

REVIEW

NMDA and AMPA Receptors: Development and Status Epilepticus**E. SZCZUROWSKA¹, P. MAREŠ¹**¹Department of Developmental Epileptology, Institute of Physiology Academy of Sciences of the Czech Republic, Prague, Czech Republic

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Summary

Glutamate is the main excitatory neurotransmitter in the brain and ionotropic glutamate receptors mediate the majority of excitatory neurotransmission (Dingeldine *et al.* 1999). The high level of glutamatergic excitation allows the neonatal brain (the 2nd postnatal week in rat) to develop quickly but it also makes it highly prone to age-specific seizures that can cause lifelong neurological and cognitive disability (Haut *et al.* 2004). There are three types of ionotropic glutamate receptors (ligand-gated ion channels) named according to their prototypic agonists: N-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propanoic acid (AMPA) and kainate (KA). During early stages of postnatal development glutamate receptors of NMDA and AMPA type undergo intensive functional changes owing to modifications in their subunit composition (Carter *et al.* 1988, Watanabe *et al.* 1992, Monyer *et al.* 1994, Wenzel *et al.* 1997, Sun *et al.* 1998, Lilliu *et al.* 2001, Kumar *et al.* 2002, Matsuda *et al.* 2002, Wee *et al.* 2008, Henson *et al.* 2010, Pachernegg *et al.* 2012, Paoletti *et al.* 2013). Participation and role of these receptors in mechanisms of seizures and epilepsy became one of the main targets of intensive investigation (De Sarro *et al.* 2005, Di Maio *et al.* 2012, Rektor 2013). LiCl/Pilocarpine (LiCl/Pilo) induced status epilepticus is a model of severe seizures resulting in development temporal lobe epilepsy (TLE). This review will consider developmental changes and contribution of NMDA and AMPA receptors in LiCl/Pilo model of status epilepticus in immature rats.

Key words

Brain • Maturation • Glutamate receptors • Epilepsy

Corresponding author

E. Szczurowska, Institute of Physiology AS CR, Videňská 1083, CZ 142 20 Prague 4, Czech Republic.

E-mail: ewa.szczurowska@epilepsy.biomed.cas.cz

NMDA receptors

NMDARs are responsible for normal brain development, they are involved in numerous physiological (neuronal growth and migration, memory plasticity) as well as pathological mechanisms (development of epilepsy, neurodegeneration associated with Parkinson, Alzheimer or Huntington diseases). NMDA receptors are ubiquitously distributed throughout the central nervous system (CNS). They are located mainly postsynaptically, but some of them are present on presynaptic membranes where they can play a role of auto- or heteroreceptors (Conti *et al.* 1997). These receptors can be also found on cortical astrocytes (Lee *et al.* 2010). NMDARs are heteromeric complexes of four various subunits surrounding central ion channel permeable for calcium. All subunits can be present in a form of different splice variants which exhibit diverse physiological and pharmacological properties. At the moment, seven different NMDA receptor subunits have been determined (NR1, NR2A–D, NR3A and NR3B). Functional NMDA receptors are assembled from at least one (more often two) constitutive, glycine-binding NR1 subunit (there are 8 known isoforms of NR1); one or two NR2A–D glutamate-binding subunits presence of which modulates ion channel functional properties (Monyer *et al.* 1994, Low and Wee 2010, Traynelis *et al.* 2010). NR3A–B subunits which do not form functional receptors alone can additionally assemble with NR1–NR2

complexes and further increase NMDARs functional diversity (Sucher *et al.* 1995, Wong *et al.* 2002).

NMDARs subunits and their ontogeny

During rat ontogeny, especially between postnatal day 7 and 14 (P7 and P14), CNS exhibits increased sensitivity to the toxic effects of glutamate. It has been suggested that the reason for this enhanced vulnerability may be an increased expression of specific NMDA receptor subunits (Miyamoto *et al.* 2001). Soon after birth cortical neurons exhibit large, long-duration NMDAR-mediated excitatory postsynaptic currents (EPSCs), but during the first postnatal week EPSCs of the NMDAR becomes shorter and faster (Barth and Malenka 2001, Lu *et al.* 2001, Liu *et al.* 2004). These fluctuations in the receptor kinetics are accompanied by the developmental changes in level of expression of specific NMDARs subunits, and their composition varies in different brain regions. Longer currents duration and larger amplitude of EPSCs can be blocked by ifenprodil, a specific antagonist of NMDARs containing NR2B subunit. NR2B dominated currents probably cause greater Ca^{2+} entry through NMDAR central channel in developing synapses, assisting establishment of thalamocortical circuitry (Constantine-Paton *et al.* 1998, Cull-Candy *et al.* 2001). It was shown that the level of expression of NR2A subunit dramatically increases during the first week of postnatal development in rodents, become predominant, and in this way taking over the role of NR2B subunit. Overall levels of NR2B expression do not change dramatically throughout development (Zhong *et al.* 1995, Stocca and Vicini 1998). Therefore, a change in ratio of the main NMDARs subunits, the synaptic NR2B/NR2A is responsible for the developmental shortening of NMDARs mediated current (Lu *et al.* 2001).

NMDARs subunits and developmental changes in their expression profiles

NR1 subunit

Formation of eight different NR1 splice variants is possible thanks to the insertion or deletion of three short exon cassettes: exon 5 in the N terminal (N1) and exons 21 and 22 in the C terminal (C1, C2) domains of the NR1 molecule (see Fig. 1). NR1-1 is the full-length clone containing both C-terminal exons, NR1-2 lacks exon 21, NR1-3 lacks exon 22, and NR1-4 lacks both, letter (a) indicate presence and letter (b) absence of

exon 5 (Durand *et al.* 1993). These splice variants differ in their spatial and temporal expression patterns, they have also different properties in interaction with protein kinases (Bradley *et al.* 2006).

