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NMDA Receptor-mediated Synaptic Plasticity in Developing Mammalian Visual Pathways

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List of Abbreviations

ATP . . . . . Adenosine Triphosphate

Ca++ . . . . . Calcium ion

CNS . . . . . Central Nervous System

D-APV . . . . . (D)-Amino-5-phosphonovaleric Acid

DNA . . . . . Deoxyribonucleic Acid

EAA . . . . . Excitatory Amino Acid

EPSC . . . . . Excitatory Post-synaptic Potential

EPSP . . . . . Excitatory Pos-synaptic Current

GABA . . . . . Gamma-aminobutyric Acid

GluR . . . . . Glutamate Receptor

IPSP . . . . . Inhibitory Post-synaptic Potential

LGN . . . . . Lateral Geniculate Nucleus

LTP . . . . . Long-term Potentiation

MAP . . . . . Myelin-associated Protein

Mg++ . . . . . Magnesium Ion

Na++ . . . . . Sodium Ion

NMDA . . . . . N-Methyl-D-Aspartic Acid

NMDAR . . . . . N-Methyl-D-Aspartic Acid Receptor

PKC . . . . . Protein Kinase C

PTK . . . . . Protein Tyrosine Kinase

RGC . . . . . Retinal Ganglion Cell

Abstract

NMDA RECEPTOR-MEDIATED SYNAPTIC PLASTICITY IN DEVELOPING MAMMALIAN VISUAL PATHWAYS

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Precise connections in many mammalian nervous systems require a great deal of remodeling during development. In the visual system, many excess synapses are originally formed in the lateral geniculate nucleus and striate cortex. Only the correct set of axon terminals are retained during normal development, while imprecise ones withdraw. The mechanism by which only correct axons are retained requires neural activity, and may be regulated by specific receptors at synapses.

The transmission of neural signals at these synapses is carried out in part by the glutamate-activated NMDA receptor. It is hypothesized that NMDA receptor activation plays a crucial role in enhancing only those

connections in the immature system which will form a retino-topically correct map in the LGN and cortex. NMDA receptor activation requires depolarization of the neuron membrane. Possibly, only neurons transmitting information from nearby areas in the retina summate to produce NMDA receptor- mediated currents. The result is an influx of Ca++ ions that has been shown to cause trophic effects within the cell that could enhance the synaptic connection. Thus, NMDA receptors may act to detect coincident neural activity in immature animals, thereby allowing only visuo-topically related axon terminals to undergo enhancement of synaptic transmission and structure. As development proceeds, NMDA receptor function decreases, possibly reducing these intracellular effects.

Blocking NMDA receptor activation experimentally does alter the normal set of connections in the visual system. Yet, is there a direct cause-and-effect relation between NMDA receptor activity and anatomical changes? Many cellular events probably result from NMDA-mediated currents. Intracellular changes in phosphorylation states and protein levels could eventually alter a synapse at the anatomical level. Study of the changing NMDA receptor subunit types making up the receptor within visual system structures could reveal, in part, the means by which plasticity is down-regulated. The experimental regulation of these subunits in vivo could reveal important information concerning their specific function if plasticity and development were to be altered as a result. A summary of previous studies, and proposals for further research

concerning the role of the NMDA receptor and its various types in developing visual pathways are presented in this manuscript.

INTRODUCTION

The adult mammalian visual system is characterized by highly complex and precise patterns of neural connectivity. Remarkable examples of such precision in connectivity are the topographic ordering of projections and segregation of inputs from the two eyes at successive levels of visual information processing. Ganglion cells from each eye project to the lateral geniculate nucleus (LGN) on both sides of the brain. Retinal axons within the LGN terminate in separate eye-specific layers that receive afferents from one single retina. Relay neurons in the LGN then project to neurons in layer 4 of the primary visual cortex, where information from each eye is again kept segregated in alternating patches. These patches represent the system of ocular dominance columns at the level of Layer IV. How are these highly ordered sets of axonal connections present in the adult central nervous system formed during development?

