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N-Methyl-D-Aspartate Receptors in the Retina

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Abstract

The vertebrate retina is a "genuine neural center" (Ramón y Cajal), in which glutamate is a major excitatory neurotransmitter. Both *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors are expressed in the retina. Although non-NMDA receptors and/or metabotropic glutamate receptors are generally thought to be responsible for mediating the transfer of visual signals in the outer retina, there is recent evidence suggesting that NMDA receptors are also expressed in photoreceptors, as well as horizontal and bipolar cells. In the inner retina, NMDA receptors, in addition to other glutamate receptor subtypes, are abundantly expressed to mediate visual signal transmission from bipolar cells to amacrine and ganglion cells, and could be involved in modulation of inhibitory feedback from amacrine cells to bipolar cells. NMDA receptors are extrasynaptically expressed in ganglion cells (and probably amacrine cells) and may play physiological roles in a special mode. Activity of NMDA receptors may be modulated by neuromodulators, such as D-serine and others. This article discusses retinal excitotoxicity mediated by NMDA receptors.

Index Entries: NMDA; NMDA receptor; glutamate; retina; information processing; excitotoxicity.

Introduction

As a full-fledged part of the central nervous system (CNS), the retina is generally thought to be an excellent model for the understanding of the neural mechanisms underlying elementary neural information processing in the brain. This is because the retina is easily accessible and it comprises only a few classes of neurons, which are organized into clearly distinct sublayers. The schematic diagram shown in Fig. 1 gives a general idea of cellular organization of the retina and the orderly arrangement of retinal neurons. There are five basic types of neurons in the retina: photoreceptors, bipolar cells, ganglion cells, horizontal cells, and amacrine cells. Signals generated in photoreceptors are transmitted to ganglion cells via bipolar cells, forming the direct line of information flow in

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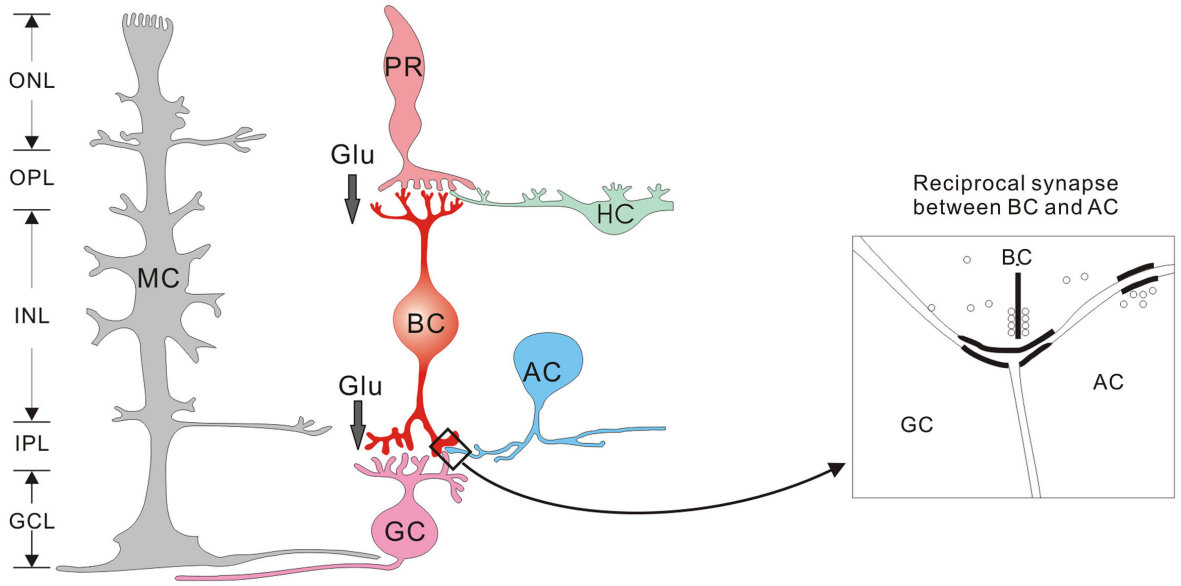

Fig. 1. A schematic diagram showing major cell types of the vertebrate retina and the orderly arrangement of these cells. Signals produced by light in photoreceptors (PRs) are transmitted through bipolar cells (BCs) to ganglion cells (GCs), forming a direct line of information flow in the retina. Information flow along this direct route is modulated by horizontal cells (HCs) and amacrine cells (ACs) in the outer and inner plexiform layers (OPL and IPL), respectively. Both photoreceptors and bipolar cells release glutamate (Glu) as a neurotransmitter. Müller cells (MCs) are the principal retinal glia, spanning the entire neural retina. Note that reciprocal synapses are formed between bipolar cells and amacrine cells in the IPL. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.

the retina. Information processing along this line is further modulated by horizontal and amacrine cells, which mediate lateral interactions in the outer plexiform layers (OPLs) and inner plexiform layers (IPLs), respectively.

