The Contribution of NMDA and Non-NMDA Receptors to the Light-Evoked Input-Output Characteristics of Retinal Ganglion Cells

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Summary

To examine how light-evoked excitatory synaptic inputs to retinal ganglion cells are transformed into output patterns of activity, action potentials were recorded with cell-attached patch-clamp techniques, and then EPSCs and EPSPs were recorded from the same cell in the whole-cell configuration. AP7, an NMDA antagonist, reduced the light-evoked peak spike frequency 36% ± 21% (mean ± SD) and reduced the EPSC amplitude, indicating a major role for NMDA receptors in the light response. CNQX, a non-NMDA receptor antagonist, reduced the light-evoked peak spike frequency 28% ± 22%. CNQX also caused a voltage- and magnesium-dependent delay in spike onset. AP7 and CNQX, however, did not differ significantly in their effect on the EPSC time course, indicating that postsynaptic cellular properties are responsible for the delay observed in the presence of CNQX. These results show that the NMDA receptor contribution to the excitatory response is increased as the cell is depolarized from rest by non-NMDA input.

Introduction

Both N-methyl-D-aspartate (NMDA) and non-NMDA ionotropic glutamate receptors mediate excitatory synaptic input to many neurons (for review, see Watkins and Collingridge, 1989, and Mayer and Westbrook, 1987). The two receptors have been characterized pharmacologically by selective antagonists (Davies et al., 1980; Honoré et al., 1988), and the associated ion channels have been distinguished biophysically in single-channel recordings (Cull-Candy and Usowicz, 1987; Jahr and Stevens, 1987). A striking difference between the two receptors is a voltage dependence conferred uniquely onto the NMDA receptor's ion channel by external magnesium, which blocks the channel at hyperpolarized potentials (Mayer et al., 1984; Nowak et al., 1984). This voltage dependence has been observed in the NMDA receptor-mediated component of excitatory synaptic inputs recorded in many cells, including those in the hippocampus (Forstyh and Westbrook, 1988; Hestrin et al., 1990; Randall et al., 1990), spinal cord (Dale and Roberts, 1985), and retina (Mittman et al., 1990). The effects of NMDA and non-NMDA receptor agonists and antagonists on output patterns of action potentials, recorded extracellularly, have also been investigated (Boos et al., 1990; Fox et al., 1990; Kwon et al., 1991; Massey and Miller, 1988; Miller et al., 1989; Nishigori et al., 1990; Salt and Eaton, 1989). The present study addresses a question to which relatively little attention has been paid: in a single cell, how do NMDA and non-NMDA synaptic inputs, evoked by a physiological stimulus, contribute to the generation of action potential responses? In particular, how is the output spike response influenced by the voltage dependence of the NMDA receptor-mediated inputs?

Glutamate is thought to be the major neurotransmitter at excitatory synapses in the retina (for review, see Massey and Redburn, 1987). Glutamate released from photoreceptors excites OFF bipolar cells and horizontal cells through non-NMDA receptors (Slaughter and Miller, 1983); glutamate drives ON bipolar cells through metabotropic 2-amino-4-phosphonobutyrate receptors (Slaughter and Miller, 1981). Bipolar cells provide excitatory, glutamatergic input to amacrine cells and ganglion cells through NMDA and non-NMDA receptors (Dixon and Copenhagen, 1992; Massey and Miller, 1988; Slaughter and Miller, 1983). Amacrine cells, in turn, inhibit ganglion cells through glycine and γ-aminobutyric acid type A (GABA_A) receptors (Belgum et al., 1984; Frumkes et al., 1981). Understanding the processing of visual information in vivo requires the characterization of the complex interplay between excitation and inhibition in the inner retina. This study focuses only on the glutamatergic excitation of ganglion cells, a first step toward that complete characterization.

Using a combination of recording methods in the same cell, we measured light-evoked synaptic inputs and spike outputs from salamander retinal ganglion cells. Light-evoked spike activity in intact, unruptured cells was measured with cell-attached patch-clamp techniques. The contributions of the NMDA and non-NMDA inputs to the output spike pattern were investigated using selective antagonists. Light-evoked postsynaptic currents (EPSCs) and postsynaptic potentials (EPSPs) were recorded in the whole-cell configuration with the same patch pipette used to record cell-attached spike responses. The time course, voltage dependence, and magnitude of the NMDA and non-NMDA synaptic inputs were characterized using selective antagonists, as well as voltage-clamp and current-clamp protocols. The combination of recordings enabled us to study how synaptic input currents are transformed into excitatory voltage responses and output patterns of action potentials.

Our results indicate that, even though the time courses of the NMDA and non-NMDA inputs to ganglion cells in dark-adapted retina overlap considerably, the two inputs play different roles in the generation of the cells' physiological output. As a result of the voltage-dependent magnesium block of NMDA channels, currents mediated by non-NMDA receptors underlie the initial light-evoked depolarization in
these cells. In addition, at more depolarized levels, the relief of the magnesium block on NMDA channels appears to offset the decline in response that would result from the excitatory inputs approaching their reversal potential. This interaction tends to linearize excitatory synaptic inputs over a wide response range.

Results

Light-Evoked Spike, Voltage, and Current Responses in Ganglion Cells

Light-evoked action potentials were recorded from ganglion cell somata using the cell-attached patch-clamp technique. In these and all other recordings described in this study, the excitatory component of the response was isolated by blocking inhibitory inputs mediated by GABA, and glycine receptor channels with 50 μM picrotoxin and 500 nM strychnine, respectively. Light stimuli of 2 s duration elicited excitatory responses either at light onset and extinction (ON–OFF cells), during the light stimulus (ON cells), or only at light extinction (OFF cells). OFF ganglion cells were encountered rarely and were not studied. The duration of ON cell responses varied over a wide range. Some ON cells responded transiently, with spike trains lasting 200–300 ms, whereas others fired action potentials for nearly the entire duration of the light stimulus. The analysis in this paper is confined to all types of ON cell responses and the ON portion of ON–OFF cell responses.