Remarkably, neither the layers in the LGN nor the cortical columns are present initially during development. Thus, the laminar organization present in the adult LGN of ferrets, cats, nonhuman primates, and humans is not present during early development, when retinogeniculate

connections are diffuse and display extensive overlap of the contra- and ipsilateral retinal fibers (Rakic, 1976; Linden et el., 1981; Shatz, 1983). Complete segregation of retinal afferents from each eye occurs concurrently with the cytoarchitectonic differentiation of the LGN layers. The eye-specific layers emerge as axons from the two eyes gradually remodel by withdrawing branches that are formed in inappropriate territory and further growing branches that have formed in the appropriate territory (Sretavan and Shatz, 1986).

Ocular dominance columns in the visual cortex also form from an initial condition of intermixed LGN inputs related to the two eyes. Thus, initially neurons in cortical layer IV receive functional inputs from LGN afferents representing both eyes (Levay et al., 1978, 1980) and ocular segregation emerges subsequently. Interestingly, the formation of the LGN layers occurs before ocular dominance columns appear within the cortex. Thus, formation of layers in the LGN occurs even before eye-opening, and, thus, before visual activity is possible, during approximately the first two postnatal weeks in the ferret (Linden et al., 1981) and during fetal life in cats (Shatz, 1983) and primates (Rakic, 1976). In contrast, formation of ocular dominance columns occurs postnatally in cats and monkeys (Levay et al, 1978, 1980), at a time when visually driven activity has already started. Patterned visual activity is critical for the formation of ocular dominance columns. In summary, acquisition of the precise pattern of adult connectivity in the visual system requires extensive remodeling of

connections probably involving elimination of inappropriate synapses (Campbell and Shatz, 1992). How do inputs representing the two eyes segregate to form eye-specific layers in the LGN and ocular dominance columns in the primary visual cortex?

Activity-Dependent Mechanisms During Development

In this manuscript, evidence will be reviewed that indicates neuronal electrophysiological activity, especially involving the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptor, regulates remodeling of visual connections during development. It has been appreciated for several years that the electrical activity of neurons is fundamental for maturation of form and function in the mammalian brain (for a review, see Goodman and Shatz, 1993). The classical work of Wiesel and Hubel on the effects that visual deprivation (Wiesel, 1963) has on the maturation of form and function in the visual cortex has provided critical insight into the role of activity in neural remodeling. These authors have discovered that if one eye is deprived of vision by closing the eyelids for at least a few days, the majority of neurons in the visual cortex respond only to the eye that remained open (in contrast, in the normal cortex, the majority of neurons are binocularly driven). In addition to this physiological shift in ocular dominance, it has been shown that the anatomical organization of LGN axons within layer IV also changes. Following a period of monocular deprivation, axons representing the open eye occupy most of layer IV,

whereas in normal animals they would occupy approximately half of this layer (Hubel et al, 1977). Activity-dependent competition for synaptic contacts between axon terminals from the two eyes for layer IV neurons was proposed to drive the formation of ocular dominance columns during normal and abnormal development. The greater activity displayed by neurons driven by the open eye provides them with an advantage in the competition for synaptic terminals within the cortex. As a result, the deprived eye terminals lose connections within layer IV while connections from the open eye are stabilized.

In the LGN in particular, recent results have indicated that neuronal electrophysiological activity plays a critical role in binocular segregation. Thus, the reduction of activity by blockade of voltage-dependent sodium channels (Shatz and Stryker, 1988) or blockade of N-methyl-D-aspartic acid receptors (Hahm et al., 1991) in LGN neurons prevents normal maturation of connectivity. Since eye-specific layers in the LGN are formed before the retina is mature enough to detect light stimuli, it is likely that spontaneous visual activity plays a critical role in neural remodeling. Consistent with this possibility, recent results have shown that the retinal ganglion cells are spontaneously active during early fetal life (Galli and Maffei, 1988; Meister, et al, 1991). These findings raise the questions of how does activity influence the process of neural remodeling during development, and what role do NMDA receptors play in activity-dependent neural remodeling?

