potentials in this cone.

[Figure 2 near here]

SNC suppressed Ca channel currents in cones by decreasing the maximum conductance. Compared to the control conditions, maximum Ca channel ensemble conductance was reduced to 78±3% (n=5, p<0.01) in 0.1-0.2 mM SNC and to 77±6% (n=17, p<0.01) in 0.4-1 mM SNC. When the Boltzmann equation was fit to leak subtracted data, no statistically significant shift in $V_{1/2}$ of cone Ca channels was observed: $1.8±2.4$ mV (n=5, p>0.4) in 0.1-0.2 mM SNC and $0.9±1.1$ mV (n=17, p>0.4) for 0.4-1 mM SNC. As was the case in rods, there was no significant change in the slope factor: $1.12±0.05$ (n=5, p>0.05) and $1.03±0.04$ (n=17, p>0.5) of the control value for 0.1-0.2 mM and 0.4-1 mM SNC, respectively.

Similar to the results in rods (Kurenny et al. 1994), we found that SNC increased the slope of the current-voltage relationship at negative potentials (below –50 mV), where Ca channels are deactivated (Fig. 2). We studied properties of this conductance while other currents were blocked by cadmium, cesium, and TEA. Under these conditions, conductance was significantly increased from $1.89±0.22$ nS (n=32) in control to $3.68±0.24$ nS (n=16) in 2 mM SNC (p<0.001). The SNC-
induced conductance was voltage independent and its reversal potential was 0.8±1.6 mV (n=16).
The membrane conductance recorded in SNC solutions that had been allowed 1-2 days to de-gas
NO from the solution, 2.50±0.44 nS (n=4), did not differ significantly from the control (p>0.3),
indicating that NO must be present in the solution to activate this conductance.

We excluded the possibility that this conductance was due to activation of sodium dependent
 glutamate transporter (Eliasof and Werblin, 1993) by substituting lithium for sodium (n=4) and
by using the transporter blocker DL-threo-β-hydroxyaspartate, βHA (n=4). In both cases, SNC
activated a conductance that did not differ statistically from the conductance activated by SNC in
control solution (p>0.25). We also tested the hypothesis that the SNC-induced conductance was
due to activation of cGMP-gated channels. As found previously with rods, diltiazem (20 μM), a
blocker of cGMP-gated channels, reduced the SNC-induced conductance in cones. On average,
the conductance decreased from 4.03±0.47 nS (n=4) to 2.27±0.46 nS (n=4). In another series of
experiments we applied 8-bromo-cGMP, a membrane permeable analog of cGMP, to activate
cGMP-gated channels. In the presence of 8-bromo-cGMP (1 mM), membrane conductance was
increased to 3.92±1.60 nS (n=4), and diltiazem also blocked this conductance.

**Blocking NO production favors cone over rod input to horizontal cells (HCs).** HCs in tiger
salamander retina receive input from both rods (peak sensitivity, ~500 nm) and red cones (peak
sensitivity, ~600 nm) (Yang and Wu 1996). We recorded the responses of HCs to 500 ms flashes
of light of different wavelengths in eyecup preparations in control and after incubation in N^G-
nitro-L-arginine (NNA, 0.5 mM), a selective inhibitor of nNOS. Whereas we had used the NO
donor, SNC, in our imaging and electrophysiological studies of isolated rods and cones, now in
the intact retinal system we felt it best to use an inhibitor of endogenous NO production. Since
the the rods and cones in the previous studies were isolated from the retinal network and most of the endogenous NO production sites, it seemed that adding NO to the isolated cells would offer the best modulatory signal. In the dark-adapted, intact retinal eyecup, where it can be argued that endogenous NO levels would be at their peak, we felt that the most prominent modulatory signal would be the elimination of NO. This approach also minimized contributions from the by-products of the NO donors, which are easily tested for in isolated systems using degassed solutions, but present significant obstacles in difficult eyecup recording conditions.

Fig. 3A shows voltage responses to 500, 550, and 600 nm light, normalized to the response at 550 nm to allow comparisons between the responses in control and in NNA from different cells. NNA reduced the relative response amplitude at 500 nm and increased it at 600 nm. A reduction of the depolarizing rollback during the light step also occurred in the presence of NNA. The effect of NNA on the spectral response curve (Fig. 3B) was to sharpen the peak, essentially increasing the red (cone-driven) while reducing the green (rod-driven) inputs. In a sample of 10 horizontal cells recorded under control conditions, the 600 nm to 500 nm ratio was 1.58±0.09, whereas in 4 cells treated with NNA, the ratio was increased significantly by ~20% to 1.90±0.2 (p<0.05). Since the red/green strengthening occurs when NO signaling is blocked, the inverse would be that NO should tend to enhance green input and reduce the red input to the HC, consistent with the effects of NO on Ca channels in isolated rods and cones.