Figure 1A shows a cell-attached recording of a light-evoked response from an ON ganglion cell. During the response, the cell fired 13 action potentials. The average number of spikes evoked by each stimulus increased monotonically with light intensity. Light-evoked action potentials were eliminated reversibly by 10 nM tetrodotoxin, a blocker of voltage-gated sodium channels (5 of 5 cells tested; data not shown). The potassium channel blocker tetraethylammonium (500 μM) slowed the repolarization of each action potential (4 of 4 cells tested; data not shown). This repolarization is known to be mediated, in large part, by delayed-rectifying potassium channels (Hodgkin and Huxley, 1952a). These results confirmed that spikes measured in the cell-attached configuration were generated via voltage-gated sodium and potassium channels.

The whole-cell voltage-clamp technique was used to record light-evoked, synaptically driven input currents. In some of these cells, light-evoked voltage responses—both EPSPs and action potentials—generated by the synaptic inputs were measured under whole-cell current clamp, in addition to voltage-clamped current recordings. To obtain whole-cell recordings from the same cell after cell-attached spike recordings were completed, the membrane spanning the electrode was ruptured. Figures 1B and 1C show whole-cell current- and voltage-clamped light responses, respectively, in the same ganglion cell from which the cell-attached recording in Figure 1A was obtained. In the current-clamped recording (Figure 1B), the light-evoked voltage response includes an EPSP leading to a series of action potentials at the baseline depolarization. Voltage-clamped response (Figure 1C) demonstrates the time course of the light-evoked synaptic input from bipolar cells. The 57 ms difference between the times to the first spike in the current-clamped recording (Figure 1B) and the cell-attached recording (Figure 1A) is due to the fact that negative current was injected under current clamp to hold the baseline membrane potential near −80 mV. Because this potential was probably more hyperpolarized than the normal resting potential of this ganglion cell, more time was required in the current-clamped recording for the cell to reach spike threshold.

In ganglion cells in which both cell-attached and whole-cell recordings were obtained in control saline, cell-attached spike responses matched well with whole-cell voltage-clamped current responses in terms of classification of response type (e.g., ON versus OFF versus ON–OFF) and, to a first approximation, the duration and magnitude of the light response. This correspondence between synaptic currents and spike out-
put suggests that the time course of the synaptic input current was not altered significantly by the voltage-gated conductances in and near the cell soma.

**CNQX and AP7 Reduce the Light-Evoked Spike Response**

The contribution of pharmacologically isolated non-NMDA or NMDA inputs to the spike output of ganglion cells was studied by recording light-evoked spike responses in the cell-attached patch-clamp configuration. Figure 2A shows a raster plot of multiple light responses in a single ganglion cell. Each dot in the raster represents the occurrence of one action potential; a horizontal line of dots depicts the spike response to a single 2 s light stimulus. The non-NMDA component of the excitatory response was isolated by blocking the NMDA receptors with the competitive antagonist 6-cyano-7-nitroquinoxaline (CNQX, 1 uM), also a competitive antagonist. CNQX and AP7 together abolished the light-evoked EPSC in whole-cell voltage-clamped recordings (data not shown).

As a method to quantitate the strength of the NMDA and non-NMDA inputs, we compiled instantaneous spike frequency histograms, from which we obtained the peak firing frequency (see Experimental Procedures). The histograms in Figure 2B summarize the raw spike data plotted in Figure 2A. Each histogram represents the average of the last 10 light responses in each experimental condition. In the illustrated cell, 20 uM AP7 decreased the peak light-evoked spike frequency by 60% versus control; 1 uM CNQX caused a 46% reduction. In a sample of cells in which recovery was obtained, AP7 reduced the peak light-evoked fir-

![Figure 2. Effects of AP7 and CNQX on the Light-Evoked Spike Responses of Ganglion Cells](image-url)

(A) Raster summarizing light-evoked spike responses recorded from an ON ganglion cell in the cell-attached configuration. Each dot on the raster represents 1 action potential; single responses to light stimuli, recorded every 10 s, are plotted in each horizontal row. In control saline, the cell began firing spikes approximately 313 ms after light onset. In most cells this spike delay was 250-400 ms, similar to delays reported in turtle thalamic and retinule (Baylor and Fettiplace, 1979). Both 20 uM AP7 and 1 uM CNQX reduced the light-evoked spike frequency, but CNQX increased the spike delay by 165 ms, whereas AP7 increased the spike delay by only 12 ms. Light intensity was $1.6 \times 10^7$ photons $\mu m^{-2} s^{-1}$.

(B) Spike frequency histograms summarizing data in (A). In each case, histograms were constructed with a sliding window (100 ms wide, 10 ms steps); each histogram represents an average of the last 10 responses from each experimental condition (see Experimental Procedures).
ing rate by 36% ± 21% (mean ± SD; n = 18) and CNQX reduced the peak rate by 28% ± 22% (n = 18). The significant reduction (p < .001; paired t test) by both AP7 and CNQX of the light-evoked spike response argues strongly that both NMDA and non-NMDA inputs play physiologically important roles in the generation of light-evoked action potentials in ganglion cells.

To compare response delays in spike recordings, we measured the time between light onset and the first action potential, a value referred to here as the spike delay. In each case, spike delays in 10 responses were averaged together. In the illustrated cell (Figure 2), when 1 µM CNQX was added to the bath the spike delay increased by 165 ms, from 313 ms to 478 ms. In 18 cells analyzed, CNQX caused a 101 ± 53 ms increase in the spike delay. In the cell shown in Figure 2, AP7 increased the spike delay by only 12 ms, from 306 ms to 318 ms. On average, AP7 increased the spike delay 9.6 ± 13.4 ms (n = 18). To determine the significance of the increased spike delays, the variability of the spike delays in control responses was compared with the changes induced by either CNQX or AP7 in the same cell. CNQX increased the spike delay by an amount exceeding the standard deviation of the control spike delay in all 18 cells analyzed; this effect of CNQX did not vary significantly with cell type or time course of response. The increase in spike delay induced by AP7 exceeded the control spike delay standard deviation in only 3 of 18 cells, consistent with the notion that AP7 exerted only a small effect on the spike delay as compared with CNQX.