In general, NR1 subunits expression begins already at embryonic day 14 (E14), reaches the peak at 3rd postnatal week and then slightly declines towards the adulthood (Laurie *et al.* 1994, Paupard *et al.* 1997). The NR1-b variant of NR1 (without the N-terminal N1 exon) is expressed mainly in neonatal sensorimotor cortex, caudate nucleus, and thalamus and CA3 layer in hippocampus, while NR1-a (which contains the N1 exon 5 cassette) is expressed abundantly throughout the brain and is found in all principal cells in hippocampus. Expression of NR1-1 variant is restricted to rostral parts (caudate, hippocampus, cortex) and NR1-4 to more caudal parts (thalamus, cerebellum). Therefore, the composition and number of NMDAR signaling complexes may be dynamically regulated through the splicing of NR1 throughout development (Hollmann and Heinemann 1994, Laurie *et al.* 1994, Hoffmann *et al.* 2000).

NR2A subunit

Determination of regional distribution by means of in situ hybridization analysis (Wenzel *et al.* 1997, Liu *et al.* 2004), followed by RT-PCR and then western blotting (Liu *et al.* 2004) of NR2A and NR2B subunits expression levels have been performed. Results of analysis of different rat and mice brain areas showed that from P0 to P2, levels of synthesized mRNA and protein of NR2A were very low in cerebral cortex and striatum, while in CA1 region of hippocampus expression of mRNA of NR2A subunit at P0 was easily detectable. Expression of NR2A between P5 to P10 increases significantly in the whole brain on both mRNA (see Table 1) and protein levels. It reaches a peak at P12 to P15 and remains at these adult levels (Wenzel *et al.* 1997, Liu *et al.* 2004). NR2A levels are the highest in hippocampus, cerebral cortex and thalamus. In hippocampus NR2A mRNA signals are already strong at the time of birth but its protein levels remain low at least up to P10. This delay is caused by posttranscriptional mechanisms which regulate NR2A protein synthesis (Wood *et al.* 1996, Wenzel *et al.* 1997).

NR2B subunit

At birth, NR2B mRNA subunit expression is very strong in cortex and hippocampus while in the

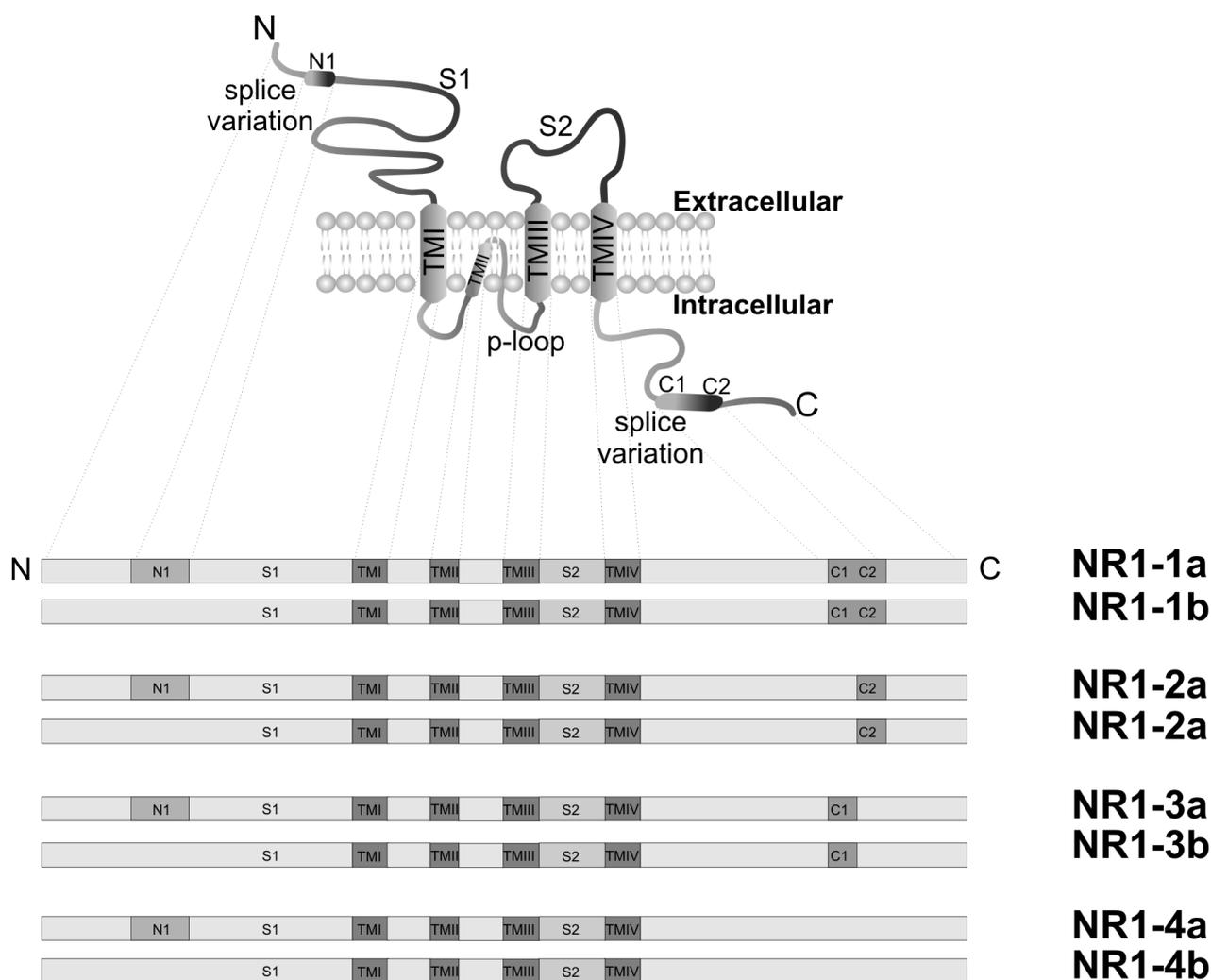


Fig. 1. Schematic representation of NR1 subunit structure and its splice variants. TMI-IV transmembrane domains; N1-exon 5 splice variation site; C1 and C2-exons 21 and 22 splice variation sites; S1 and S2-agonist binding domain (NR1-glycine-binding, NR2-glutamate-binding, and NR3-glycine-binding) (According to Stephenson *et al.* 2008)