[Figure 3 near here]

**Synaptic model.** We constructed models of rod and cone photoreceptors using NEURON software to predict how changes in Ca channels would affect the light response in these cells and
in postsynaptic HCs. When fed with a simulated photocurrent signal (waveforms taken from Baylor et al. 1984 for rods and from Attwell et al. 1982 for cones), this model recreated a photovoltage that corresponded well to the recorded light responses. The HC response was simulated using the transfer function: \( V_{HC} = 39 \left( \exp \left( \frac{V_R - 40}{2.1} \right) - 1 \right) \) for the rod input (Attwell et al., 1987) and \( V_{HC} = 33.4 \left( \exp \left( \frac{V_C - 39.5}{8.1} \right) - 1 \right) \) for the cone input (our fit to Fig. 3E in Rieke 2001), where \( V_{HC} \), \( V_R \) and \( V_C \) are voltages in the HC, the rod and the cone, respectively.

We simulated the effects of SNC in rods by shifting \( V_{1/2} \) for Ca channels by \(-4.3\) mV and in cones by reducing \( G_{max,Ca} \) to 77% of the control value. Other voltage gated channels are not affected by SNC (Kurenny et al. 1994). Fig. 4 illustrates that the predicted amplitude of the HC response is increased for the rod input and decreased for the cone input. The postsynaptic rod response more than doubled (~220% of control) and there was a modest (~9%) reduction in the postsynaptic cone response, amounting to a rod/cone ratio change much greater than the ~20% change observed during NOS inhibition in the intact retinal eyecup.

[Figure 4 near here]

The non-specific conductance increase during SNC application to rods and cones could not be included in the photoreceptor voltage simulations or in the synaptic transfer functions since the added conductance strongly depolarized the photoreceptors. Thus, the model indicates that a conductance increase of this magnitude may not normally occur in photoreceptors and that conditions associated with patch clamp recording may tend to amplify such membrane conductance responses to the NO-donor.
Discussion

We show that the NO donor SNC has important actions on the Ca channels of cone photoreceptors, that these actions are dramatically different than those in rods, and that together, these changes in calcium signaling alter the balance of rod and cone synaptic strength measured postsynaptically in retinal neurons. In a previous investigation of the actions of NO on rod photoreceptors, we showed that SNC facilitated the activation of L-type Ca channels by shifting the channel open probability (activation) curve to more negative potentials (Kurenny et al. 1994). Not only is the functional effect of opposite polarity in cones, in that Ca channel activity is suppressed, but the modulatory mechanism appears to be different as well. While the negative-shift in channel activation in rods implied changes in channel gating properties, the decrease of the maximum calcium conductance in cones suggests a drop in the number of available channels or a reduction of the conductance of individual channels.

Such an opposing effect of NO on rod and cone Ca channels could contribute to the mechanism that alters the balance of rod and cone input to the second-order retinal neurons. A change in relative rod-cone strength is known to occur when the level of ambient illumination changes and may be under the control of a circadian clock (Witkovsky et al. 1988; Witkovsky et al. 1989; Mangel et al. 1994; Yang and Wu 1996; Wang and Mangel 1996). Rod signals are enhanced and cone signals suppressed in second-order neurons following dark-adaptation or during the subjective night. We show that the selective nNOS inhibitor NNA changes horizontal cell responses to rod- and cone-dominating wavelengths, decreasing rod and increasing cone signals recorded postsynaptically. Our modeling of rod, cone and horizontal cell responses predicts that changes of the appropriate Ca channel parameters ($V_{1/2}$ for rods and $G_{\text{max}}$ for cones)
alters the synaptic transfer functions and results in an increase, in the case of rods, or a decrease, in the case of cones, of the HC response produced by a fixed change of the presynaptic photoreceptor voltage.

It is interesting to consider that the most proximal source of NO production in the retina that could modulate photoreceptor Ca channels is the photoreceptors themselves. Photoreceptors contain nNOS, the activity of which is positively correlated with intracellular levels of calcium (Mayer et al. 1992; Bredt and Snyder 1994; Hu and el-Fakahany 1996; Goldstein et al. 1996). In darkness, calcium is elevated in photoreceptors and this could result in elevated NOS activity and NO production. In rods this would in turn facilitate Ca channels and allow even more calcium to enter the cell, producing a positive feedback system in rods that could increase the sensitivity of the rod pathway by increasing the output of rods onto second-order neurons. For cones, whose Ca channels are suppressed by NO, the production of NO in darkness would have the opposite effect, yielding a negative feedback loop that would stabilize calcium levels.

