Localization of NMDA receptors in the cerebral cortex: a schematic overview

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Abstract

The fundamental role of N-methyl-D-aspartate (NMDA) receptors in many cortical functions has been firmly defined, as has its involvement in a number of neurological and psychiatric diseases. However, until recently very little was known about the anatomical localization of NMDA receptors in the cerebral cortex of mammals. The recent application of molecular biological techniques to the study of NMDA receptors has provided specific tools which have greatly expanded our understanding of the localization of NMDA receptors in the cerebral cortex. In particular, immunocytochemical studies on the distribution of cortical NMDA receptors have shown that NMDA receptors are preferentially localized on dendritic spines, have disclosed an unknown fraction of presynaptic NMDA receptors on both excitatory and inhibitory axon terminals, and demonstrated that cortical astrocytes do express NMDA receptors. These studies suggest that the effects induced by the activation of NMDA receptors are not due solely to the opening of NMDA channels on neuronal postsynaptic membranes, as previously assumed, but that the activation of presynaptic and glial NMDA receptors may mediate part of these effects.

Key words
- Glutamate
- Ionotropic receptors
- Autoreceptors
- Heteroreceptors
- Astrocytes
- Neuron-glia signaling

Glutamate (Glu) receptors of the N-methyl-D-aspartate (NMDA) type have a fundamental role in the functions of the cerebral cortex, being implicated in developmental processes, transmission of sensory information, synaptic plasticity, learning and memory, neurotoxicity, and in a number of neurological and psychiatric diseases (1,2).

NMDA receptors are formed by different subunits belonging to two classes: NMDAR1 (NR1) and NMDAR2 (NR2) (3-7). The first subunit to be characterized, NR1, exhibits the basic features of the NMDA receptor when expressed in Xenopus oocytes (8), and can exist in several isoforms generated by alternative splicing (9). It has recently been shown that targeted disruption of the NR1 gene abolishes classical NMDA neuronal responses (10), thus demonstrating that NR1 is an essential subunit of the NMDA receptor, and confirming previous suggestions based on expression studies of cDNA in heterologous cells and on the widespread distribution of NR1 mRNA in the central nervous system (4). The second class of NMDA receptor subunits, NR2, includes four different subunits, NR2A-D, encoded by separate genes (3,11-13). No splice variants have been reported for NR2A, B, or C, whereas NR2D exists in two forms, NR2D1
and NR2D2 (13). Electrophysiological experiments indicate that NR2 subunits produce detectable currents only when they are coexpressed with NR1 (3,11-13), and *in situ* hybridization shows that they are differentially expressed in the brain (3,11-13) and during development (3,6,14). These findings have generated the notion that NR2 subunits play a modulatory role. Indeed, it has been shown that the combination of NR1 with different NR2 subunits modifies both electrophysiological and pharmacological responses (3,4,11-13,15-20).

Advances in the knowledge of the molecular biology of NMDA receptors have made available, among others, potent and specific tools for studying the localization of these receptors at the cellular level. This minireview succinctly describes the results of a series of recent anatomical investigations on the localization of NMDA receptors in the cerebral cortex of adult mammals. Notwithstanding the limitations inherent in each of the anatomical techniques employed to generate the data reported here (which have been discussed in the original publications), the availability of these probes has made it possible to define with sufficient detail the basic features of the pattern of NMDA receptor localization in the mammalian cerebral cortex.

The outstanding features of NMDA receptor localization and their functional implications can be summarized as described below.

**NMDA receptors are present in many but not in all neurons of the cerebral cortex.** Moriyoshi et al. (8) reported that “(NMDAR1)...mRNA is expressed in almost all the neuronal cells throughout the brain regions” (p. 36). Since then it has been assumed that virtually all cells express NMDA receptors. Subsequent *in situ* hybridization and immunocytochemical studies have shown that NMDA receptors exhibit a widespread distribution in the cerebral cortex (21-24), but semiquantitative analyses suggest that the population of cortical neurons not expressing NMDA receptors is likely to be much larger than previously assumed (about 20%) (22,24).

*In situ* hybridization and immunocytochemical studies have shown that neurons expressing NMDA receptors appear to be less numerous in layer IV than in layers II-III and V-VI (24). Since the afferent input reaches the cerebral cortex through Gluergic thalamocortical axons mostly in layer IV (25), this observation is in agreement with the notion that thalamocortical transmission is largely mediated by non-NMDA receptors (26-30), and suggests that the impact of NMDA receptor activation on cortical function is more important in late rather than in early stages of cortical processing.

**In cortical neurons NMDA receptors are mostly formed by NR1 and NR2A and/or B subunits.** This statement is supported by the observations that i) NR1, NR2A, and NR2B are highly expressed by cortical neurons, whereas NR2C and D are weakly expressed (3,4,11-13,15,21-24,31-33); ii) NR1 and NR2A and B exhibit similar distribution patterns, both at the light and at the electron microscopic level (3,11-13,15,24,31-33); iii) NR1 and NR2A/B immunoreactivity (IR) is colocalized in most cortical neurons (24), and iv) “triple subunit” heteromeric NMDA receptors (NR1 + NR2A + NR2B) are present in the cerebral cortex (6).