thalamus, striatum, midbrain, cerebellum, and brainstem expression is moderate or very low. NR2B mRNA levels do not dramatically change since P5, but its protein level increases notably in cerebral cortex and olfactory bulb. At that time, an NR2B mRNA subunit expression level in hippocampus is already high and comparable with the levels in cerebral cortex or olfactory bulb (see Table 1). By P7 to P10, both NR2B mRNA and protein levels increase in whole brain to reach a peak at P21 and then slightly decrease toward adulthood (Wenzel *et al.* 1997). Marked differences between mRNA and protein levels of NR2B subunit can be explained by the fact that under conditions of low activity, NMDARs are able to stimulate local translation of NR2B subunits (Yashiro *et al.* 2008, Chen *et al.* 2007). The gradual decrease in the NMDA mediated EPSCs decay time constant (P1-P16) at thalamocortical synapses in ventroposterior nucleus of the thalamus (VP) have been first reported by Golshani *et*

al. 1998). What might be important in thalamocortical circuitry, a “switch” in synaptic NR2A and NR2B subunits at VP has very similar developmental profile as that in somatosensory cortex (Liu *et al.* 2004). In general, changes in NR2B/NR2A subunits ratio occurs about two days earlier in VP (P7) than in cortex (P9) (Liu *et al.* 2004). Timing of this change in hippocampus (even within separate CA regions and dentate gyrus) differs also from other brain structures and occurs at last 7 days later than in thalamus or cortex (Guilarte and McGlothan 1998). In adult hippocampus, NR2B are present at the connections of the perforant path, but usually not detected at mossy fibers synapses (Paoletti *et al.* 2013). The NR2A and NR2B subunits are the predominant NR2 subunits in the adult hippocampus and neocortex (Monyer *et al.* 1994, Sheng *et al.* 1994) and new synapses rich in NR2A are added to those in which predominate NR2B instead of switching subunits (Liu *et al.* 2004).

Table 1. Developmental expression profiles of NIMARs subunits in selected brain regions.

Subunit	P0				P3-P5				P7-P9				P10-P15				P20-P25				Adult				References
	Thalamus	Hippocampus	Neocortex	Whole brain	Thalamus	Hippocampus	Neocortex	Whole brain	Thalamus	Hippocampus	Neocortex	Whole brain	Thalamus	Hippocampus	Neocortex	Whole brain	Thalamus	Hippocampus	Neocortex	Whole brain	Thalamus	Hippocampus	Neocortex	Whole brain	
<i>NR2A</i>	*	**	*	**	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	*****	*****	*****	*****	Wood <i>et al.</i> 1996 Wenzel <i>et al.</i> 1997 Liu <i>et al.</i> 2004 Paoletti <i>et al.</i> 2013
<i>NR2B</i>	**	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	Golshani <i>et al.</i> 1998 Wood <i>et al.</i> 1996 Wenzel <i>et al.</i> 1997 Liu <i>et al.</i> 2004 Guilarte and McGlothan 1998 Paoletti <i>et al.</i> 2013
<i>NR2C</i>	**	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	*****	*****	*****	*****	Monyer <i>et al.</i> 1994, Carter <i>et al.</i> 1988 Wenzel <i>et al.</i> 1997 Watanabe <i>et al.</i> 1992 Paoletti <i>et al.</i> 2013
<i>NR2D</i>	**	**	**	**	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	Monyer <i>et al.</i> 1994, Watanabe <i>et al.</i> 1992, Paoletti <i>et al.</i> 2013
<i>NR3A</i>	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	*****	Sucher <i>et al.</i> 1995 Pachernegg <i>et al.</i> 2012 Henson <i>et al.</i> 2010
<i>NR3B</i>	—	—	—	*	—	—	—	*	—	—	—	*	—	—	—	*	—	—	—	—	—	—	—	Sun <i>et al.</i> 1998 Matsuda <i>et al.</i> 2002 Wee <i>et al.</i> 2008 Henson <i>et al.</i> 2010 Pachernegg <i>et al.</i> 2012	

Relative mRNA expression levels: ***** Very High; **** High; *** Moderate; ** Low; * Very low; — Not detected

There is general agreement that activity and visual experience increases the NR2A/NR2B ratio at the protein level, (Chen *et al.* 2007, Yashiro *et al.* 2008). On the other hand, NR2B-containing receptors may be removed from their localization by several different mechanisms, i.e. (i) involving activity of casein kinase 2 (CK2), (ii) activation of repressor element 1-silencing transcription factor (REST), responsible for long lasting repression of GRIN2B gene transcription, and (iii) activation of metabotropic glutamate receptors group I (mGluRs) which play an important role in the triggering of NMDARs “subunit-switching” (Paoletti *et al.* 2013). In addition the ratio of NR2A/NR2B subunits can be regulated by means of ubiquitination and subsequent proteasomal degradation (Ehlers 2003, Jurd *et al.* 2007).

NR2C and NR2D subunit

NMDA receptors containing NR2C or NR2D subunits are activated by glycine and glutamate with higher potency, showing also lower maximal opening probability and a lower sensitivity to extracellular Mg^{2+} than receptors containing NR2A or NR2B (Mosley *et al.* 2010). NR2D-containing NMDA receptors have been hypothesized to exhibit exceptionally slow deactivation following removal of glutamate (Carter *et al.* 1988, Monyer *et al.* 1994). These marked differences controlled by a single GluN2 residue in the M3 segment of receptor molecule significantly affect the relative contribution of NMDAR subtypes to synaptic integration and plasticity (Paoletti *et al.* 2013). NR2D subunits present at the synapse, are co-assembled with other NR2 subunits forming triheteromeric receptors (NR1/NR2B/NR2D) which may not exhibit the slow deactivation of pure NR1/NR2D receptors but may display other NR2D-like properties (Traynelis *et al.* 1998, Cull-Candy *et al.* 2001). NR2C and NR2D subunits also show localization patterns distinct from NR2A and NR2B, with prominent expression in cerebellum, discrete nuclei within the basal ganglia and select populations of interneurons (Carter *et al.* 1988, Monyer *et al.* 1994). NR2D and NR2C mRNAs are expressed in hippocampal and cortical interneurons but are barely expressed in principal cells (Monyer *et al.* 1994). NR2C mRNA subunit signals can be already detected at P0 in cerebellum, but no NR2C protein could be detected even at P10 (Monyer *et al.* 1994, Watanabe *et al.* 1994, Wenzel *et al.* 1997). It has been reported that the late appearance of the NR2C protein coincides with the termination of granule cell migration and the

completion of the cerebellar circuitry. On the other hand, the time course of the appearance of NR2C mRNA and protein in the thalamus and olfactory bulb is very similar. NR2C levels increase dramatically during postnatal week 3, when the mature circuitry is established (Wenzel *et al.* 1997). NR2D subunit is abundantly expressed in the caudal region of the embryonic brain and its expression decrease rapidly in first two postnatal weeks. During adulthood NR2D expression is low and restricted mostly to mesencephalon and diencephalon. Both, NR2C and D are expressed in cortical and hippocampal interneurons but not in principal cells (Watanabe *et al.* 1992, Monyer *et al.* 1994, Wenzel *et al.* 1997).