In the cell shown in Figure 2, both AP7 and CNQX caused the duration of the light-evoked spike pattern to increase, an effect that was observed in a number of cells. One possible explanation for this phenomenon is that CNQX and AP7, in addition to blocking glutamatergic input to the ganglion cell, also inhibited interneurons that function to truncate the duration of the ganglion cell spike response. Inhibition of such interneurons would cause the ganglion cell spike response to become longer. The response prolongation was observed in the presence of picrotoxin and strychnine, suggesting that the putative truncation mechanism was mediated independently of GABA<sub>A</sub> or glycine receptors. Preliminary experiments suggest that GABA<sub>A</sub> receptors may underlie this truncation. Application of CGP 35348 (500 µM), a GABA<sub>A</sub> antagonist, caused reversible elongation of the light-evoked spike response, similar to that observed here in the presence of AP7 or CNQX. The mechanisms responsible for prolonging the spike response were not addressed further in this study.

Light-evoked EPSPs and/or action potentials were recorded under whole-cell current clamp in 27 cells. It was apparent in these recordings that the increase in the spike delay in the presence of CNQX was a result of a slower membrane depolarization preceding the first action potential. Figure 3 shows voltage responses from one of these cells. As with cell-attached recordings, spike delay is defined here as the period of time between light onset and the first action potential. In control saline, after a 395 ms spike delay, the cell fired 14 action potentials over a 375 ms period. Because of the lower number of light-evoked responses recorded under whole-cell current-clamp, spike frequency statistics were not compiled as in the cell-attached recordings. In the presence of 20 µM AP7, the spike delay was comparable to control, and 13 action potentials were fired over a 595 ms period. In the presence of 1 µM CNQX, the light-evoked depolarization leading to the initial action potential was clearly more gradual than in control or AP7. The first of 11 spikes was observed after a 622 ms spike delay, 227 ms longer than the spike delay observed in control. This increase in spike delay was greater than that observed in most cells. During the experiment described in Figure 3, a negative holding current was injected into the cell, hyperpolarizing the baseline membrane potential to ~80 mV, which exaggerated the spike delays.

Two possible effects of CNQX that would account for the increased spike delay observed in the presence of CNQX were checked experimentally: CNQX could have acted directly on the cell's action potential-generating mechanisms, or CNQX could have reduced the cell's input conductance, thereby increasing the membrane time constant and, therefore, the amount of time required to depolarize the membrane to threshold.

To check the first possibility, we injected depolarizing, suprathreshold current steps into ganglion cells under whole-cell current clamp and saw no effect of 1 µM CNQX on the spike patterns, which argues against an action of CNQX on voltage-gated, spike generation mechanisms (data not shown). Furthermore, CNQX caused no change in the threshold or waveform of individual action potentials in light-evoked voltage responses (Figure 3). To check the second possibility, the passive membrane time constant (τ<sub>m</sub>) of ganglion cells was calculated with whole-cell current-clamped recordings by injecting a current step and fitting the resulting voltage response with a single exponential function. This technique demonstrated that τ<sub>m</sub> was on the order of 80 ms. CNQX certainly reduced the light-evoked reduction of τ<sub>m</sub> by blocking the non-NMDA synaptic conductance, but in order for CNQX to cause a delay similar to that observed in Figures 2 and 3 via effects on passive membrane characteristics, it would have had to increase τ<sub>m</sub> by more than an order of magnitude. This value corresponds to an input resistance exceeding 20 GΩ, an unrealistically high value. Under whole-cell current clamp, CNQX induced no significant change in τ<sub>m</sub> in ganglion cells (data not shown).

The results described above indicate that both NMDA and non-NMDA receptor-mediated inputs contribute to the production of light-evoked action potentials in retinal ganglion cells. Both inputs contribute throughout the spike response (Figure 2), but
the increase in the spike delay induced by CNQX indicates that the non-NMDA component mediates a particularly large portion of the membrane depolarization prior to the first spike. While NMDA input alone was sufficient to produce action potentials, in the absence of non-NMDA input the attainment of spike threshold was delayed substantially.

NMDA and Non-NMDA Synaptic Inputs Have Similar Time Courses

Another explanation that could account for the observation that CNQX, and not AP7, increased the spike delay stems from reports in many systems that NMDA and non-NMDA synaptic inputs have different time courses (Dale and Roberts, 1985; Forsythe and Westbrook, 1988; Hestrin et al., 1990; Mittman et al., 1990). Typically, the non-NMDA component of the response is much faster than the NMDA component, owing to differences in single-channel kinetics (Lester et al., 1990). In such a system, CNQX would be expected to increase the spike delay, because it would block the fast component of the EPSC.

To test whether there were temporal differences between the NMDA and non-NMDA light-evoked synaptic inputs, we measured light-evoked EPSCs under whole-cell voltage clamp. For the purpose of description, we define synaptic latency to be the period of time between onset of the light stimulus and the beginning of the EPSC. In 44 cells analyzed, the light-evoked EPSC in control saline peaked in 261 ± 89 ms, following a synaptic latency of 245 ± 73 ms. Figure 4A shows light-evoked EPSCs recorded in the same cell from which the raster in Figure 2 was obtained. The synaptic latency and time course of the EPSC recorded in the presence of AP7 was almost identical to that recorded in the presence of CNQX, even though the spike delay in the same cell was affected differently by the two drugs (Figure 2). These results argue against the hypothesis that the delay induced by CNQX was either a result of actions at sites presynaptic to ganglion cells, or due to the blockade of a faster non-NMDA component of the synaptic input.

Even though the increased spike delay was not attributable to selective presynaptic effects of CNQX, in the presence of either AP7 or CNQX, the light-evoked EPSCs in this cell peaked and decayed more slowly than in control (Figure 4A). The observation that AP7 and CNQX slowed the decay of the synaptic input is consistent with the elongation by AP7 or CNQX of the light-evoked spike train in this cell (Figure 2). As mentioned earlier, we speculate that this elongation was the result of a mechanism presynaptic to the ganglion cell.