In the retina, chemical transmission is predominantly responsible for cell–cell communication in the neuronal circuitry, although electrical transmission through extensive electrical coupling by gap junctions between retinal neurons may also play a role *(1)*. Signal transfer through synaptic contacts located in the OPL and IPL along the major direct route is mediated by glutamate, which is released by photoreceptors (rods and cones) in the outer retina and bipolar cells in the inner retina *(2,3)*.

Glutamate induces the activity of the postsynaptic neurons (horizontal and bipolar cells for photoreceptors in the outer retina; amacrine and ganglion cells for bipolar cells in the inner retina), an action that may come about by directly changing membrane permeability to ions or by activating intracellular signal pathways through activation of ionotropic and metabotropic glutamate receptors, respectively *(4,5)*. Notably, glutamate is tonically released from photoreceptors in the dark; therefore, most of the postsynaptic neurons, including horizontal cells and OFF-type bipolar cells, are depolarized *(6)*. Light reduces the release of glutamate from photoreceptors, thus causing changes in membrane potentials in different ways (hyperpolarization or depolarization), depending on subtypes of horizontal and bipolar cells. It should be noted that, in addition to the above signal feedforward, signals could be fed back from horizontal cells to cones, as well

as from amacrine cells to bipolar cells, through reciprocal synapses.

Müller cells are a major type of retinal glia, which that span the entire neural retina. Characteristic morphological features and geometric position of Müller cells provide the privilege to precisely modulate chemical transmission by transporters of several neurotransmitters expressed on these cells *(7)*, although uptake of glutamate by these cells may be rather limited at the synapse between rods and rod-driven bipolar cells *(8)*.

This article focuses on the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors in the vertebrate retina. It concisely describes recent advances in two important topics: characterization and modulation of NMDA receptors expressed in retinal neurons and possible physiological roles of these receptors, and NMDA-receptor-mediated retinal excitotoxicity. We do not attempt to make a comprehensively treatment describe of the topics. Prior reviews should be consulted for a detailed discussion of more extensive aspects of NMDA receptors *(4,9–16)*.

General Considerations of NMDA Receptors

Glutamate receptors are categorized into two main classes: ionotropic and metabotropic. The ionotropic glutamate receptors, which are all non-selective cation channels, are described as either NMDA or non-NMDA subtypes. NMDA receptors are characterized by a high permeability to Ca^{2+}, voltage-dependent block by Mg^{2+}, and slow gating kinetics. These receptors are known to be involved in a variety ofphysiological processes in the CNS *(17–21)*.

Cloning experiments have demonstrated that there are at least five NMDA receptor subunits: NR1 and NR2A through NR2D *(4)*. Most recently, novel subunits NR3A and NR3B have been cloned *(9,22,23)*. These subunits assemble as heterotetramers in the endoplasmic reticulum to form functional channels through distinct combinations, producing various postsynaptic responses. In the mature nervous system, NMDA receptors are composed primarily of NR1 and NR2A through NR2C *(5)*. The receptor in some neurons may contain only NR1 combined with either NR2A or NR2B. The NR1 subunit in rat has at least eight splice variant forms *(24,25)*, and splice variants are also found in NR2B-D and NR3A subunits *(9)*. On the other hand, unique genes code for each NR2 subunit *(20)* and for NR3A *(25,26)*. NR1 serves a fundamental subunit of the NMDA receptor, without which the receptor can not function, whereas NR2A through NR2D could may be regarded as modulatory subunits *(27,28)*.