Activity-Dependent Mechanisms and the Role of NMDA Receptors

As discussed in the previous section, the amount and pattern of activity at the retinogeniculate and geniculocortical pathways may determine whether synapses are eliminated or stabilized during development of the LGN and visual cortex, respectively. According to a model initially proposed by D. O. Hebb (Hebb, 1949) to explain mechanisms of learning, synapses are strengthened where activity coincides with target cell depolarization sufficient to trigger action potentials. Stent (1973) has argued that synchronous synaptic activation and firing of action potentials by the postsynaptic neuron protects neurotransmitter receptors within the active synaptic area. In contrast, synapses would be weakened where activity is not correlated with post-synaptic activation (Stent, 1973).

The Hebbian model of potentiation as further elaborated by Stent may explain ocular dominance plasticity. In the monocular deprivation experiments of Wiesel and Hubel (1963), a sufficient level of synchronous activity would occur at geniculocortical synapses driven by the open eye to generate action potential activity in cortical neurons. This would in turn lead to strengthening of the activated synapses. In contrast, synapses driven by the deprived eye would not be activated in synchrony with action

potential activity in the target neurons. These synapses would be weakened and eventually lost.

Evidence supporting the Hebbian model of synaptic plasticity during visual system development has been reported recently. In animals that had received intraocular injection of tetrodotoxin to block retinal ganglion cell activity, it has been shown that direct electrical stimulation of optic nerves may lead to rearrangements in geniculocortical connectivity (Stryker and Strykland, 1986). When right and left eye retinal fibers were stimulated at slightly different times, asynchronously, separation of geniculocortical terminals into eye-specific columns was found to occur. In contrast, concurrent stimulation of the two sides did not lead to the segregation.

The NMDA subtype of excitatory amino acid receptors may play an important post-synaptic role in Hebbian mechanisms of plasticity. Consistent with this possibility, it has been shown that blocking NMDA receptor activation in cortical layer IV by continuous application of a specific NMDA receptor antagonist, D-APV, can block the effects of monocular deprivation (Kleinschmidt, et al, 1987). Thus, most neurons in the cortex of untreated animals responded only to stimulation of the open eye, while in the D-APV treated animals, a large percentage of neurons were driven by the deprived eye. If NMDA receptors are indeed involved in visual plasticity, how can they detect the relative amounts of activity at synapses? Furthermore, does their activation contribute to the strengthening of a synapse?

Biophysical Properties of NMDA Receptors

The biophysical properties of the NMDA subtype of excitatory amino acid receptors suggests that it may play a post-synaptic role in activity-dependent increases in synaptic strength (for a review, see Constantine-Paton, et al., 1990). The most unique property of the NMDA receptor is that the associated ion channel is blocked by Mg++ at the resting membrane potential and hyperpolarized voltage potentials (Nowak, et al., 1984; Mayer, et al., 1984). Glutamate binds the receptor, but activation also requires removal of the magnesium ion within the associated channel. Whole-cell recordings conducted in nominally-free Mg++ solution revealed that NMDA receptor-induced inward and outward currents vary linearly with the voltage. In contrast, 550uM Mg++ added to the bathing medium greatly diminished the inward currents at negative but not positive potentials. Single channel properties similarly changed markedly at negative recording potentials when Mg++ was applied to the medium (Nowak, et al., 1984). Thus, the mean open time of channels was lowered from 4.7ms in Mg++ free solution to 1.2ms in 10uM Mg++, and to .7ms in 100uM Mg++. However, the number and duration of short closings of the channels

increased during application of magnesium. The average burst time (a group of channel openings occurring less than 5ms apart from each other) decreased from 5.7ms in Mg++ free solutions to 3.2ms in 100uM Mg++. The Mg++ ion block may make the NMDA receptor ideally suited as a detector of the amount of activity occurring at a synapse. Thus, NMDA receptors would become optimally activated when a critical amount of neural activity causes first a substantial depolarization of the post-synaptic membrane through other ion channels, and the release of glutamate. When the membrane is sufficiently depolarized by strong synaptic activation of non-NMDA excitatory receptors, such as may occur when neighboring retinal ganglion cells connected to the same LGN neuron (or neighboring LGN neurons connected to the same cortical neuron) fire synchronously, Mg++ blockade of the NMDA channels is relieved and the cell is further depolarized. When this happens, intracellular Ca++ enters the cell via the ionic channel linked to the NMDA receptor and the concentration of intracellular Ca++ has been shown to increase (MacDermott et al., 1986). This increase is due specifically to the activation of NMDA receptors, since when Mg++ ion were added to the extracellular milieu, the Ca++ build-up was found to take place only at depolarized potentials (MacDermott, et al., 1986). The influx of Ca++ ions may have important trophic effects on the neuron. Since Ca++ regulates many intracellular cascades, including phosphorylations, NMDA receptor mediated Ca++ influx might affect synaptic survival during development.