Though the NMDA portion of the light-evoked synaptic input had a time course similar to the input mediated by non-NMDA receptors, the NMDA conductance did display a distinctive voltage dependence similar to that described previously (Mayer et al., 1984; Nowak et al., 1984). The current–voltage plot in Figure 4B, summarizing whole-cell, voltage-clamped recordings from an ON ganglion cell, demonstrates that AP7 reduced the nonlinear conductance associated with the voltage-dependent magnesium block of the NMDA receptor channel. AP7, however, had little effect on the time course of light-evoked EPSCs (Figure 4C). Similar results were obtained in 72 cells. The difference current (dashed line in Figure 4B), calculated by subtracting from the control curve the curve obtained in the presence of AP7, strongly resembles the current–voltage relation associated with NMDA receptors (Mayer et al., 1984; Nowak et al., 1984). Current–voltage plots similar to this difference curve were obtained in other cells in the presence of 1 μM CNQX (data not shown).

In agreement with previous reports (Forsythe and Westbrook, 1988; Hestrin et al., 1990; Mayer et al., 1984; Mittman et al., 1990; Nowak et al., 1984), the results described in Figures 4B and 4C demonstrate that blocking NMDA receptors reduces a voltage-dependent synaptic conductance. The data also indi-
Figure 4. NMDA and Non-NMDA Inputs Exhibit Similar Time Courses

(A) Whole-cell voltage-clamped recordings of light responses in the same ON ganglion cell from which the spike raster in Figure 2 was obtained. Responses have been scaled and smoothed to facilitate comparison of time courses. The light-evoked EPSCs (V_m = -36 mV) in 20 μM AP7 and 1 μM CNQX overlapped in time almost completely, indicating that the NMDA and non-NMDA inputs had similar time courses. Both drugs caused a slight slowing of the EPSC time course as compared with control and recovery. Light intensity was 1.6 × 10^3 photons·μm^-2·s^-1.

(B) Current-voltage plot summarizing voltage-clamped recordings obtained from an ON ganglion cell. The peak value of each EPSC, relative to the prestimulus baseline, was plotted. At 20 μM, AP7 (triangles) reduced the region of negative slope conductance conferred onto the control curve by NMDA receptor-mediated input. The AP7 curve was subtracted from control, revealing the NMDA-mediated portion of the EPSC (dashed line).

(C) Some of the voltage-clamped EPSCs (V_m = -66, -21, and 24 mV) used to construct the current-voltage curve in (B). These recordings demonstrate that, over a wide range of membrane potentials, AP7 caused very little change in the time course of synaptic input to this cell. Light intensity was 1.6 × 10^4 photons·μm^-2·s^-1.

cate, however, that the time course of the EPSC mediated by NMDA receptors was similar to that mediated by non-NMDA receptors. This result is in contrast with a large body of work in other systems demonstrating that EPSCs mediated by NMDA receptors are many times slower than those mediated by non-NMDA receptors. In most systems, neurotransmitter release is triggered by the arrival of an action potential to the presynaptic terminal. As a result, the time course of release is fast enough that the differences in kinetics between the NMDA and non-NMDA receptors are reflected in the time course of the EPSC, which is a temporal convolution of the time course of vesicle release with the postsynaptic response to the release of a single vesicle (i.e., the miniature EPSC).

In dark-adapted retina, however, neurotransmitter release from bipolar cells depends on the slow, graded depolarization of the presynaptic terminal (Tachibana and Okada, 1991). As a result, the time course of neurotransmitter release is approximately 2 orders of magnitude slower than that observed in the hippocampus (Hestrin et al., 1990) and many times slower than that in light-adapted retina (Mittman et al., 1990). The release time course is so slow that it "smears out" the kinetic differences between the NMDA and non-NMDA receptors and leads to EPSCs of similar time
course. This interpretation is supported by the observation that non-NMDA miniature EPSCs recorded in light-adapted retina (Taylor, Chen, and Copenhagen, unpublished data) have time courses similar to those recorded in dark-adapted retina (data not shown).

The CNQX Effect on the EPSP Time Course Is Voltage Dependent

A likely explanation for the CNQX-induced synaptic delay increase takes into account the voltage dependence of the NMDA receptor. It suggests that, because most of the NMDA receptors are blocked at the cell's resting potential by magnesium, non-NMDA receptors mediate most of the initial portion of the excitatory response. Kinetic studies have suggested that, in the presence of 1 mM magnesium, almost 96% of the NMDA receptors on a hippocampal neuron are blocked at -70 mV (Jahr and Stevens, 1990). If non-NMDA receptors were blocked by CNQX, at the resting potential excitatory current would be conducted only by that 4% of NMDA channels that were not blocked by magnesium. An NMDA receptor-mediated EPSC would produce a small depolarization that would relieve the magnesium block slightly. Subsequently, more NMDA receptors would be free to conduct current, and the depolarization would grow. The time required to recruit enough NMDA receptors to produce a significant EPSP would depend on the membrane potential of the cell: At hyperpolarized potentials, the magnesium block would be stronger, and fewer NMDA receptors would be available to conduct current. In addition, because the magnesium block displays a sigmoidal dependence on voltage, at more hyperpolarized potentials a small depolarization would have a lesser effect on the magnesium block (Jahr and Stevens, 1990).

To test our hypothesis, we recorded whole-cell current-clamped, light-evoked responses from ganglion cells (see Figure 5). We included 10 nM tetrodotoxin in the bathing saline and 84 mM cesium in the pipette solution in an effort to reduce voltage-gated conductances and isolate the EPSP. The holding current was varied between responses to change the baseline membrane potential of the cell. In control saline (Figure 5A) the time course of the rising phase of the EPSP was not affected significantly by changes in baseline membrane potential. However, in 1 μM CNQX, the rising phase of the EPSP slowed as the baseline membrane potential was hyperpolarized (Figure 5B). This result indicates that the CNQX-induced delay is dependent on the ganglion cell's membrane potential (Figure 5C) and supports the notion that the NMDA receptors' inability to contribute to the early phase of the voltage response is due to the voltage-dependent magnesium block.

Removing Magnesium Reduces the CNQX-Induced Delay

The experiments described above suggest that NMDA receptors do not participate strongly in the light-evoked response until the cell is depolarized sufficiently to relieve a magnesium block. This hypothesis was tested in another way by observing the effects of CNQX in saline that was nominally magnesium free.