The NMDA receptor channel is highly permeable to Ca^{2+} *(29,30)*. The increase in intracellular calcium levels ($[Ca^{2+}]_i$) in neuronal cells resulting from activation of the NMDA receptor channel has been shown to be responsible for modulating neuronal activity and producing neurotoxicity *(4,9,11,29,31–34)*. There is recent evidence that inclusion of an NR3A subunit attenuates the calcium current *(22,26)*. A unique feature of the NMDA receptor channel is the voltage-dependence of the receptor-mediated inward ionic currents *(5)*. This is because the channel becomes clogged by Mg^{2+} at negative membrane potentials and Mg^{2+} is driven out of the channel pore when the membrane is depolarized. Actually, the NMDA-receptor-mediated inward current is maximal between –20 and –30 mV in the external medium containing physiological concentrations of Mg^{2+} (approx 1 m*M*), and it is reduced at more hyperpolarized potentials and becomes neglected at –80 mV *(4,35)*. Therefore, at resting membrane potentials of most spiking neurons (–70 to –90 mV), NMDA receptors undergo great channel blockage by Mg^{2+}, and the block is relieved in a voltage-dependent manner when the neurons are depolarized by activation of co-localized postsynaptic non-NMDA receptors. In other words, the NMDA receptor could serve as a molecular apparatus detecting the presynaptic signal in concord with postsynaptic depolarization at the synapse. This voltage dependence is important for synaptic integration in the CNS.

The NMDA receptor channel shows desensitization with a time constant (τ), greatly depending on the subtype of the NR2 subunit that is assembled with NR1. Generally, the rate of desensitization in the native NMDA receptor is much slower (τ ~ 100 ms) than in the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (several milliseconds) *(4)*. This suggests that this receptor is mostly involved in slow and long-lasting neurotransmission.

Glycine is absolutely required for activation of the NMDA receptor channel and works as a co-agonist, without involving the strychnine-sensitive glycine receptor *(36,37)*. The fact that current responses to NMDA application are detectable in nominally glycine-free solution in various preparations may result from background glycine contamination *(38)*. It is generally thought that the NR1 subunit is a binding site for glycine, whereas the NR2 subunit is one a binding site for glutamate *(11)*. Although the binding site for glycine is located on the NR1 subunit, the affinity of the receptor for glycine depends on which subtypes of the NR2 subunit are assembled. For example, the affinity in receptors containing the NR2A subunit is about 10-fold lower than in those containing other NR2 subunits *(39,40)*. In this connection, there is recent evidence that D-serine released by glia could work as an agonist at the glycine site, playing a regulatory role for the glutamate activation of NMDA receptors *(41)*.

The classical competitive antagonists of NMDA receptors are D-AP5 and D-AP7, which are phosphorous derivatives of short-chain amino acids and act on the glutamate site *(42)*. The antagonists acting on the glycine site include halogenated quinoxalinediones and kynurenic acid derivatives, such as 5,7-dichlorokynurenic acid (DCKA). More recently, certain phthalazinedione derivatives *(43)* and benzazepinedione derivatives *(44)* are have been found to be highly potent, selective antagonists at the glycine site.

NMDA Receptors in the Outer Retina

Photoreceptors

It has been thought believed that NMDA receptors appear to be absent or rare on photoreceptors. No immunoreactivity for NMDA receptors was found in photoreceptors *(45)*, and no currents to NMDA could be induced from these cells *(46–48)*. However, recent immunocytochemical studies demonstrated the localization of some NMDA receptor subunits in photoreceptors. Using the polyclonal antiserum NR1C2′ directed against the alternative C-terminus of the NR1 subunit that recognizes splice variants 3a, 3b, 4a, and 4b in rats, Wässle and his colleagues *(49)* reported that immunolabeling for NR1C2′ is was restricted to the photoreceptor terminals (rod spherules and cone pedicles) in the outer retina, whereas the NR2A and NR2B subunits were not found at these positions *(49)*. The presence of immunolabeling for NR1C2′ in cone pedicles was confirmed by later work of this group in both rats and rabbits *(50)*. It is possible that this subunit might function as an autoreceptor for glutamate at the photoreceptor terminal. Of course, receptor subunit expression studies do not necessarily mean that the receptor must be functional, and the functional consequences of this expression remain to be explored. In fact, no change in AGB entry in cone photoreceptors was found when using 1-amino-4-guanidoutane (AGB), a sensitive indicator of changes in neurotransmitter-dependent neuronal activity *(51)* secondary to NMDA activation *(50)*.

It is particularly interesting that the NR2A and NR2B subunits are immunocytochemically localized to the outer segments of red/green and blue cones and to the tips of rod outer segments *(52)*. Because NMDA receptors are highly permeable to Ca^{2+}, such localization of these subunits suggests that the NMDA receptor may provide a possible alternative pathway (other than the cyclic guanosine

monophosphate-gated Ca^{2+} channel) for Ca^{2+} entry into the outer segments, thus modulating the Ca^{2+}-mediated phototransduction cascade. However, immunolabeling for NR2A and NR2B subunits in cone outer segments were not confirmed by other studies *(49,50)*.