For this reason, it has been proposed that Hebbian synaptic consolidation occurs when the Ca++ flux exceeds a threshold level (Bear, et al., 1987).

One example of synaptic plasticity that may involve NMDA receptors is long-term potentiation (LTP) of synaptic transmission, which has been shown at synapses in the hippocampus (Bliss, et al., 1973). When an afferent fiber is tetanically stimulated at a certain frequency (10-100Hz), synaptic transmission may be enhanced such that excitatory post-synaptic potentials (EPSP) are potentiated for hours or even days (Bliss, et al., 1973), a phenomenon that may play a role in memory processes (Morris, et al., 1990). This phenomenon appears to result from increased Ca++ currents flowing through NMDA receptors as a result of the tetanic stimulation (Lynch, 1983). Whether such a process contributes to plasticity in the visual system is still unclear. Synaptic potentiation has been revealed in the visual thalamus (Mooney, et al., 1993) and cortex (Teyler, 1993; Kirkwood, 1994). This potentiation is, however, surprisingly difficult to elicit and much weaker than that present in the hippocampus.

Developmental Changes in NMDA Receptor Function

The proposal that NMDA receptors may underlie Hebbian plasticity has motivated some recent work aimed at learning the role of NMDA receptors in LGN and visual cortex synaptic transmission and plasticity during development. These studies have indicated that NMDA receptors play a critical role in synaptic transmission in the developing LGN (Mooney, et al., 1993; Ramoa and McCormick, 1994) and cortex. Moreover, the functional role of NMDA receptors changes during the course of visual system development. In both LGN (Ramoa and McCormick, 1994a, b) and cortex (Agmon, 1992; Carmignoto, 1992) NMDA receptor-mediated synaptic transmission is markedly enhanced in the CNS during early development. Thus, application of the NMDA receptor antagonist D-APV was found to block the visual responses of neurons in layer IV of kittens to a greater degree than in adult cats (Tsumoto, et al., 1987). Recordings of visual responses of cortical neurons revealed that D-APV suppressed the visual responses of only 33% of adult cortical cells, in contrast to 71% of kitten cortical cells. The non-specific glutamate receptor antagonist kynuremic acid blocked about 71% of cells in both cats and kittens.

Several mechanisms may contribute to facilitation of the NMDA receptor response in immature LGN and cortical neurons. The first reason is that changes occur in the kinetic properties of the NMDA receptor response. The NMDA component of excitatory post synaptic currents (EPSC) recorded in layer IV neurons of rats shows a longer lasting current at days 9-14 than at day 35 (Carmignoto and Vicini, 1992). EPSC's are described as having a double exponential curve, with fast and slow components of 21-63ms and 177 to 321 ms, respectively. At the early, age, the slow component comprised 92+/- 12% of the EPSC, but at the later age, less than 35%. Similar findings have been reported at the level of the LGN (Ramoa and McCormick, 1994). Interestingly, the NMDA receptor-mediated responses in both cortex and LGN were enhanced during the initial periods of synaptic plasticity for remodeling of retinogeniculate and corticogeniculate connections.