Light responses were recorded in the cell-attached configuration, first in normal saline and then in nominally magnesium-free saline (Figure 6). The effect of CNQX on the spike delay was reduced significantly by removing magnesium from the superfusion saline.
Figure 6. The CNQX-Induced Delay to Spiking Is Magnesium Dependent

Raster summarizing cell-attached recordings from an ON gan-
glion cell. In the presence of 1 mM magnesium, 1 μM CNQX
increased the spike delay by 160 ms. When magnesium in the
superfusion saline was replaced with calcium (0 Mg²⁺), the spike
train became longer, but CNQX increased the spike delay only
38 ms, 77% less than in saline containing magnesium. Light inten-
sity was 1.6 x 10⁴ photons μm⁻² s⁻¹.

In control saline, the addition of CNQX to the bath
increased the spike delay by 160 ms, from 353 ms to
513 ms. When magnesium in the bathing solution was
replaced with calcium, CNQX increased the spike de-
lay by only 38 ms, 77% less than in control saline. On
average, removing magnesium from the superfusion
saline reduced the CNQX effect on the spike delay
by 89% ± 10% (n = 4).

These results in magnesium-free saline support the
hypothesis that the voltage-dependent magnesium
block prohibits NMDA receptors from participating
in the initial stages of the light-evoked spike response,
but we are cautious in assuming that the removal of
magnesium affected only ganglion cell NMDA recep-
tors. Removing magnesium greatly increases ganglion
cell excitability (Massey and Miller, 1990). If magnesium
was not replaced with calcium, ganglion cells showed
increased firing rates, larger EPSCs, and changes in im-
pulse response patterns (data not shown). When mag-
nesium was replaced with calcium to maintain the
divalent concentration, cells displayed relatively nor-
mal firing rates, in agreement with previous reports
(Massey and Miller, 1990), but the length of the im-
pulse pattern increased (Figure 6). These observations
suggest that magnesium acts pre- and post-
synaptically at sites other than ganglion cell NMDA
receptors. Nonetheless, the reduction of the CNQX-
induced delay in magnesium-free saline did not de-
pend on whether or not magnesium was replaced
with calcium, an observation that lends support to
our hypothesis.

A Simple Somatic Model Mimics the CNQX Effect

The experimental results described above suggest
that the distinct contributions of NMDA and non-
NMDA inputs to the generation of the light-evoked
spike response are a result of postsynaptic processing
performed in the ganglion cell itself. As another way
to deduce whether it is plausible that such voltage-
dependent processing of NMDA- and non-NMDA-
mediated synaptic inputs might affect the spike out-
put, we constructed a somatic membrane model of
a spiking neuron.

This simplified model included only conductances
that are common to all spiking cells which receive
NMDA and non-NMDA excitatory input. Passive mem-
brane parameters and Hodgkin-Huxley formulations
for classic voltage-gated sodium channels and de-
layed-rectifying potassium channels were taken, with
only minor modifications, from a more extensive
model of lamprey spinal cord neurons (Ekeberg et al.,
1991), as was a similar representation of the voltage-
dependent NMDA receptor-mediated synaptic con-
ductance (Figure 7A). The only other conductance,
that mediated by non-NMDA synaptic input, was set
linear with respect to voltage (Figure 7A).

Although the anisopotentiality of ganglion cells has
been demonstrated (Taylor, Mittman, and Copenhagen,
unpublished data), for the sake of simplicity we
modeled the cell as a sphere with synaptic inputs di-
rectly onto the cell body. The time courses of both
EPSCs were identical in the model, represented by the
function \( t^2 \exp(-t/\tau) \) with \( \tau = 125 \) ms. Thus, both
EPSCs peaked in 250 ms (Figure 7B), which agreed
well with our experimental results (see Figure 4 for
examples). In the model, the simulated voltage re-
sponse to combined synaptic input (Figure 7C) elic-
ted a train of action potentials similar to current-
clamped light responses (Figure 3). In this simple
model, however, the membrane potential did not re-
main at a depolarized plateau during spike intervals,
and the spike train exhibited little accommodation.
Previous work in salamander ganglion cells (Lukasie-
wicz and Werblin, 1988) demonstrated a series of volt-
age-gated potassium currents that may be responsible
for timing and truncation of the spike response, but
in the interest of generality they were excluded from
our model.

The simulated cell took longer to initiate a spike
when the non-NMDA input was removed (Figure 7D).
The response driven by both inputs and that driven
only by non-NMDA input fired initial spikes within
10 ms of each other; the first spike in the NMDA case
occurred after an extra 165 ms delay. The addition to
our model of noninactivating calcium channels and
calcium-dependent potassium channels, both of which
are present in tiger salamander ganglion cells (Luka-
Figure 7. A Somatic Model Mimics the Voltage-Dependent Delay Induced by CNQX

(A) Current-voltage plots of simulated excitatory inputs. The NMDA input, as measured at +40 mV, was configured to be 50% larger than the non-NMDA input, consistent with the ratio observed in most ganglion cells. Ordinate is in picocoulombs.

(B) Time course of both excitatory synaptic inputs. A function was chosen to produce a waveform that peaked in 250 ms (see Experimental Procedures), which was in good agreement with voltage-clamped, light-evoked EPSCs obtained experimentally.

(C) Simulated light response. Synaptic input with the time course described above elicited a pattern of action potentials. The time course of the excitatory input (shown in (B)) was inverted and plotted below.

(D) Simulation of added delay to spike onset induced by CNQX. Removal of the NMDA input (dashed line) caused only a 10 ms increase in the spike delay versus control (smooth line). When the non-NMDA input was removed (dotted line), however, the spike delay increased 165 ms versus control.