NR3A and NR3B subunits

Unlike NR2 subunits, which bind glutamate NR3A form a glycine binding structure (Yao *et al.* 2008, Henson *et al.* 2010). NR3A containing receptors exhibit also reduction in Ca^{2+} permeability, ionic currents, and Mg^{2+} sensitivity which may be explained by the constriction a ring of threonines present in the external vestibule of the channel (Wada *et al.* 2006). NR3 subunits assemble into a heterodimers of NR1 and NR3 and a heterotrimeric complex of NR1, NR2, and NR3 subunits that forms an NMDAR with novel properties and attenuated currents compared to NR1/NR2 NMDARs (Henson *et al.* 2010). NR3A subunit play an important role in preventing the premature synapse formation and NR3A removal allow for the insertion of mature NMDARs that in turn trigger introduction of AMPAR (Sucher *et al.* 1995). NR3A subunits are expressed at high level already at early stages of development. NR3A mRNA appears in the rat CNS by E16, peaks at second postnatal week and its expression gradually decreases during maturation (Henson *et al.* 2010, Paoletti *et al.* 2013). NR3B expression exhibits opposite developmental profile. Its levels are low at early life and progressively increase till adult life (Fukaya *et al.* 2005, Paoletti *et al.* 2013).

AMPA receptors

The AMPA receptors mediate most of the fast excitatory synaptic transmission in the mammalian CNS. Their prolonged activation is highly neurotoxic and plays a key role in generation and spreading of seizure activity. AMPA are heteromeric glutamate receptors consisting of four major subunits: GluR1, 2, 3, and 4 with varying stoichiometries (Sommer *et al.* 1991, Hollmann and Heinemann 1994). Their diversity is further increased by

such mechanisms as an alternative splicing or RNA editing which results in formation of the receptors with different biophysical properties of their channels (Lilliu *et al.* 2001, Kumar *et al.* 2002). Each of AMPA receptor subunits is encoded by the separated gene and shows unique area- and temporal-expression pattern, therefore AMPA receptor subunits transcripts appear to be independently regulated during development (Lilliu *et al.* 2001). If an AMPAR lacks a GluR2 subunit, then it will be permeable to sodium, potassium and calcium, but presence of a GluR2 subunit will almost always render the channel calcium impermeable (Hsu *et al.* 2010). An exonic glutamine (Q) codon in a pore-forming segment of the AMPARs GluR2 subunit protein may be changed to an arginine (R) codon by RNA editing (Sommer *et al.* 1991). The edited GluR2-containing AMPARs, exhibit outwardly rectifying currents and little Ca^{2+} permeability, whereas those containing not edited GluR2 generate inwardly rectifying currents and are permeable to Ca^{2+} ions (Verdoorn *et al.* 1991, Burnashev *et al.* 1995). These two forms of GluR2 have been called respectively flip and flop (Standley *et al.* 1995).

AMPARs and their Ca^{2+} permeability

Calcium-permeable AMPARs have been reported to play important role in various physiological and pathological processes (synaptic transmission, plasticity, or neurological diseases including epilepsy) (Washburn *et al.* 1997, Konig *et al.* 2001, Cull-Candy *et al.* 2006, Kwak and Weiss, 2006), and are likely to have important functional implications in developmental (Lawrence and Trussell 2000) and activity-dependent forms of synaptic plasticity (Liu and Cull-Candy 2000). In different cell types of the CNS, AMPARs are functionally and molecularly distinct (see Table 2). The specific expression of rapidly gated, Ca^{2+} -permeable AMPARs in interneurons and relay neurons could be responsible for the shortening of EPSPs and for reduction of the time interval between individual EPSP (Jack and Redman 1971). Calcium entering through AMPARs may activate Ca^{2+} dependent K^+ channels and thereby can contribute to the termination of the EPSP (Geiger *et al.* 1995). Glutamate released from the same presynaptic neuron will exert very different effects on postsynaptic target neurons, depending on the functional and molecular characteristics of the postsynaptic AMPARs. For example AMPAR subunit expression profiles in hippocampal principal neurons (CA3 pyramidal cells, hilar mossy cells) differs from that in interneurons (DG

basket cells, hilar interneurons) (Amaral *et al.* 1990).

In the case of Bergmann glial cells (radial glia, which are present still during adulthood) and DG granule cells, GluR2 and GluR4 transcripts are almost completely edited at the R/Q site in most cell types. The low degree of R/Q site editing in DG granule cells is a reason for more rapid desensitization of AMPARs than those in CA3 pyramidal cells or hilar mossy cells. This could be also due to the virtual absence of GluR2 and the high level of GluR4 in Bergmann glial cells (Geiger *et al.* 1995).