These changes in NMDA receptor-mediated synaptic transmission may be regulated by sensory experience. Thus, dark-rearing was found to delay the changes in NMDA receptor function in kitten visual cortex (Fox et al, 1991). Moreover, when kittens were reared in the dark, the EPSC's retained their longer duration through day 45 (Carmignoto and Vicini, 1992). Total block of retinal activity by intraocular application of TTX produced rat cortical cells with even slower EPSC's than dark-reared cells, showing that spontaneous retinal activity may produce some decrease in NMDA EPSC duration. In conclusion, light stimuli appear to have some

regulatory control over development of cortical cell visual response characteristics. How activity causes changes in the properties of NMDA receptors is not known. Electrical stimulation at critical periods may cause up-regulation of various channel subunits, changing the intrinsic physiological characteristics of the channel itself.

Second, GABAergic mechanisms, which may also be involved in the control of NMDA receptor-mediated activity in mature and developing cortex (Luhmann and Prince, 1990) and LGN (Ramoa and McCormick, 1994), follow a protracted course of development in several central nervous system structures (Luhmann and Prince, 1990; Harris and Teyler, 1983; Schwob et al., 1984; Ramoa and McCormick, 1994). Hyperpolarizing IPSP's might bring the summation of EPSP's below the threshold for NMDA receptor activation and action potentials. Without this inhibition, depolarizations may occur, relieving the Mg++ block of NMDA receptors. Thus, late functional maturation of inhibitory connectivity may represent a general mechanism to enhance NMDA receptor activation in the developing CNS. Third, development of the intrinsic membrane properties in the immature neurons appear to be coordinated to enhance excitatory synaptic transmission during development (Ramoa and McCormick, 1994a, b). For instance, both the increased input resistance (Ramoa and McCormick, 1994a) and lack of intrinsic oscillatory behavior (Ramoa and McCormick, 1995) of immature thalamic neurons raise the probability that excitatory neurotransmission is enhanced. Another important factor may

be the depolarized levels of the resting membrane potential at early developmental stages in the LGN (Ramoa and McCormick, 1994a) and cortex (McCormick and Prince, 1987). Finally, the voltage-dependent block of the NMDA receptor-associated channel also appears to change during development (Burgard and Hablitz, 1994).

Molecular Biology of Developmental Changes of the NMDA Receptor

The previous results showing that developmentally relevant changes occur in the functional properties of NMDA receptors may be explained by modifications in their subunit composition. Consistent with this possibility, recent molecular cloning studies have shown that the NMDA receptor is composed of different subunits, which can be classified into two subfamilies, the NMDAR1 and NMDAR2A, B, C, and D that determine its functional properties (Ishii, et al., 1993; Kutsuwada, et al., 1992; Meguro, et al, 1992; Monyer, et al., 1992; Monyoshi, et al., 1991). There is 50-70% sequence homology within the NR2 subfamily, but only about 20% between NR1 and NR2 (Monyer, et al., 1992). NMDAR1 is expressed throughout the mammalian brain, leading to the idea that it participates in many functions (Kutsuwada, et al., 1992). NR2A is expressed in forebrain and the cerebellum, NR2B in the forebrain, and NR2C mostly in the cerebellum (Kutsuwada, et al., 1992). When the NR1 subunit is expressed in Xenopus oocytes with a member of the NR2 type they are able to form functional receptors (Meguro, et al, 1992). Since expression of a single subunit type does not lead to normal NMDA receptor currents, it is thought that the in

vivo channel probably exists as a combination of two or more subunits (Meguro, et al., 1992). In fact, recent studies have shown that NMDA receptors may be composed of three different types (Sheng, et al., 1994).

Depending on the combination of subunits, different NMDA receptor subtypes may form which display different response properties. The physiological properties of the channels are studied after one or two subunit genes are transfected within a vector (a DNA plasmid which has a gene promoter connected to the gene, allowing for transcription in the cells) into xenopus oocytes. Ion currents are much stronger when NR1 was expressed with an NR2 subunit (rather than only the NR1) (Meguro, et al, 1992). Application of 10uM L-glutamate and 10uM glycine activated NR1-NR2A channels currents of 364+/-62nA; NR1-NR2B reached 667+/- 187nA; and NR1-NR2C was 191 +/- 67nA. Also, Mg++ blocks the channels in a voltage-dependent manner. But NR1-NR2A channels show almost no current at hyperpolarized voltages in .5 and .1mM Mg++, whereas NR1-NR2B and C channels still produce current upon addition of .1mM Mg++. Furthermore, addition of Ca++ extracellularly increased the reversal potential of all receptor types from 0mv to about 20mv, showing that the ion permeates the channels (Kutsuwada, et al., 1992).