(E) The delay to spike onset showed a greater dependence on the baseline membrane potential when the non-NMDA input was removed. Responses to combined (smooth line) and isolated NMDA (dotted line) inputs are plotted. The baseline membrane potential was varied from −80 to −50 mV. With both inputs present, the 30 mV variation caused only a 25 ms change in time to the first spike. With the NMDA input isolated, the difference between the delays at −50 mV and −80 mV increased to 117 ms.
siewicz and Werblin, 1988), had little effect on the results, so they were not included. These channels, as well as the A-type inactivating potassium channel, have been incorporated into a model of a repetitively firing salamander ganglion cell (Fohlmeister et al., 1990). When we employed the latter model and isolated the NMDA and non-NMDA synaptic inputs, we obtained results similar to those described above (data not shown). The basic model also mimicked the voltage dependence of the CNQX-induced delay that was observed experimentally (in Figure 5). When the membrane potential of the model cell was set to −80 mV (Figure 7E), removing the non-NMDA input increased the spike delay by 120 ms versus control. When the resting membrane potential was changed to −50 mV, this increased spike delay was only 27 ms. In addition, magnesium-free conditions were simulated by removing the voltage-dependent term from the expression representing the NMDA synaptic conductance. When this was done, removing the non-NMDA input increased the spike delay only 13 ms, 92% less than in control conditions (data not shown). This effect was qualitatively similar to our experimental data.

The aim of this model was to reproduce qualitatively our experimental observations with a minimum number of specialized conductances, thereby illustrating that the interaction between NMDA and non-NMDA inputs may not be unique to ganglion cells and therefore may resemble interactions in other neurons that receive similar excitatory input. It should be stated here that it was not our intent to simulate in vivo ganglion cell activity, nor to predict how interactions between the two inputs might affect other aspects of ganglion cell behavior. Parameters describing the voltage-gated conductances were taken from a simulation of a different system (Ekeberg et al., 1991); these parameters were changed only slightly to increase the distance between the resting and spike threshold potentials, and once in place they were not changed or tuned to optimize the performance of the simulations.

The simulation results described above were robust to broad changes in magnitude and time course of synaptic input and, to a lesser extent, voltage dependence of the NMDA conductance. For the simulations illustrated in Figure 7, the ratio of peak NMDA conductance to peak non-NMDA conductance \( G_{\text{NMDA}} : G_{\text{non-NMDA}} \) was set to 1.5, which was in good agreement with experimental data. We observed longer spike delays in the “NMDA alone” case than in the “non-NMDA alone” case as long as \( G_{\text{NMDA}} : G_{\text{non-NMDA}} \) was less than 4.

Our model mimicked qualitatively the increased spike delay observed experimentally in the presence of CNQX, even when the synaptic latencies and time courses of the NMDA and non-NMDA inputs were identical. Furthermore, the simulated “CNQX effect” was sensitive to changes in resting membrane potential and to the presence of voltage dependence in the NMDA conductance. These results were analogous to those observed experimentally. The results from this general model suggest that the voltage-dependent interaction between NMDA and non-NMDA synaptic inputs observed in retinal ganglion cells also may occur in other spiking neurons that receive similar, temporally overlapping inputs.

**Discussion**

The results presented here indicate that both NMDA and non-NMDA receptor-mediated inputs participate in light-evoked spike responses in salamander ganglion cells. Our findings also suggest that, although NMDA receptors are able to generate a delayed spike response by themselves, they do not contribute significantly to the normal light response until the cell has been depolarized sufficiently by non-NMDA receptor-mediated input.

Numerous studies have shown that glutamate excites retinal ganglion cells via NMDA and non-NMDA receptors (Boos et al., 1990; Coleman and Miller, 1989; Lukasiewicz and McReynolds, 1985; Massie and Miller, 1988; Mittman et al., 1990; Slaughter and Miller, 1983; Yazejian and Fain, 1992). Some authors, however, have concluded that NMDA input does not contribute significantly to the light-evoked spike response (Slaughter and Miller, 1983; Coleman et al., 1986; Coleman and Miller, 1988, 1989; Massie and Miller, 1988, 1990). The present study reports that the removal of AP7 of NMDA receptor-mediated input reduced the light-evoked frequency of spike discharges by 36% ± 21% \( (n = 18) \). Furthermore, when the non-NMDA input was blocked by CNQX, the NMDA component of the EPSC was sufficient to elicit action potentials (Figures 2A, 3, and 6). We conclude from these observations that NMDA receptors contribute to a significant portion of the physiological excitatory light response in salamander retinal ganglion cells.

Earlier studies that are contradicted by our findings in salamander were performed in rabbit and mud-puppy retina, and it is possible that species differences underlie our conflicting conclusions. However, one important difference between previous work and that presented here is that all of our experiments were carried out in the presence of strychnine and picrotoxin, which block the amacrine cell-mediated glycinergic and A-type GABAergic inhibition of ganglion cells, respectively. The effects of glutamate receptor antagonists on ganglion cell light responses in the presence of inhibition may be more difficult to dissect and certainly are more difficult to interpret, because amacrine cells in tiger salamander also are driven by NMDA and non-NMDA receptors (Dixon and Copenhagen, 1992). With inhibition present, CNQX and AP7, in addition to their direct effects, partially disinhibit ganglion cells by reducing amacrine cell-mediated inhibition. Nonetheless, the CNQX-induced increase in the spike delay described here also was observed in the absence of strychnine and picrotoxin (data not shown), indicating that interaction between NMDA...
and non-NMDA inputs persists in the presence of inhibition.

Interaction of Non-NMDA and NMDA Receptor-Mediated Inputs

Combined non-NMDA and NMDA receptor activation contributes significantly to neuronal signal processing in spinal cord (lamprey swimming, Grillner et al., 1990; rat nociception, Dickenson and Sullivan, 1990), thalamus (rat somatosensation, Salt and Eaton, 1989; cat vision, Kwon et al., 1992), somatosensory cortex (rat, Armstrong-James et al., 1993), auditory cortex (bat biosonar, Suga et al., 1990), and visual cortex (rat, Fox et al., 1990). The present study examines more closely how interactions between NMDA and non-NMDA inputs contribute to signal processing in a single cell. Our results indicate that, in dark-adapted ganglion cells, the initial light-evoked depolarization is mediated primarily by non-NMDA receptors, because near the resting potential a high percentage of the NMDA receptors are blocked by magnesium. After the cell has been depolarized sufficiently, however, NMDA receptor-mediated input contributes substantially to the excitatory response. The voltage dependence of the CNQX-induced delay across a physiological range of potentials (Figure 5) is consistent with the idea that the magnesium block of the NMDA conductance is reduced, and therefore the NMDA current is increased during light-evoked membrane depolarization from rest.