Developmental changes in AMPARs subunits expression

Low expression levels of AMPARs GluR2 subunit (therefore high Ca^{2+} permeability) at early stages of CNS development (Durand and Zukin 1993, Monyer *et al.* 1994) might participate in the increased seizure susceptibility in the immature brain. Increased excitability seems to be caused by Ca^{2+} -permeable AMPARs conducting hyperexcitation and subsequent excitotoxic death of inhibitory GABA-ergic interneurons (Moshé *et al.* 1983). Additionally, AMPARs lacking GluR2 may still be formed even in the presence of GluR2 mRNA (Greger *et al.* 2003, Cull-Candy *et al.* 2006). AMPA receptor subunits are abundantly distributed in the ventral mesencephalon and striatum where they play a crucial role in controlling basal activity and plasticity of both ventral midbrain and striatal neurons. GluR1 and GluR4 mRNAs are expressed at higher levels in mesencephalon than in striatum which develops later during prenatal development (E15-P0) (Lilliu *et al.* 2001). Immunoreactivity for GluR2/3 subunits is concentrated postsynaptically at corticothalamic synapses and their activation by cortical stimulation results in fast EPSPs and slower NMDA receptor-based EPSPs (Salt and Eaton 1996). The greatest amount of expressed proteins of GluR2 and GluR3 directed to corticothalamic synapses, underlines the role of corticothalamic input in gating sensory transmission through the thalamus (McCormick and Bal 1994, Liu 1997) and in setting up reciprocal thalamocortical oscillations (Liu 1997). During the early stages of life GluR3/4 subunits are broadly expressed in hippocampus. Later in development GluR4 expression decrease in that structure, while GluR2 expression increase and it becomes dominant AMPAR subunit in the adult rat hippocampus. AMPARs density in ventral hippocampus (VH) is much lower when compared with dorsal hippocampus (DH) due to the lesser

Table 2. Functional properties and subunit composition of AMPARs in different cell types (according to Geiger *et al.* 1995).

Cell type	Deactivation time	Desensitization time	Ca ²⁺ permeability	Relative abundance			
				GluR1	GluR2	GluR3	GluR4
<i>Neocortex</i>	+	++++	*	*	*****	**	*
<i>CA3 pyramidal cells</i>	++	+++++	*	*****	****	*	—
<i>Bergman glial cells</i>	+	+++	***	*****	*	**	***
<i>DG granule cells</i>	+	++++	*	*****	****	**	—
<i>Interneurons DG basket cells</i>	+	++++	**	*****	**	**	**

Relative deactivation and desensitization time: + very fast, ++ fast, +++ moderate, ++++ slow, +++++ very slow. Relative expression level and Ca²⁺ permeability: * very low, ** low, *** moderate, **** high, ***** very high, — not detected

expression of the mRNAs of all three AMPAR subunits (Pandis *et al.* 2006). In rat cortical pyramidal neurons, synaptic AMPARs switch from GluR2-lacking to GluR2-containing, occur between P13 and P21 (Kumar *et al.* 2002), whereas AMPARs on dendrites of the same neurons exhibit characteristics of those GluR2-containing AMPARs, already at P6 till P40 (Brill and Huguenard 2008, Hsu *et al.* 2010) therefore dendrites of cortical pyramidal neurons at early stages of postnatal development are probably Ca²⁺ impermeable. The expression of the flop (not edited R/Q i.e. Ca²⁺ permeable variant) version of GluR1-4 remain constant until P14, whereas the flip variants (edited R/Q i.e. Ca²⁺ impermeable) are expressed at low levels at birth, and increase considerably between P8 and P14 (Standley *et al.* 1995). There are also some reports (Pellegrini-Giampietro *et al.* 1992) showing that most GluR transcripts are transiently overexpressed, when compared to adult levels, with a peak expression at about P14 in hippocampus (Standley *et al.* 1995).

NMDARs and AMPARs: general signaling and trafficking

NMDARs interact with numerous different intracellular signaling pathways by cooperation with several cellular kinases including Protein Kinase A (PKA), Protein Kinase C (PKC), Extracellular signal-regulated kinase 1/2 (ERK1/2) or Ca²⁺ Calmodulin-dependent Protein Kinase II (CaM II kinase) which directly interact with C-terminal domain of NR2B subunit. The ERK1/2 (extracellular signal-regulated

kinase 1/2) signaling pathway is responsible for activation of different transcription factors and plays an important role in synaptic plasticity and cell survival (Sweatt 2004, Thomas and Huganir 2004). It is stimulated (phosphorylated) and differentially regulated by calcium influx through NMDARs, depending on their subunit composition (Sava *et al.* 2012). In general, activation of ERK is coupled to cellular survival (Hetman and Kharebava, 2006). An excessive NMDAR activation evokes neuronal degeneration by excitotoxic mechanism during early stage of neuronal development (Sava *et al.* 2012). On the other hand, different pools of NR2B-containing NMDA receptors are coupled to ERK in a different way: synaptic receptors are positively coupled, while extrasynaptic NR2B-containing receptors are negatively coupled to ERK. In developing hippocampal neurons, NR2B-containing NMDA receptors have been shown to mediate both pro-death and pro-survival signaling (Martel *et al.* 2009, Sava *et al.* 2012).

ERK1/2 pathway involves small GTPase: Ras, which is activated by specific guanine nucleotide exchange factors (GEFs) and inhibited by GTPase activating proteins (GAPs) (Thomas and Huganir 2004). Both RasGEF and RasGAP can directly interact with NR2B subunit of NMDA receptor. Synaptic RasGAP (SynGAP) is present in high concentrations in postsynaptic densities and associate with NMDARs *via* PSD-95 proteins. The other signaling molecule acting downstream of NMDA receptors is the tyrosine phosphatase: STriatal-Enriched Phosphatase (STEP). Phosphorylation of the serine residue in STEP kinase-domain decreases STEP activity and reduces its affinity

for substrates. Dephosphorylation of this same serine residue activates STEP. This activation can occur *via* calcineurin activity promoted by NMDA receptor-mediated Ca^{2+} influx. In basal conditions STEP activity is low, but when activated, STEP can down-regulate ERK2 by its dephosphorylation and influence excitotoxicity mediated *via* NMDARs (Paul *et al.* 2010). Both phosphorylation and dephosphorylation of ERK2 is Ca^{2+} influx-dependent. It was reported that induction of large, delayed Ca^{2+} influx (characteristic for NR2B subunit containing NMDAR) cause dephosphorylation and therefore STEP activation. As a result regulation of activation of the STEP by NR2B can explain NR2B involvement NMDARs in the inhibition of ERK activity (Paul *et al.* 2010) and influence excitotoxicity mediated *via* NMDARs (Paul *et al.* 2010).