An important characteristic of NMDA receptors, the slow decay of EPSP's due to activation by glutamate, differed between NR1-NR2A and NR1-NR2C. Exposure to glutamate and glycine produced time constants of about 31ms for NR1-NR2A and 51ms for NR1-NR2C (Monyer, et al., 1992).

The differences in physiological properties of the various NMDA subunits appear to be due to the specific amino acid make up of the receptor. Each NMDA receptor subunit has an aspartate residue within the second transmembrane sequence which is important for Ca++ permeability and the Mg++ blockade (Burnashev, et al., 1992). However, each subunit's physiological properties is differentially affected by mutations at this aspartate. For example, the NR2 aspartate has a more critical role than the NR1's residue in the Mg++ ion block of NMDA receptor activation. These changes in NMDA receptor properties according to subunit composition may be crucial in the enhancement of their function seen during early development.

Indeed, recent evidence suggests that NMDA receptors undergo age-related alterations in their subunit composition (Williams, 1993; Sheng, et al, 1994). MRNA studies of each subunit, NR1, NR2A, NR2B, NR2C, NR2D, show that in cortex NR1 rises from a low level at birth, reaches its highest level during the second week, and drops to an intermediate level throughout adulthood (Sheng, et al, 1994). NR2B remains at a higher level throughout, while NR2A does not exist at birth, and rises to adult during the second and third weeks. Immunoprecipitation using subunit-specific studies show the same patterns of channel formation for NR2A and NR2B with NR1. Also, NR2A and NR2B can co-precipitate each other, meaning that a tri-subunit channel may exist. These results suggest that the various physiological and pharmacological properties which have been reported to

change during development (increased Mg++ block, duration of evoked currents, decrease in glycine sensitivity) may result from changes in the ratio of NR2A to NR2B making up NMDA receptors.

Intracellular Effects of NMDA Receptor Activation

Many intracellular effects have been shown to result from NMDA receptor activation that could contribute to changes in synaptic efficacy. The increased intracellular calcium concentration that results from NMDA receptor activation induces diverse intracellular effects that may play a role in synaptic plasticity. For instance, NMDA receptor activation has been shown to cause the phosphorylation of a tyrosine side chain in myelin-associated protein II (MAP II) in rat hippocampal cells (Bading and Greenberg, 1991). An increase in phosphotyrosine occurs on a 39kD protein, which is inhibited by D-APV. Ca++ influx may cause this phosphorylation because chelation of Ca++ ions within the cells with 2mM EGTA also inhibited the reaction.

The NMDA receptor-induced Ca++ influx also may act to regulate the activation-state of the receptor. If intracellular Ca++ concentration reaches too high a level it will inhibit NMDA receptor function (Mayer, et al., 1985). But, the rundown of the receptor's activity occurs independent of Ca++ concentrations in the absence of high-energy phosphates provided by ATP (MacDonald, et al., 1989). Therefore, it has been proposed that the Ca++

influx acts upon Ca++-dependent phosphatases, causing changes in the phosphorylation state of some protein (McBain and Mayer, 1994). In contrast, addition of protein tyrosine kinases (PTK) intracellularly can increase conductances through NMDA channels, while a PTK inhibitor caused a reduction of currents (Wang and Salter, 1994; Lieberman and Mody, 1994; Wang, et al., 1994). Whether these kinases and phosphatases act directly on NMDA receptors or on other proteins which regulate NMDA receptors was not shown. However, at least one study has shown that one type of glutamate receptor, GluR6, is directly phosphorylated by cyclic AMP-dependent protein kinase (Raymond, et al, 1993).