Horizontal Cells

It is believed that the transfer of visual information from photoreceptors to horizontal cells is almost exclusively mediated by the AMPA receptor subtype *(53–58)*. Most of the immunocytochemical studies did not show immunoreactivity for the NMDA receptor subunits in horizontal cells of various species (goldfish *[45]*, rat *[50]*, and rabbit *[59]*). However, a few recent studies provided morphological evidence, suggesting the existence of NMDA receptors in horizontal cells. Faint labeling of NR1 was detected in cell bodies but not processes of rat horizontal cells *(49)*. Furthermore, Gründer et al. *(60)* reported in the same species that both NR1 and NR2 labeling was seen observed in horizontal cells *(60)*. Immunoreactivity for the NMDA receptor subunits is was also reported in adult human horizontal cells in culture *(61)*.

No current responses could be induced by NMDA in horizontal cells *(62,63)*. Although NMDA was found to uncouple horizontal cells of goldfish and to reduce their light responses, these effects of NMDA were thought to be mediated by dopamine released from interplexiform cells *(64)*. The lone exception is was the catfish horizontal cell, from which currents could be evoked by NMDA *(65)*. The NMDA-induced currents were suppressed by D-AP5 and potentiated by glycine. They were blocked by Mg^{2+} in a dose-dependent manner. Furthermore, activation of the NMDA receptors in catfish horizontal cells produces produced a long-term downregulation of voltage-gated sodium and calcium currents and may play a role in modulating information about light/dark transition in the retina *(66)*. This effect of NMDA requires calcium release from a ryanodine-sensitive intracellular calcium store and activation of a calmodulin-dependent signaling pathway *(67)*.

In a recent work of the authors' in our laboratory *(68)* it is shown in the carp retina that immunolabeling for NR1 is was seen in cone-driven (H1) but not rod-driven (H4) horizontal cells. In accordance with the immunocytochemical data, NMDA induces inward currents from the H1 cells, and the currents are completely blocked by D-AP5. NMDA also increases $[Ca^{2+}]_i$ in these cells. No such effects are have been observed in the H4 cells. These results raise an intriguing possibility regarding whether the expression of NMDA receptors may have been largely overlooked. Therefore, it would be interesting to re-examine whether horizontal cells in other species may also express functional NMDA receptors.

Bipolar Cells

Inception of the ON and OFF pathways that convey information related to the onset and offset of a light step occurs at the bipolar cell level *(69)*. In addition, signals from rods and cones are exclusively (in mammals) or relatively (in non-mammals) separated at this cell level *(70)*.

Physiologically, current responses to NMDA could be recorded from neither ON type bipolar cells *(71–73)*, nor OFF type bipolar cells *(74)* (but *see* below). In mammals, NMDA receptors appear not to be associated with ON type bipolar cell axon terminals *(49)*. Although basal AGB labeling is noted in ON type bipolar cells, this labeling is not modulated by NMDA *(50,59)* and is completely abolished by L-AP4, (an metabotropic glutamate receptor antagonist), suggesting no involvement of NMDA receptors *(50)*. However, there is also evidence provided by immunocytochemistry and *in situ* hybridization suggesting the localization of NMDA receptor subunits on bipolar cells. In primates, NR1C2' is specifically localized with flat midget bipolar cell axons *(75)*. In the rat, retina labeling for NR2C is found in the somata

of bipolar cells by *in situ* hybridization *(76)*. In both rat and rabbit, rod bipolar cells are NR2D-immunoreactive, and the labeling is moderate in dendrites arborizing in the OPL, intense at the pole of the soma facing the OPL, but low in the part of the soma in the INL *(77)*. In particular, labeling for NR2D is strongest in proximal axonal segments and axonal endfeet in the IPL *(77)*, suggesting that these receptors modulate lateral interactions in the IPL and/or work as presynaptic autoreceptors for controlling glutamate release *(78)*. Accordingly, the NR1 subunit is present in mouse rod bipolar cells *(79,80)*. Results provided by electophysiological recording from rod bipolar cells in the mammalian retina are apparently inconsistent. Although isolated rat rod bipolar cells are responsive to NMDA *(81)*, recording from these cells in the retinal slice preparation produces no evidence of NMDA-receptor-mediated responses *(72)*. It is unclear why NMDA receptors are present in these depolarizing cells because glutamate acts to close channels of these cells by activation of metabotropic glutamate receptors. Moreover, rat cone bipolar cells appear to be unresponsive to NMDA *(82)*.