Production of functional NMDA receptors is limited, at least in part, by the availability of NR2 subunits (Prybylowski and Wenthold 2004). NMDARs assemble in the endoplasmic reticulum and interact with membrane-associated guanylate kinases (MAGUKs) which are a family of proteins (including SAP102-synapse associated protein 102, SAP97 synapse associated protein 97, PSD-93- postsynaptic density protein 93kDa, and PSD-95- postsynaptic density protein 95kDa) that play a role in scaffolding of the postsynaptic density (PSD). The NMDAR/MAGUK interaction is mediated by the PDZ-binding domain of the NR2 subunit and Sec8, a protein of the exocyst complex. This interaction is necessary for the delivery of the NMDAR to the synapse. NMDARs are internalized by clathrin-mediated endocytosis (McGee and Brecht 2003, Sans *et al.* 2003). Synaptic and extrasynaptic receptors differ in their subunit composition and are also differentially regulated in response to phosphorylation changes (Li *et al.* 2002). The developmental subunit switch is dependent upon preferential MAGUK binding to either GluN2A or GluN2B subunits (Sans *et al.* 2003). NR2B-containing receptors delivery to the dendrite is possible thanks to binding of the motor protein KIF17 to a multiprotein complex containing the NR2B (Prybylowski and Wenthold 2004). The extracellular matrix (ECM) protein: reelin modulates NR2B surface diffusion, and reelin overexpression cause decreases in synaptic NR2B expression and reduces NR1/NR2B-mediated synaptic currents (Gladding and Raymond 2011, Groc *et al.* 2007).

AMPA receptors undergo kinesin and/or dynein mediated vesicular trafficking in dendrites. The Ca^{2+} sensitive motor protein, Myosin Vb, is also involved in

the dendritic vesicular trafficking of GluA1- containing AMPARs (Henley *et al.* 2011). Influx of the Ca^{2+} *via* activated NMDARs stimulates CaMKII which in turn, phosphorylates GluR1 subunit of AMPARs. MyoVa is Ca^{2+} -activated motor protein, which recognizes and phosphorylated GluR1 connected with Rab11 adaptor complex. Activated by Ca^{2+} MyoVb transports AMPAR along the actin cytoskeleton to sites of exocytosis. Cytoskeletal adaptor protein 4.1N links AMPARs with actin cytoskeleton. When GluR1A is phosphorylated by PKC at serine 816 and 818 its affinity for 4.1N increases and AMPA receptor can be inserted into membrane. On the other hand, phosphorylation of GluR1 at serine 845 by PKA, is responsible for insertion of AMPAR at extrasynaptic and perisynaptic sites where syntaxin4 mediates membrane fusion events at the sites of exocytosis. Later these receptors are replaced by edited GluR2- containing AMPA receptors (Yang *et al.* 2008). PKC isoform protein kinase M zeta (PKMz) (Ling *et al.* 2002), maintains AMPARs at synapses by downregulating GluR2-containing receptor internalization. RNA editing of GluR2 has been shown to be important for exocytosis (Araki *et al.* 2010). The signaling of Ras/ER pathway leads to AMPAR insertion into the dendritic membrane up to 3 mM away from the synaptic site of potentiation (Ling *et al.* 2002, Hoogenraad *et al.* 2010, Patterson *et al.* 2010, Henley *et al.* 2011). In mature neurons NR2A-NMDARs promote Ras/ERK activation as well as surface expression of GluR1 subunit of AMPARs while NR2B-NMDARs inhibit both processes. On the other hand, changes in NR2A or NR2B expression levels and signaling do not markedly influence GluR2 AMPA receptor expression subunit. Loss of GluR1/GluR2 subunits combination mediated by NR2B is rather balanced by the gain in GluR2/GluR3 therefore preventing calcium permeability of AMPARs (Kim *et al.* 2005). Reversible posttranslational modifications, including phosphorylation, palmitoylation and ubiquitination, have been shown to control various aspects of AMPA receptor trafficking and functional modulation (Lu *et al.* 2012).

Ictogenic and anticonvulsant action of drugs influencing NMDA and AMPA receptors

Agonists of NMDA receptors (NMDA, homocysteic acid) elicit epileptic seizures. Their efficacy is high at early stages of development and decreases with age (Mareš and Velišek 1992, Mareš *et al.* 1997a). Similar but not so marked attenuation of efficacy during

maturation was observed with the anticonvulsant action of some antagonists of NMDA receptors and their anticonvulsant action (Velíšek *et al.* 1990, 1991, Mikolášová *et al.* 1994, Kubová and Mareš 1995). Ifenprodil, a selective antagonist for NR2B subunit-containing NMDA receptors, exhibits anticonvulsant action only during the first three postnatal weeks in rats (Mareš and Mikulecká 2009). Antagonists of AMPA receptors CNQX, DNQX and NBQX have only moderate anticonvulsant action against pentetrazol-induced seizures expressed during the first three postnatal weeks (Velíšek *et al.* 1995). NBQX but not noncompetitive antagonist of AMPA receptors GYKI52466 exhibited decreasing action against cortical epileptic afterdischarges during development (Kubová *et al.* 1997, Mareš *et al.* 1997b).

Status epilepticus in immature brain

The incidence of status epilepticus (SE) is highest in patients during the first and after the 60th year of life (Haut *et al.* 2004). High incidence of status epilepticus in infants and children might be due to immaturity of mechanisms arresting seizures, what was demonstrated in amygdala-induced afterdischarges (Moshé and Albala 1983) as well as in hippocampal afterdischarges (Velíšek and Mareš 1991) in rats. There is little information concerning effects of convulsive generalized SE on the immature brain due to a high mortality mainly as a result of respiratory failure after long lasting tonic-clonic seizures (Haut *et al.* 2004). Therefore several animal models of SE with long-lasting clonic seizures and low mortality were developed (Ben-Ari 1985, Turski *et al.* 1986, Pitkanen *et al.* 2002). A great variability of the results has been reported in animal models of SE induced by chemoconvulsants such as lithium-pilocarpine model (LiCl/Pilo).