Many intracellular effects of NMDA receptor activation have been related to LTP of synaptic transmission in the hippocampus. In the hippocampus, protein kinase C has been shown to play a role in the initiation and maintenance of LTP (Abers, et al., 1984). Ca++ concentration and ATP have also been linked to the effectiveness of LTP. Too great of an intracellular Ca++ concentration increase due to NMDA receptor activation inhibits LTP (Clark, et al., 1990). Since ATP is required to maintain NMDA receptor function in the hippocampus (MacDonald, et al., 1989), another hypotheses suggests that ATP serves as a source of energy to prevent excessive Ca++ build-up. Ca++ may act on the NMDA receptor itself or on other regulatory proteins, while ATP may support the function of Na+/Ca++ exchangers to maintain normal cytosolic levels of Ca++ (Blanstein, 1988; Miller, 1991).

NMDA receptors located within membranes of visual system neurons may interact with both intracellular regulatory proteins and the cytoskeleton. Intracellular Ca++ increases cause the depolymerization of filamentous actin, which is followed by NMDA receptor activity rundown (Rosenmund and Westbrook, 1993). Furthermore, 25% of NMDA receptors in visual system neuron somas and dendrites are mobile, not located at a specific site within the cell membrane (Benke, et al., 1993). Synaptic plasticity might be aided by the movement of available NMDA receptors (MacBain and Mayer, 1994). Long-term modification of regulatory proteins, the cytoskeleton, or intracellular Ca++ may have the effect of altering a neuron's synaptic capabilities.

EXPERIMENTAL APPROACHES TO TEST THE HYPOTHESIS THAT NMDA RECEPTORS UNDERLIE NEURAL PLASTICITY

A General Hypothesis for Development of the Visual System

Do NMDA receptors play a specific instructive role in neural plasticity? Although many studies have addressed this important issue, the role of NMDA receptors in visual plasticity has remained elusive. One especially popular approach to study the role of NMDA receptors in visual plasticity has involved the use of pharmacological agents. Thus, many of the pharmacological experiments suggesting that blockade of NMDA receptors disrupts visual plasticity can be interpreted in favor of the idea that NMDA receptors play a significant role in synaptic plasticity. However, an alternative interpretation is also available. The findings that NMDA receptors transmit a large portion of the excitatory response in immature neurons also raises the possibility that the effects of NMDA receptor antagonists are due to a reduction of synaptic activity (for a discussion, see Daw, 1994). What remains to be answered is whether the properties of the NMDA receptor participate in Hebbian, or other, synapse mechanisms which have a direct role in the stability of synapses.

It is possible that NMDA receptor activation plays an instructive role during development, allowing for changes in synaptic connections in the LGN and cortex.As described earlier, the unique biophysical properties of the NMDA receptor could signal when strong synchronized firing of input fibers occurs that leads to postsynaptic firing. The question arises as to why NMDA receptors, although still functional in adult animals, no longer contributes to synaptic plasticity in the mature LGN and cortex. The initial period of plasticity in the developing visual system provides us a remarkable opportunity to study the role of NMDA receptors in synaptic plasticity. Looking at developmental changes in NMDA receptor function and how these changes correlate in time with the critical periods of plasticity in the LGN and cortex has been another approach which provided us with important insight into specific mechanisms of visual plasticity. Other approaches that may be applied to reveal the role of NMDA receptors in visual plasticity are described below.

Future Research: Genetics

The knowledge now available about the characteristics of the NMDA receptor genes may help elucidate the exact role of NMDA receptors in neural plasticity. The complete knock-out of an NMDA receptor subunit, using a mutant gene transfected into embryonic stem cells, was shown to reduce the magnitude of LTP in the hippocampus (Sakimura, et al., 1995). Mice that lacked the NR2A subunit showed a moderate reduction in

hippocampal NMDA receptor currents, and a decreased NMDA receptor-mediated LTP of synaptic transmission. It would be valuable to study the visual system in similar animals at various ages. Would the initial period of plasticity be altered in duration or, even be present in these animals? Would LTP in visual cortex be affected or abolished in these animals?