NMDA Receptors in the Inner Retina

Amacrine Cells

More than 20 types of amacrine cells have been described by physiological, biochemical criteria *(83–85)*, and all these cells receive glutamatergic synaptic input from bipolar cells. Many studies have reported the existence of NMDA receptors in amacrine cells. Early intracellular recordings have shown that many subtypes of amacrine cells are depolarized by NMDA, suggesting that they possess NMDA receptors *(86,87)*. In lower vertebrates, labeling for NR1 has been shown to be confined to amacrine and ganglion cells *(45)*. Electrophysiological experiments have indeed demonstrated that light-induced responses of amacrine cells in the tiger salamander retinal slice preparation are mediated by both non-NMDA and NMDA receptors *(88)*. However, there are also reports showing that NMDA receptors contribute little or nothing to light-induced responses of amacrine cells *(89–91)*. Notably, isolated amacrine-like cells in the carp retina have been shown electrophysiologically to express AMPA receptors predominantly because glutamate-induced currents are completely suppressed by GYKI56355, a specific AMPA receptor antagonist *(56)*. A possible explanation for the difference is that papain digestion may have eliminated NMDA receptors of in these cells.

In the mammalian retina, the NMDA receptor subunits are expressed in subsets of amacrine cells by *in situ* hybridization and immunocytochemistry *(49,50,76,77)*. Interestingly, it is has been shown in the cat retina that all γ-aminobutyric acid (GABA)ergic amacrine cells and displaced amacrine cells express the NR2A subunit *(77)*. Together with the fact that NMDA directly stimulates the release of GABA from amacrine cells *(92–96)*, this result suggests that the NMDA receptor may play a crucial role in modulating GABAergic inhibitory feedback to rod and cone bipolar cells through the reciprocal synapse (*see* Fig. 1). Most recently, the NR3A subunit has been found in mouse displaced amacrine cells, which could modulate NMDA-receptor-mediated calcium influx in these cells *(22)*. It is suggested that the expression of NMDA receptors in mammalian amacrine cells may depend on photoreceptor signal types by which these cells are driven. The NR1, NR2A, and NR2B subunits are present on cone-driven amacrine cells, but are not expressed at the rod bipolar cell dyads *(49,50,76,97)*. It is further speculated, based on the data obtained using AGB, that the NMDA receptor is functional in the cone-driven pathway in the inner retina *(50)*. Whether or not the NMDA receptor is involved in the rod-driven pathway is not certain. Electrophysiological experiments conducted on AII amacrine cells that receive input from rod bipolar cells actually yielded different results. Whereas Boos et al. *(97)* found no evidence for NMDA receptors in these cells, Hartveit and Veruki *(98)* found that these cells

were responsive to NMDA and the responses could be blocked by the specific NMDA receptor antagonist CPP *(98)*, although activation of NMDA receptors was shown not to not be essential for the rod signal feedforward from bipolar cells to amacrine cells *(99)*.

Ganglion Cells

Similarly to amacrine cells, ganglion cells appear to express NR1 and NR2A-C *(45,100, 101)*. Again, NR3A is expressed in mouse ganglion cells *(22)*. In compliance with the morphological data, physiological experiments have demonstrated that most of ganglion cells are responsive not only to kainate, but also to NMDA *(86,89,90,102,103)*. Researchers have generally concluded that ganglion cells express both NMDA and non-NMDA receptors, and that there is interplay between the responses mediated by these two receptor types. Blockade of NMDA receptors tends to result in a more transient light response of primate ganglion cells that appears to be mediated by non-NMDA receptors *(104)*. In salamander ON type ganglion cells, the effect of blockade of NMDA receptors on the light response is most prominent for the sustained component *(105)*. Furthermore, the contribution to the excitatory response of ganglion cells mediated by NMDA receptors is increased by an activation of non-NMDA receptors in salamander *(106)*, which could, at least partly, result from a relief of the Mg^{2+} block of the NMDA receptors. Conversely, an enhanced contribution mediated by NMDA receptors is observed in ganglion cells of the same species when non-NMDA receptors are blocked, a phenomenon that is supposed to be result from a suppression of inhibitory input to these cells from amacrine cells *(107)*. Interestingly, for directionally sensitive ganglion cells of primate and rabbit, blockade of both nicotinic acetylcholine receptor and NMDA receptors considerably suppresses the light response of these cells and eliminates the directional selectivity, whereas the light response is intact with AMPA receptors being blocked *(108,109)*.