Mechanism of lithium-pilocarpine model

Mechanism of action of pilocarpine in induction of SE depends on activation of M1 muscarinic receptor leading to subsequent stimulation of NMDA receptors. Activation of ERK1/2 (MAP kinase-mitogen-activated protein kinases) triggered by phospholipase C (PLC)-dependent IP₃ release occurs independently to Ca²⁺-mediated oxidative stress. IP₃-mediated surge in intracellular Ca²⁺ and its increased influx through NMDARs leads to activation of NOX (NADPH oxidase) and generation of superoxide. It results in oxidative modification of cell surface NMDARs with impairment

of receptor function. Mentioned events may trigger pathological states that lead to the local activation of neuronal nitric oxide synthase (nNOS) in close association with the NMDA receptor, and NADPH oxidase. Thus, Ca²⁺ influx through NMDAR channels leads to production of superoxide *via* NOX activation, and Ca²⁺ uptake by mitochondria in combination with NO production triggers cell death (Di Maio *et al.* 2011).

It was repeatedly demonstrated that young animals are more sensitive to epileptogenic stimuli as well as to development of epilepsy, but resulting brain injury differs from that observed in mature animals (Cavalheiro *et al.* 1987, Lado *et al.* 2002, Kubová *et al.* 2004). Status epilepticus can be induced by LiCl/Pilo administration in rats since the second postnatal week, which (when compared to human development) is considered as a newborn/infant/toddler (Haut *et al.* 2004). Previous study shows that the LiCl/Pilo elicited SE at early developmental stage (e.g. at P12) induce plastic changes and have long lasting structural and functional consequences (Nairismagi *et al.* 2006).

Early changes after LiCl/Pilo induced status epilepticus

Li/Pilo induced status epilepticus including its acute, silent and of recurrent seizures phase is a very complicated process difficult to study in detail. Application of pilocarpine triggers a long lasting cascade of changes including alterations of ionic gradient across the cell membrane, activation of different types of kinases, modification of gene expression, activation of trophic factors, enzymes etc. Due to its complexity and involvement of huge scale of factors here we will focus on changes occurring mostly during the acute phase of pilocarpine model (Scorza *et al.* 2009).

Involvement of NMDARs in SE

Due to the well characterized neuronal damage, synaptic reorganization, well determined morphological and biochemical changes as well as an easy access, most of the research concerning developmental changes in NMDARs subunits expression pattern were focused on hippocampal formation (Haut *et al.* 2004). In hippocampal area mRNA levels of NMDARs subunits change dynamically during development. In models of epilepsy most marked changes in hippocampal mRNA levels occur during transition from the latent phase to chronic limbic seizures. The area of maximal hippocampal neuronal injury after lithium-pilocarpine induced SE in the immature brain (P20) is ventral

hippocampus (VH) what is consistent with previous records from the mature brain (Ekstrand *et al.* 2011). Proper function of the mitochondrial electron transport system relies on disulfide oxidation or reduction (Di Maio *et al.* 2012). Thiol residues (functional side groups in cysteine) are important in regulation of signal transduction cascades, protein import, and regulation of the activity of transcription factors. Impairment of antioxidant systems causes a redox imbalance and reduction of thiol residues in neuronal proteins. In *in vitro* pilocarpine-induced epileptic activity, Reactive Oxygen Species (ROS) production cause NMDA-mediated oxidative injury leading to apoptosis.

In the LiCl/Pilo model of SE induction, IP3 synthesis is necessary for activation of two independent pathways, which together cause abnormal NMDAR subunit expression: Ca²⁺-dependent NADPH oxidase activation and ERK1/2 phosphorylation. These pathways are together responsible for short-term up-regulation (overexpression) of the NR2B subunit *in vitro* and *in vivo*. Short-term exposure to pilocarpine induces early overexpression of functional NR2B subunits in hippocampal neuronal cultures, while no changes in the expression of NR2A subunit were detected after 24 h of exposure to pilocarpine (Di Maio *et al.* 2011). On the other hand, later after Pilo application NR2B subunits can regulate NR2A subunits expression level in two ways: by hyperactivation of NR2B, which is induced *via* ERK1/2 activation or, independently by oxidative stress-mediated NR2B overexpression. It was reported that NR2A but not NR2B subunit containing NMDARs activation was required for development of limbic epilepsy in kindling and pilocarpine models (Chen *et al.* 2007). Several investigators (Kubova *et al.* 2001, 2002, Wasterlain *et al.* 2002, Nairismagi *et al.* 2006) described in detail morphological changes occurring in different brain structures after LiCl/Pilo-induced SE in immature brain. In TLE NR2B containing NMDARs are restored in adult brain, so neuronal hyperexcitability in epilepsy may be caused by mechanisms similar to those during early development, a stage of high susceptibility to epileptogenesis (Di Maio *et al.* 2011). On the other hand proteolysis of NR2B subunit by calpain was observed in hippocampus in animals after SE (Araujo *et al.* 2005). Susceptibility of the NMDA receptor cleavage by calpain varies within neonatal period (Dong *et al.* 2006).