NMDA and Non-NMDA Receptors Combine to Linearize Ganglion Cell Responses

While an NMDA receptor-mediated EPSC contributes a highly nonlinear component to a cell’s current-voltage curve, it actually helps to linearize the cell’s response with respect to strength of synaptic input. This linearization can be inferred from current-voltage curves obtained from individual ganglion cells (Figure 4B). From whole-cell voltage-clamped recordings, it is evident that the NMDA component of the light response is small near the resting potential (approximately -70 mV) and increases as the cell depolarizes. In contrast, the non-NMDA component is largest at hyperpolarized potentials; as the cell depolarizes and the driving force on the permeant ions is reduced, the non-NMDA current decreases monotonically. When these two components are combined, light evokes a relatively constant amount of current at membrane potentials between -90 and -40 mV. (In the cell shown in Figure 4B, the current is actually boosted between -60 mV and -40 mV.) This flat region of the current-voltage curve occurs when the reduction in driving force is offset by the relief of the magnesium block, and it indicates that a unit excitatory signal from bipolar cells elicits EPSPs of approximately the same amplitude over this range of membrane potentials. Conversely, if the cell possessed only non-NMDA receptors, responses to larger signals would be attenuated, because the resulting depolarization would cause the membrane potential to draw closer to the reversal potential (Martin, 1955; Bekkers and Stevens, 1990; Mittman, 1990). With both NMDA and non-NMDA inputs present, larger inputs would elicit proportionally larger responses. This linearization of the response with respect to the input, caused by the unmasking of the “occult” NMDA conductance (Bekkers and Stevens, 1990), is consistent with the NMDA receptor-mediated amplification of responses that has been reported in the visual cortex (Fox et al., 1990).

Significance of Similar EPSC Time Courses in Single-Cell Processing

In many systems, NMDA receptor-mediated inputs display a much slower time course than non-NMDA inputs (Bekkers and Stevens, 1989; Dale and Grillner, 1986; Dale and Roberts, 1985; Forsythe and Westbrook, 1988; Hestrin et al., 1990; Jones and Baughman, 1988; McBain and Dingledine, 1992; Mittman et al., 1990). This distinction was accentuated by stimulating the presynaptic neuron with single, brief depolarizing pulses (Dale and Grillner, 1986; Dale and Roberts, 1985; Forsythe and Westbrook, 1988; Hestrin et al., 1990; Jones and Baughman, 1988) or by observing single quantal events (Bekkers and Stevens, 1989; McBain and Dingledine, 1992), although analogous results have been reported in physiological responses from ganglion cells in salamander retina (Mittman et al., 1990).

We observed, in dark-adapted retina, that light responses were much slower than those reported in the light-adapted preparation (Mittman et al., 1990). We believe this to be due, in large part, to the slower kinetics of the rod-driven pathway, which predominates at low light levels, as compared with the faster cone pathway, which mediates responses to higher illumination (Fain and Dowling, 1973). This distinction is evident in the difference between ganglion cell EPSCs reported in light-adapted retina, which peaked in 28 ± 10 ms (Mittman et al., 1990), and those described here, which peaked in 261 ± 89 ms. Postsynaptic receptor mechanisms in ganglion cells appear to be unaffected by the state of light adaptation: Non-NMDA receptor-mediated miniature EPSCs recorded from ganglion cells in dark-adapted retina (data not shown) displayed similar rise and decay times to those recorded in light-adapted retina (Taylor, Chen, and Copenhagen, unpublished data). Thus, in dark-adapted retina the time course of the light-evoked EPSC in ganglion cells is rate limited not by the kinetics of the postsynaptic receptors, but by the time course of release from the presynaptic bipolar cells. Glutamate release from bipolar cells has been shown to be dependent on the slowly changing membrane potential of the presynaptic terminal (Tachibana and Okada, 1991). As a result, the time courses of the EPSCs mediated by NMDA and non-NMDA receptors are similar, even though the receptor kinetics may be different.

In many areas of the brain, physiological stimuli...
from presynaptic neurons probably do not consist of simultaneous, brief pulses of neurotransmitter release, but more sustained release from numerous, asynchronous inputs. As a result, physiological responses probably are more sustained than those observed in response to single pulse stimulation (Dale and Grillner, 1986; Dale and Roberts, 1985; Forsythe and Westbrook, 1988; Hestrin et al., 1990; Jones and Baughman, 1988). For example, visually evoked responses in the cortex consist of sustained responses, some lasting almost 2 s, during which action potentials are fired from atop a plateau of depolarization (Baughman, 1988). For example, visually evoked responses in the cortex consist of sustained responses, some lasting almost 2 s, during which action potentials are fired from atop a plateau of depolarization (Baughman, 1988).

This situation is quite analogous to that observed here in retinal ganglion cells.

Experimental Procedures

Retinal Slices and Solutions

Neotenous tiger salamanders (Ambystoma tigrinum from Charles D. Sullivan Co.) were maintained under a 12 hr light-dark cycle in 3°C-4°C water. Animals were dark adapted for 90 min before being despatched and double-pithed under dim red light. Under infrared (850 nm) illumination, both eyes were removed and hemisected, and retinal slices were prepared as described (Werblin, 1978).

Solutions were delivered from an array of bottles to a 700 Il recording chamber by a gravity superfusion system at a rate of 1-2 ml/min. Superfusion saline contained 104 mM NaCl, 2 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 5 mM glucose, 5 mM hemisodium HEPES, 0.025 mM picrotoxin, and 0.0065 mM strychnine hydrochloride (pH adjusted to 7.4 with NaOH). Picrotoxin (100 mM stock in dimethyl sulfoxide) and strychnine (1 mM stock) were added just prior to the experiment. In magnesium-free solutions, MgCl2 was replaced with 1 mM CaCl2. Pipette solutions contained 84 mM CsF (or KF), 11 mM EGTA, 15 mM hemisodium HEPES, 0.4 mM MgCl2, 0.4 mM CaCl2, and 7.2 mM NaCl (pH adjusted to 7.6 with CsOH or KOH). All saline components were of reagent grade (Sigma). KF pipette solutions were used for whole-cell current-clamped recordings, and CsF pipette solutions were used for cell-attached experiments. After the experiment, digitized raw data were run through a slide window algorithm.