However, given that few studies have been devoted to the analysis of NR2C and D expression in the cerebral cortex, we cannot rule out a contribution of NR2C and D, and since both NR2C and NR2D determine important biophysical properties (15,20), it follows that the functional properties of cortical NMDA receptors cannot be inferred on the basis of present knowledge.

**The large majority of NMDA receptors are located postsynaptically on dendrites and dendritic spines.** Electron microscopic immunocytochemical studies have shown that both NR1 and NR2A/B IRs are mostly
NMDA receptors in the cerebral cortex

present on dendrites and dendritic spines (21,23,24,31,32). This observation is consistent with i) the location of axon terminals forming asymmetric synapses (34) and the nature and location of Glu+ axon terminals (35,36); ii) previous indications from radioligand binding (37-41) and in situ hybridization (8; for a discussion, see 22) studies, and iii) the results of electrophysiological (42) and combined electrophysiological-Ca2+ imaging investigations (43). Overall, this evidence indicates that the bulk of the effects of NMDA receptor activation is generated at distal dendrites and spines, and supports the view expressed by several investigators that dendritic spines in cortical neurons are the site of biophysical events underlying complex integrative properties of cortical neurons (44-48).

**NMDA receptors are preferentially expressed by pyramidal neurons.** Analysis of the morphology of NR1 and NR2A/B+ neurons showed that in rat neocortex the large majority (about 70%) of all labeled neurons are pyramidal, and that this proportion is higher in layers II, III, V and VI (21,23,24). This conclusion is supported by the following observations: i) as reported in the preceding paragraph, NMDA receptors are preferentially located on dendritic spines, which is a typical, though not exclusive, attribute of pyramidal neurons (49-51), and ii) Thomson and collaborators (52) studied excitatory synaptic connections between pairs of cortical neurons recorded in cortical slices from adult rats, and characterized the receptor(s) mediating excitatory postsynaptic potentials (EPSPs). They showed that connections between pyramidal neurons exhibit properties typical of NMDA-mediated processes, even though they are not exclusively mediated by NMDA receptors (53-56). Even though much caution is required when comparing results obtained with different techniques, these results are consistent with the present conclusion in indicating that in all likelihood NMDA receptors display a preferential relation to pyramidal neurons.

Some NMDA receptors are presynaptic auto- and heteroreceptors. Immunocytochemical studies have shown that some axon terminals contain NR1 or NR2A/B IR. Some NR1-NR2A/B+ axon terminals form asymmetric synapses (57; see also 21,23,32), and given that these axon terminals are either Glu- or aspartate (Asp)-positive (35,36), it follows that NMDA receptors in axon terminals forming asymmetric synapses are autoreceptors that can facilitate Glu (or Asp) release. An unexpected result of our studies has been the identification of NR1 and NR2A/B in some axon terminals forming symmetric synapses (57). Combining pre- and post-embedding immunocytochemistry, we have shown that all NR1 and NR2A/B+ axon terminals forming symmetric synapses are selectively enriched in gold particles coding for GABA, thus providing the first evidence that some NMDA receptors are heteroreceptors (57). These data suggest that NMDA receptors play a role in the regulation of GABAergic transmission. Overall, these data are consistent with previous demonstrations that presynaptic NMDA receptors contribute to NMDA-mediated phenomena in other regions of the nervous system (58-64).

**NMDA receptors are expressed by astrocytes.** Whereas the notion that cortical astrocytes express Glu receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate (KA) types has been firmly established (for reviews, see 65-68), there has been considerable debate on whether astrocytes express NMDA receptors (see 64,69). Compelling evidence for astrocytic expression of NMDA receptor subunits, however, has been provided only recently in immunocytochemical studies using specific antipeptide antibodies (69). The demonstration by electron microscopic immunocytochemistry that some cortical astrocytes do indeed express NR1 (21,22,69) and NR2A/B (69) subunits of the NMDA recep-
tor indicates that at least part of the effects of NMDA receptor activation in the cerebral cortex may well be due to astrocytic receptors. These receptors can monitor Glu release by neighboring axon terminals (35,36) of thalamic (25) and corticocortical (70) origin, as well as from axon collaterals of cortical Gluergic neurons (71), and therefore they can mediate part of the neuron-glia signaling mechanisms that regulate gene expression and responses to pathological elevations of Glu levels of astrocytes, and may participate in the mechanism(s) subserving activity-dep

dependent cortical plasticity (72).

The data reviewed here indicate that in three years much has been learnt about the cellular and subcellular localization of NMDA receptors in the cerebral cortex. From the present analysis, it appears that in some cases anatomical studies have been confirmatory of previous findings, whereas in other cases immunocytochemical studies have disclosed features such as astrocytic and presynaptic localization that had not been described earlier. Electrophysiological and/or pharmacological analyses are needed to understand their functional role in health and disease.

Acknowledgments

I thank all the colleagues who participated in the studies cited in the references for their contribution and those who provided information, antibodies, and cDNAs.

References

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