NMDA receptors are traditionally assumed not to be much activated at rest in neurons because of the voltage-dependent Mg^{2+} blockade. However, it is reported that background activation of NMDA receptors is present at the resting membrane potential (approx –70 mV) in salamander ganglion cells in Ringer's containing 1 m*M* Mg^{2+}, suggesting contribution of these receptors to the baseline noise *(110)*. This background NMDA channel activity may be caused by an endogenous level of glutamate, although the source remains to be determined.

NMDA-receptor-mediated responses of ganglion cells are modulated by modification of the glycine-binding site of these receptors. In the tiger salamander retinal slices, excitatory postsynaptic currents (EPSCs) of ganglion cells, induced by potassium puffs or light stimulation, are reduced by application DCKA *(111)*. On the other hand, D-serine functions as an agonist at the glycine-binding site of NMDA receptors *(38)*. It is proposed that D-serine released by glia may play a regulatory role as a co-agonist necessary for the glutamate activation of NMDA receptors in the CNS *(112)*. In the vertebrate retina, D-serine and its synthesizing enzyme, serine racemase, are localized to retinal glia cells (Müller cells and astrocytes), and because D-AAO, (a D-serine-degrading enzyme) significantly reduces NMDA-induced currents of ganglion cells and proximal negative components, D-serine may be a necessary component of full activation of the NMDA receptor *(41)*. Background levels of D-serine, measured by capillary electrophoresis, are at least in the low micromolar range *(113)*, so that D-serine may contribute to modulation of the baseline noise for which NMDA receptors are partly responsible *(110)*. Moreover, D-serine was found to cause a suppression of light-evoked EPSCs in ganglion cells, an effect that may result from the suppressive action of D-serine at a site presynaptic to the ganglion cells *(111)*.

There is a puzzle concerning functional expression of NMDA receptors in ganglion cells (and probably amacrine cells). It is shown that evoked EPSCs comprise two components

mediated by non-NDMA receptors and NMDA receptors, respectively *(102,106,114–116)*. However, spontaneous EPSCs are solely mediated by AMPA receptors *(116,117)*. In other words, only non-NMDA receptors are responsive to quantal release of glutamate *(116–119)*. These results suggest that NMDA receptors may be located extrasynaptically and could be activated only by the concomitant release of many vesicles. Several important studies have supported this notion. Using paired recording of bipolar cells and ganglion cells (or displaced amacrine cells) in the newt retina, Tachibana and colleagues *(116)* demonstrated in the newt retina, that using paired recording of bipolar cells and ganglion cells (or displaced amacrine cells), NMDA receptors on ganglion cells are mainly responsible for mediating prolonged glutamate release from bipolar cells, suggesting that these receptors may be localized slightly away from the glutamate release sites *(116)*. They further showed that light-evoked EPSCs of mouse ON-type transient amacrine cells are predominantly mediated by non-NMDA receptors when $GABA_C$-receptor-mediated inhibitory feedback via the reciprocal synapse between these cells and bipolar cells (*see* Fig. 1) is intact, and blockade of $GABA_C$ receptors results in dramatic changes in the amplitude and time-course of the light-evoked EPSCs in ON-type transient amacrine cells, which could result from the activation of NMDA receptors *(120)*. This result is consistent with the idea that the activation of presynaptic $GABA_C$ receptors may limit the extent of glutamate release from bipolar cells and blockade of $GABA_C$ receptors could lead to a spillover of glutamate that activates NMDA receptors located slightly away from the release sites of glutamate.

Researchers have found that glutamate encountered by NMDA receptors of rat ganglion cells and displaced amacrine cells is less than by that by AMPA receptors during evoked EPSCs. A NMDA-receptor-mediated component in spontaneous EPSCs could be revealed by reducing glutamate uptake *(114)*. A recent work about the interplay between AMPA receptors and NMDA receptors on goldfish amacrine cells mediated through $GABA_A$ and $GABA_C$ receptors in bipolar cells also favors of the notion that NMDA receptors may be located further away from glutamate release sites and therefore, thus may only be activated by glutamate spillover *(121)*.