Further genetic studies should also aim at elucidating the promoter and regulatory sequences, and transcription factors of NMDAR genes. Upstream sequences could be matched among already known NMDAR genes. DNA "footprinting" might locate them as well. Cellular proteins are mixed in vitro with the upstream sequence. Then, a restriction endonuclease digestion is performed. A transcription factor protein for the gene would bind its promoter sequence and not allow it to be digested. This would result in a long stretch of DNA on a Southern blot. A transcription factor might be located by running cellular proteins within an affinity column which has the promoter sequence attached to beads. The transcription factor would bind the sequence and not filter through the column. These factors could provide very specific tools for altering NMDA subunit expression, and therefore receptor function. An exciting experiment would alter NMDAR genes in only a specific region of the brain at various ages. For example, manipulation of NMDAR expression on only one side of the posterior lobe during, or after, the critical plasticity period could expose changes resulting from the experimentally altered levels of a subunit.

Also, in contrast to a gene knock-out experiment, in which a completely non-functional gene is introduced into animals, an NMDAR gene which has only a certain region, or amino acid, altered could be transfected. For example, a mutated NMDA receptor subunit which, when composing part of the receptor, decreases its binding of a trophic molecule could be introduced into cells. A certain region of NMDA receptor subunits contains a possible sequence for the binding of arachidonic acid (Petro, 1993). If the mutation were shown to alter arachidonic acid's positive regulatory effects on NMDA receptors in in vitro expression systems, then the gene could be introduced into animals in which any changes in plasticity and development could then be studied. One study has already shown the role of specific amino acids in the voltage-dependent Mg++ blockade of NMDA receptors in vitro. What would be the developmental changes seen in an animal which matured with an NR2A subunit with less affinity for Mg++ ions?

Neural Activity And Changes In NMDA Receptor Function

Developmental changes in both NMDA receptor currents (Carmignoto and Vicini, 1992; Hestrin, 1992; Ramoa and McCormick, 1994) and subunit types (Ramoa, et al., 1995; Sheng, et al., 1994) of NMDA receptors present in the mammalian visual system and cortex have been shown. What mechanisms regulate these changes? Dark-rearing experiments (Fox, et al, 1991) have suggested that visually-induced activity regulates some of

these developmental changes. To study this idea, additional manipulation of activity should be attempted, and then the NMDA receptor subunit content of membranes studied. Would dark-rearing affect the changes in subunit composition during development? Furthermore, about 25% of NMDA receptors are mobile in visual system neurons (Benke, 1993). Possibly, receptor migration away from synapse is a mechanism by which it is functionally weakened. Experimentally, the clustering of NMDA receptors at synapses could be compared in stimulated versus non stimulated in vitro (or dark-reared versus light-reared) neurons.

Consistent with this probability, Stent (Stent, 1973), proposed that receptors in the post-synaptic membrane would be removed, or internalized as a mechanism to reduce synaptic plasticity. If neural activity could be shown to alter receptor clustering or induce differential subunit expression, we would be one step closer to understanding the molecular mechanisms that account for synaptic strengthening or weakening. Changes in the efficacy of receptors, possibly under tight control of genetic developmental changes would be the final step in a series that finally leads to neural rearrangement.

NMDA Receptor Activity and Synapse Withdrawal

How are NMDA receptors involved in the withdrawal of inappropriate retinogeniculate and geniculocortical synapses during development? Studies on the neuromuscular junction show that a critical level of activity

at one synapse causes a neighboring inactive terminal to withdraw (Balice-Gordon and Lichtman, 1994). Another study has shown that stimulation of one synapse causes the suppression of a neighbor terminal in vitro (Lo and Poo, 1991) A similar experiment (although technically challenging) might be performed on a surface cortical area or in cultured cortical cells. If D-APV were used to block a few specific synapses in a LGN or cortical neuron, would non-NMDA channel activation be sufficient to prevent withdrawal of those synapses?

Antibodies

Antibodies already exist for each NMDA receptor subunit. Immunoprecipitation studies of cortical membranes should be performed to determine the changing subunit composition of NMDA receptors in the visual cortex. The antibodies could be used to antagonists of NMDA receptor function by blocking different subunits. In conclusion, results obtained using different experimental approaches should elucidate whether NMDA receptors play a specific role in developmental mechanisms involving remodelling of connections.

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