Involvement of AMPARs in SE

Seizures can cause rapid alterations in AMPAR

subunit composition and function (mediated by trafficking and endocytosis) in the developing brain. Hyperexcitability of hippocampal networks may cause their transition from normal to epileptic networks. GluR2 subunit expression and therefore the Ca²⁺-permeability of AMPARs changes in various seizure models. There is an increase in AMPA-mediated calcium permeability associated with an augmented AMPAR-mediated potentiation of hippocampal epileptiform activity (Sanchez *et al.* 2001). It is possible that this hyperexcitability is a consequence of a decrease in GABAergic inhibition induced by Ca²⁺ activated phosphatase, calcineurin C (CaN). The expression levels of GluR2 changes during two weeks following SE and prior to the development of spontaneous seizures which begin at 1.5 months following SE in this model (Roch *et al.* 2002, Raol *et al.* 2003). Northern blot analysis and *in situ* hybridization showed that 6 and 12 h after LiCl/Pilo-induced status epilepticus the mRNA level of some subunits of AMPA-selective glutamate receptors dramatically change (Condorelli *et al.* 1994). In the dentate gyrus an increase of GluR3 mRNA level is induced 12 h after LiCl/Pilo treatment, while a clear decrease in GluR1 mRNA level and no significant change in GluR2 mRNA level can be observed in the same area (Condorelli *et al.* 1994). Both the GluR1 decrease and the GluR3 increase are temporary and a return to basal level after 48-72 h. In the CA1 subfield of the hippocampus 12-24 h after SE, an analogous decrease in GluR-1 and GluR-3 expression levels occurred, but at 48 h the expression return to control values. There was also observed a general decrease in mRNA level for the AMPA receptor subunits (GluR-1-3) in the hippocampal layers, in particular in CA3 and CA4 subfields (Condorelli *et al.* 2004). Grooms *et al.* (2000) and Hu *et al.* (2012) found a marked decrease in GluR2 and GluR1 expression 12-16 h after SE. Consequently the reduction in GluR2 might serve as a "molecular switch", leading to the arrangement of Ca²⁺ permeable AMPA receptors and enhancing the toxicity of endogenous glutamate following a neurological insult (Hu *et al.* 2012). Calcium ions influx mediate the rapid increase in the activity of CaMKII, PKA (Protein Kinase A), and PKC (Protein Kinase C) in the hippocampal neurons, this may lead to enhanced phosphorylation of the GluR1 and GluR2 receptor subunits. Phosphorylation of GluR1 subunit immediately after seizures enhances AMPAR-mediated EPSCs, as well as phosphorylation of GluR2 that promote internalization from the synaptic surface and

cause decrease in GluR2. Additionally, inhibition of Q/R editing in GluR2 results in increased excitability of hippocampal neurons with associated spontaneous seizures (Krestel *et al.* 2004).

The seizure induced expression of Ca^{2+} -permeable AMPAR is developmentally regulated and indicates the susceptibility of the immature brain to epileptic activity (Cull-Candy *et al.* 2006). There is an increase in GluR2 mRNA in mature dentate gyrus neurons (DGNs) after SE, suggesting a decrease in Ca^{2+} permeability, which may be neuroprotective against excitotoxic injury (Liu *et al.* 2004, Porter *et al.* 2006). Interestingly, 3 h after SE in the whole brain of immature animals the subunit composition of AMPARs changes from predominantly GluR3/4 subunits to predominantly GluR2/3 subunits (Hu *et al.* 2012). For that reason it can be proposed that developing hippocampus has the ability to activate the endogenous anti-epileptic mechanism (to maintain the balance between excitation and inhibition) after SE, and consequently reduce brain damage. These findings raise the possibility that DGNs could be utilized therapeutically as a naïve population of cells to potentially inhibit epileptogenesis after SE (Porter *et al.* 2006). The increased expression of GluR2 can inhibit excessive Ca^{2+} influx and protect hippocampal neurons from excitotoxic death, conferring early but transient protection in the immature brain after SE (Rakhade *et al.* 2008, Hu *et al.* 2012).

Concluding remarks

Early upregulation of NR2B subunit may be involved in the induction and maintenance of epileptogenesis, since NR1/NR2B heterodimers are primarily expressed as facilitatory presynaptic autoreceptors on hippocampal neurons and promote NR2A overexpression (Woodhall *et al.* 2001, Di Maio *et al.* 2012). Ifenprodil, a highly specific NR2B antagonist is able to moderately block thiol oxidation in rat hippocampus and hippocampal neurons, it was also able to block NR2A overexpression, one of the major events coupled with BDNF (Brain Derived Neurotrophic Factor) expression implicated in the development of chronic epilepsy and mossy fiber sprouting. On the other hand ifenprodil treatment was not able to avoid the activation of caspase3 and cell death occurring after pilocarpine SE, and did not display neuroprotection against pilocarpine treatment. In contrast, noncompetitive NMDA antagonist MK 801 can block thiol oxidation, apoptosis and cell

death. For that reason NR2B subunit is not utterly involved in NMDAR-induced cell damage in both *in vitro* and *in vivo* pilocarpine model of SE (Choo 2012, Di Maio *et al.* 2011). It has proposed that the location of NMDA receptors influences whether they are coupled to pro-death or pro-survival signals: synaptic NMDA receptors are neuroprotective, whereas extrasynaptic receptors preferentially promote cell death pathways (Hardingham and Bading 2010). The diverse localization of different NR2 subunits increases the possibility that subunit-selective antagonists and allosteric modulators or their appropriate combination, might provide beneficial antiepileptic treatments (Mosley *et al.* 2010). Therefore the investigation concerning development of antiepileptic treatment should also be turned in direction of the signaling processes regulating overexpression of mentioned NMDARs subunits. Targeting of early events following SE insult including IP3 synthesis or ROS generation might also be advantageous.

Phosphorylation of the intracellular C-terminus of NR3A (by PKA, PKC, protein tyrosine kinase PTK, or CaMKII) could play critical roles in regulating NR3A trafficking, signaling, and channel properties (Sucher *et al.* 1995, Eriksson *et al.* 2002, Chen and Roche 2007). Due to the fact, that NR3A-containing NMDARs subunit have reduced Ca^{2+} permeability (Sucher *et al.* 1995, Henson *et al.* 2010, Pachernegg *et al.* 2012) it has been suggested that NR3A subunit plays a neuroprotective role early in development (Nakanishi *et al.* 2009). Increasing NR3A subunit expression level in epileptic brain can be an interesting option.

AMPA antagonists are highly potent anticonvulsants (e.g. topiramate, lampanel), widely used in seizure models (Traynelis *et al.* 2010, Rakhade and Jensen 2009). But AMPARs undergo also developmental changes in their subunit composition. Calcium permeable (not containing GluR2) AMPARs are highly expressed in the somatosensory cortex in immature brain (Hsu *et al.* 2009, 2010). Ca^{2+} is an important intracellular signaling molecule; therefore it has been hypothesized that the regulation of the expression of AMPA receptor subunits might participate e.g. in neuronal injury after seizures (Standley *et al.* 1995).

Taken together, these specific developmental changes in NMDA and AMPARs subunit composition and expression patterns, as well as their involvement in intracellular signaling, represent promising targets for development of highly age-specific therapeutics that influence excitotoxicity and subsequent neuronal damage.

Conflict of Interest

There is no conflict of interest.

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