NMDA-Receptor-Mediated Retinal Excitotoxicity

In addition to their physiological roles, the activity of NMDA receptors is closely related to glutamate-caused excitotoxicity in the retina. Diseases that are associated with excitotoxicity include glaucoma, retinal ischemia, and diabetic retinopathy *(122–124)*, in which excessive stimulation of glutamate receptors, including NMDA receptors, has been demonstrated to result in the degeneration of retinal neurons. It is generally thought that excess glutamate release in the retina, leading to prolonged Ca^{2+} influx, is a major mechanism for neuronal injury and death *(12,15,34,125)*. Because NMDA receptors are highly permeable to Ca^{2+}, they may play a crucial role in glutamate-induced excitotoxicity.

In animal models in vivo, intravitreous NMDA administration induces thinning of the inner retina, ganglion cell apoptosis, and a loss of vision *(34,126,127)*. Notably, chronic administration of NMDA causes changes in retinal histology that are remarkably similar to the histopathology seen in human glaucoma *(128, 129)*. In models in vitro, glutamate, NMDA, and kainate cause ganglion cell death in a Ca^{2+}-dependent manner and the effect could be blocked by NMDA receptor antagonists, indicating the involvement of NMDA receptors *(130)*. Interestingly, the toxicity caused by kainate could be largely blocked by D-AP5 *(33)*, strongly suggesting that kainate may stimulate the release of glutamate by activating non-NMDA receptors, which in turn causes ganglion cell death by activating NMDA receptors. However, there are reports indicating that both NMDA and non-NMDA receptors are required for glutamate-induced excitotoxicity *(131)* and

excitotoxicity in ganglion cells results solely from non-NMDA receptors *(132)*. Conversely, changes in amacrine cells caused by retinal ischemia may be caused by overactivation of both NMDA and non-NDMA receptors. For example, retinal ischemia reduces immunoreactivity for GABA and chlorine acetyltransferase in rabbit amacrine cells, an effect that is antagonized by NMDA and non-NMDA antagonists *(133,134)*.

Although numerous studies in vitro and in vivo have shown that the postnatal and adult ganglion cells are vulnerable to NMDA-receptor-mediated excitotoxicity *(15,34)*, a recent study re-examined the effects of glutamate and NMDA on rat ganglion cells in vitro and in vivo and showed that when ganglion cells are exposed to high concentrations of glutamate or NMDA, ganglion cells, unlike amacrine cells, were not much affected. Therefore, it is concluded that these cells are invulnerable to NMDA-caused excitotoxicity *(135)*.

This important work challenges the general view that NMDA-caused excitotoxicity contributes to death of ganglion cells in glaucoma and retinal ischemia. Two possible explanations have been proposed for the difference in vulnerability between amacrine cells and ganglion cells *(135)*. First, NMDA receptors in ganglion cells are extrasynaptic so that the effect on currents mediated by these receptors may be reduced. Second, NR3A that reduces NMDA-induced intracellular calcium responses is strongly expressed in ganglion cells, but not in amacrine cells *(22)*.

The intracellular events produced by overactivation of NMDA receptors that ultimately lead to amacrine and ganglion cell death have been extensively studied, but are still not completely clear. In other systems, possible mechanisms are that the increase in $[Ca^{2+}]_i$ during NMDA-induced excitotoxicity may lead to changes in the activity of several signaling pathways *(10)*. Among others, neuronal NO synthase (nNOS) may play a critical role in the retina. In other words, excessive Ca^{2+} influx triggers formation of NO via nNOS and NO modulates the activity of a variety of proteins that contribute to apoptosis and other biological processes that are involved in cell death. This is supported by the experiment conducted in the NOS-knockout mouse that much less loss of ganglion cells is found than in the wild-type mouse *(128)*. A recent work *(136)* further suggested that the effects of NO in the retina might be mediated, in part, by activation of matrix metalloproteinase-9 (MMP-9), an extracellular endpeptidase that modulates cell–cell extracellular matrix interaction *(137)*, via S-nitrosylation. It should be noted that NO could be neurodestructive or neuroprotective, which is explained by a redox-based mechanism *(138)*. There is also evidence indicating the involvement of the signaling pathways, other than the NO pathway, in ganglion cell death. For example, the Rho signaling pathway that has been implicated in a variety of biological processes *(139)* may be involved in NMDA-induced excitotoxicity in ganglion cells and the Rho kinase inhibitor fasudil ameliorates excitotoxicity *(140)*.