Following cell-attached recordings, suction and capacitive pulses were used to rupture the membrane and obtain whole-cell recordings. Input resistances with CsF in the pipette were 0.5-3 GΩ; access resistances typically were 30 MΩ. Voltage-clamped recordings and most cell-attached recordings were sampled at 1 kHz and low pass-filtered at 500 Hz. All current-clamp recordings and some cell-attached recordings were sampled at 10 kHz and filtered at 5 kHz. Because the resting potential varied during current-clamp recordings, a holding current was applied to maintain the baseline potential near the physiological resting potential of salamander ganglion cells (Lukasiewicz and Werblin, 1988). All voltages were corrected to account for the -6 mV liquid junction potential that occurred when whole-cell recordings were obtained. Data analysis was performed on a Macintosh IIcx with Igor analysis software (WaveMetrics).

Light Stimulation

Light stimuli were delivered orthogonally to the major axis of the photoreceptors. Light from a tungsten lamp source passed through a variable array of neutral density filters and a 500 nm interference filter. The stimulus, imaged through the microscope condenser, consisted of a spot 435 μm in diameter centered on the photoreceptors above the cell of interest. During experiments, stimulus intensity was set between 5 x 104 and 1.6 x 105 photons μm-2 s-1.

Spike Frequency Analysis

Spike frequencies were calculated from data obtained during cell-attached experiments. After the experiment, digitized raw data were run through a spike detector, and the exact location in time of each light-evoked action potential was stored on computer. The last 10 light-evoked responses in each experimental condition, during which time any superfused drug would have exerted its largest effect, were combined, and a spike frequency histogram was constructed using a sliding window algorithm. The average spike frequency in a 100 ms window was plotted at the time point in the center of the window. The window slid through time in 10 ms steps, and the average frequency across the window was plotted at every step. The magnitude of this histogram was divided by 10, yielding the average spike frequency response to a single light stimulus. Similar histograms were constructed from responses recorded between each change of the superfusion saline.

Somatic Model

The parameters and general structure of the somatic membrane model were adapted from two recent simulations (Ekeberg et al., 1991). Numerical methods were performed with STELLA II simulation software (High Performance Systems, Inc.), using Euler's method with a constant 50 μs step size, on a Macintosh IIcx computer. The parameters used to characterize the electrical properties of the simulated cell are shown in Table 1. Five membrane currents were expressed as follows:
Table 1. Electrical Properties of Model Cell

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_m</td>
<td>1.5 nS</td>
</tr>
<tr>
<td>G_m</td>
<td>0.03 nS</td>
</tr>
<tr>
<td>E_K</td>
<td>-65 mV</td>
</tr>
<tr>
<td>E_Na</td>
<td>50 mV</td>
</tr>
<tr>
<td>E_L</td>
<td>-80 mV</td>
</tr>
<tr>
<td>E_Lmax</td>
<td>0 mV</td>
</tr>
<tr>
<td>C_L</td>
<td>1000 nF</td>
</tr>
<tr>
<td>G_L</td>
<td>200 nS</td>
</tr>
<tr>
<td>C_normal</td>
<td>8.0 nS</td>
</tr>
<tr>
<td>G_NMDA</td>
<td>5.3 nS</td>
</tr>
<tr>
<td>G_NMDA</td>
<td>6.03 x 10^{-3}</td>
</tr>
<tr>
<td>t</td>
<td>125 ms</td>
</tr>
</tbody>
</table>

Table 2. Parameters Used to Calculate Ion Channel Rate Constants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>alpha</td>
<td>-8</td>
</tr>
<tr>
<td>beta</td>
<td>2.94</td>
</tr>
<tr>
<td>gamma</td>
<td>3.2</td>
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<tr>
<td>delta</td>
<td>0.4</td>
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<tr>
<td>epsilon</td>
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<tr>
<td>zeta</td>
<td>0.14</td>
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<tr>
<td>eta</td>
<td>0.7</td>
</tr>
<tr>
<td>phi</td>
<td>0.1</td>
</tr>
</tbody>
</table>

G_m is the membrane conductance; C_m, the membrane capacitance; E_m, the reversal (Nernst) potential for the leak; E_Na, the reversal potential for sodium; E_K, the reversal potential for potassium; E_L, the reversal potential for the cationic synaptic conductance; G_L, the maximum membrane conductance for potassium; G_Lmax, the maximum membrane conductance for sodium; G_NMDA, the maximum NMDA conductance; G_NMDAmax, the maximum non-NMDA conductance; t, a scaling factor; T, the time constant used to calculate the time course of the synaptic output.

\[ i_n = \frac{G_m}{C_m} (E_m - V_m) \]
\[ i_L = \frac{G_L}{C_L} (E_L - V_m) \]
\[ i_{NMDA} = \frac{G_{NMDA}}{C_{NMDA}} (E_{NMDA} - V_m) \]
\[ i_{NMDA} = \frac{G_{NMDA}}{C_{NMDA}} (E_{NMDA} - V_m) \]
\[ i_{NMDA} = \frac{G_{NMDA}}{C_{NMDA}} (E_{NMDA} - V_m) \]

In these equations, V_m represents the membrane potential and T is a unitless function, varying between 0 and 1, that describes the time course of the synaptic input mediated by both receptor types. T is expressed by the following equation:

\[ T = \psi(t - t_0) \exp[-(t - t_0)^2] \]

where \( \psi \) is a scaling factor and \( t_0 \) is the time (in milliseconds) at which the synaptic input begins (usually 0). \( m, n, \) and \( p \) are activation parameters, and \( h \) is an inactivation parameter, as described by Hodgkin and Huxley (1952b). The differential equation describing any one of these activation or inactivation parameters, \( i \), is

\[ \frac{di}{dt} = \alpha_i (1 - h - \beta_i) \]

where values for \( \alpha \) and \( \beta \) for each parameter \( i \) are calculated with the equation

\[ \alpha_i \beta_i = \frac{(A + BV_i)}{(C + \exp(V_n + D/E)}} \]

using the parameters listed in Table 2.

The membrane potential was then calculated according to the following equation:

\[ C_m \frac{dV_m}{dt} = i_{Na} + i_L + i_{NMDA} + i_{Non-NMDA} \]

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