Dopamine was previously shown to modulate the balance of rod and cone input into second order retinal neurons, enhancing cone input and decreasing rod input (Witkovsky et al. 1988; Witkovsky et al. 1989). Like NO, dopamine modulates Ca channels differently in rods (facilitation) than in cones (suppression) (Stella and Thoreson 2000) but this result was viewed as paradoxical since the enhancement of rod Ca channel activity should increase the input of these cells to second-order neurons, not decrease it. It has been suggested that calcium-activated chloride current (I_{Cl(Ca)}) in rods, altered via modulation of Ca channels by dopamine, provides an explanation for this apparent paradox (Thoreson et al. 2002). Our data show NO-induced Ca channel facilitation and increased rod neurotransmission, suggesting that additional steps may contribute to the NO- and dopamine-mediated Ca channel/synaptic strength modulations.
It has been shown that cGMP-gated channels might play a role in synaptic transmission from cones (Rieke and Schwartz 1994; Savchenko et al. 1997). Activation by NO of cGMP-gated channels introduces another important mechanism for a modulatory influence of NO in the outer retina. Activation of a non-selective conductance by NO might act in unison with the facilitation of voltage-gated Ca channels in rods, but counteract the inhibitory effect of NO on cone Ca channels. These apparently contrasting mechanisms may again highlight the different signal processing strategies demanded for the synapse by rod and cone mediated visual transduction: high amplification and sensitivity for the rod output system and stability and lower sensitivity for that of the cones. The increase in leak conductance in rods and cones, measured with patch clamp recording techniques, was not evident in calcium imaging experiments and it is not accounted for in the experiments performed in eyecup. Modelling of the synaptic transfer of rod and cone membrane potential changes during treatment with NO-donors also could not accommodate the relatively large conductance increases seen during patch clamp recording. Such conductance increases may be specific to conditions encountered during patch clamp recording.

Taken together, the results of these investigations of calcium signal modulation in photoreceptors and of synaptic transmission to horizontal cells, demonstrate opposing actions of NO on rod and cone synapses that could be relevant to the roles of these photoreceptor systems in the duplex retina. NO modulation of Ca channels may reflect an important mechanism aligning rod/cone bias in the dark and light adapted states of the retina and may contribute to signal processing strategies that are unique for the two types of photoreceptor synapses.

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Figure legends:

**Fig. 1.** The NO donor SNC differentially modulates Ca channels in rods and cones. **A.** Calcium imaging of an isolated rod using the indicator fluo-3. Periods when the cell was stimulated with 10 mM K⁺, 8 mM K⁺, and again 10 mM K⁺ are indicated by the bars under the trace, at other times the cell was bathed in 1 mM K⁺. These K⁺ exposures produce modest depolarizations of the cells. During the superfusion with 8 mM K⁺, 1 mM S-nitrosocysteine was applied and this enhanced the potassium-induced increase in intracellular calcium. The numbered images below the trace correspond to the points numbered along the trace, showing before, during and following the SNC application, all in 8 mM K⁺. Application of 10 K⁺ was used to demonstrate viability of the cell as well as the strong dependence of the calcium signal on depolarization level. **B.** Fluo-3 imaging of an isolated cone showing the opposite response to SNC, where a decrease in fluorescence was induced by the NO donor during 8 mM K⁺ application. Scale bars are 10 µm.

**Fig. 2.** SNC suppresses Ca channels in cone photoreceptors and induces a non-selective conductance during patch clamp recording. The amplitude of I_{Ca} in control and during application of 1 mM SNC was measured at the end of 25 ms duration steps to voltages between –80 and +35 mV (in 5 mV increments) and plotted against the step voltage. Ca channel currents (in the voltage range between –35 and +20 mV) were reduced during the application of SNC. Linear leak subtraction, by the amount determined from the control I-V relation (see Methods), was applied to both I-V relations and reveals the increase in conductance during the application
of SNC, most easily seen in the voltage range from –80 to –40 mV. The inset on the left shows the I-V relations prior to any leak subtraction and the inset on the right shows examples of Ca channel currents recorded in control (larger inward current) and the presence of SNC (smaller inward current), during a 25 ms step to –15 mV from a holding potential of –60 mV. The scale bars accompanying the two current traces are 10 ms and 50 pA.

**Fig. 3.** Block of NOS activity with N^G^-nitro-L-arginine favors cone over rod input to HCs. A. HC light responses recorded in eyecup preparations. The responses to 500 ms flashes of light (indicated by horizontal bars) at 500, 550 and 600 nm are shown in a control cell and in 0.5 mM NNA in a different cell. The response amplitudes have been normalized at 550 nm to emphasize opposing changes in amplitude of the responses at 600 nm and 500 nm in NNA. B. Complete spectral response curves, normalized at 550 nm, showing that NNA enhances the responses at 600-650 nm (embracing peak of red cone response) and diminishes the response at 500 nm (near peak of green rod response).

**Fig. 4.** Computer simulation of the effects of NO on rod- and cone-driven light responses in a HC. A and C. Rod and cone light responses with (●) and without SNC-induced changes in ion channel kinetic parameters. B and D. Simulated rod (B) and cone (D) driven responses in a HC with (●) and without SNC-induced changes in photoreceptor ion channel parameters.
Fig. 1.
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