NMDA-induced retinal excitotoxicity has been ameliorated by several ways. This article only discusses the ways that are related to modification of the NMDA receptor activity. In theory, NMDA receptor antagonists, such as MK-801, flupirtine, and memantine, could be used for this purpose, and this has been shown to be true, to some extent, in experimental studies *(141–143)*. However, complete blockade of the activity of NMDA receptors is not a viable therapeutic approach because normal activity of these receptors that is essential for visual information processing in the retina must be preserved. Appropriate ways therefore are to bring excessive activation of NMDA receptors back to a normal level. Among others, memantine is of particular interest. Memantine is an uncompetitive NMDA receptor antagonist*, exerting its effects on NMDA receptor activity

*An uncompetitive agonist, distinct from a noncompetitive agonist, is defined as an inhibitor that produces actions that are contingent on prior activation history of the receptor by the agonist.

by binding at or near the Mg^{2+} site of the receptor. This agent is quite promising because it has been shown to be relatively ineffective at blocking the low levels of NMDA receptor activity associated with normal neuronal function, but to become exceptionally effective at higher levels associated with excessive activation of NMDA receptors *(14)*.

Future Perspectives

Although functional roles of NMDA receptors in the vertebrate retina and possible mechanisms underlying NMDA-receptor-mediated retinal excitotoxicity have been extensively examined in recent years, much more remain to be further studied. Several topics are provided here that in our opinion may represent future directions in this research field.

1. Characteristics of a NMDA receptor largely depend on the subunit composition of the receptor. For example, it is known that the NR2 (A-D) subunits are the primary determinants of the Mg^{2+} sensitivity of NMDA receptors *(20,144)*, and the NR2C subtype exhibits a weaker Mg^{2+} block than other subtypes *(12)*. This means that NMDA receptor subtypes with different subunit compositions may be differentially expressed on different types (or subtypes) of retinal neurons, performing distinct physiological functions. Determination of which NMDA receptor subunits expressed at the synapse of a given cell type and exploration of the dependence of functional roles on the subunit composition in the retina are just at the beginning and will be further pushed further forward.
2. Application of molecular genetics in retina research has proven fruitful *(145,146)*. For example, a knockout of the metabotropic glutamate receptor subunit mGlu6 greatly reduces ON responses of retinal neurons in the rat *(145)*. However, no similar work has been done regarding retinal NMDA receptors. Knocking in and out of a special gene encoding a subunit of the NMDA receptor will definitely provide insight into functions of the subunit.
3. Physiological roles of NMDA receptors have been well-studied in the inner retina. However, considering in consideration of complex synaptic and functional organization in this region, the data that have been accumulated in these years are still fragmentary. Both amacrine and ganglion cells could be further classified into a variety of various subtypes by physiological and structural criteria. Although NMDA receptors are known to be more involved in sustained responses of these cells, how the mechanism by which functional characteristics of NMDA receptors could be related to subtypes of these cells requires further study. Moreover, the story in the inner retina becomes more complicated because of the existence of inhibitory feedback through GABAergic reciprocal synapses. This feedback could may be mediated by at least four types of GABA receptors: $GABA_A$ receptor, two pharmacologically distinct $GABA_C$ receptors, and $GABA_B$ receptor *(147–149)*. Modulation of functions of NMDA receptors by such inhibitory feedback could be important for the understanding of information processing in the inner retina.
4. Although non-NMDA receptors are believed to mediate the transfer of visual signal in the outer retina predominantly, several studies have shown the existence of NMDA receptors in photoreceptors, as well as horizontal and bipolar cells. Functional significance of these receptors is unclear and should be determined.
5. Physiological data may account for no involvement of NMDA receptors in spontaneous EPSCs of ganglion cells (and displaced amacrine cells) by assuming that these receptors are located extrasynaptically. What are physiological roles for these extrasynaptic NMDA receptors? An alternative explanation is that these receptors and non-NMDA receptors are separately at different synapses and what could be detected are only the AMPA-receptor-mediated ones receptors can be detected *(117)*. Studies are needed to determine which one (or whether both) is correct.
6. Roles of NMDA receptors during development of the retina remain unclear. The early expression of the NR2A subunit in the rat retina suggests a possible role of this subunit in the establishment of synaptic connections in the IPL *(100,150)*. However, a study conducted in the rabbit *(151)* argues against NMDA receptors and suggests that non-NMDA receptors are involved.
7. NMDA-receptor-mediated retinal excitotoxicity is of both theoretical and clinical significance. The question regarding whether or not ganglion cells are indeed vulnerable to NMDA excitotoxi-

city should be clearly determined, considering the recent work showing that ganglion cells are not quite susceptible *(135)*. On the other hand, mechanisms underlying cell death of retinal neurons—especially ganglion cells—resulting from overactivation of NMDA receptors will be further investigated and new neuroprotective ways will be developed.

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