NMDA Receptors in the Developing Brain and Effects of Noxious Insults

Karen A. Waters a–c  Rita Machaalani a

Departments of  a Medicine and  b Paediatrics and Child Health, The University of Sydney, and  c The Children’s Hospital at Westmead, Sydney, N.S.W., Australia

Key Words
Animal models  ・ Brain  ・ Hypercapnia  ・ Hypoxia  ・ Infant brain  ・ Ischemia  ・ Nicotine  ・ Sudden infant death syndrome

Abstract
This review covers normal expression of the NMDA receptor in the fetus and newborn, and then the response of the NMDA receptors within the central nervous system (CNS) during early development, to noxious stimuli. In the research setting, hypoxia is a commonly studied noxious stimulus that has been studied in a variety of contexts, including isolated hypoxia, or hypoxia combined with ischemia or hypercapnia, and delivered in single or repeated doses (intermittent stimuli). We review differences and commonalities between these experimental paradigms, and the sequelae of a common outcome, which is cell death, possibly through excitotoxic mechanisms. Finally, based on current literature, we will examine potential directions for clinical therapeutic interventions. By highlighting knowledge gaps in this field, we hope to encourage future research focusing on clinically relevant problems and outcomes in this area.

Introduction
Cellular functions within the brain are critically dependent on matching the rate of oxygen delivery with that of oxygen consumption, so that oxygen deprivation, whether acute or chronic, constitutes a noxious stimulus. Most protocols of limiting brain oxygen supply are designed to mimic specific clinical scenarios, although their unifying characteristic is cellular energy depletion causing loss of function of cellular adenosine tri-phosphate (ATP). Differences between the insults affects the rapidity of onset, the timing and adequacy of the re-oxygenation (energy restoration), and/or the clearance of other toxic cellular metabolites. Different studies also address different outcome measures, and may or may not include the study of neuroprotective strategies. Most commonly, the sequelae of these events are studied after a single insult, so this will be the primary focus of our review. Many clinical stimuli are intermittent, and the process of re-oxygenation may contribute to the detrimental effects of such a stimulus, so some recent experimental paradigms include examination of the sequelae of second and subsequent exposures to cyclical or repeated stimuli, where the likelihood of neuronal degeneration, neurological damage and/or death may be increased [1]. Although studies have looked at many diverse sequelae of such insults, the focus of this
review is how these insults affect the N-methyl-D-aspartate (NMDA) neurotransmitter system within the developing (immature) brain.

A body of evidence exists to support the contention that noxious insults such as hypoxia and ischemia induce changes in NMDA receptor expression and function in the developing brain [2–5]. This evidence includes fetal and neonatal insults, and is largely derived from animal models of brain injury during the perinatal period. The major role of the NMDA system in response to noxious insults is in excitotoxicity, which is a form of active cell death occurring as the result of excessive and abnormal activation by glutamate of NMDA receptors [6].

Our focus in this review is on changes pertaining to the NMDA receptor in the developing brain after exposure to noxious stimuli that include hypoxia (hypoxia, hypercapnic-hypoxia (HH), or hypoxic-ischemia (HI)), or nicotine because of the relevance of the nicotinic receptor system to ventilatory and therefore hypoxic responses, as well as its purported role in the sudden infant death syndrome (SIDS), an area of research of particular interest in our laboratory.

**The Developing Brain**

In the perinatal period, the human fetus undergoes a critical period of brain development. This period corresponds to 6–10 postnatal days for rats and 2 weeks before to 4 weeks after birth in piglets. This critical period is defined by the peak rate of brain growth [7], enhanced synaptogenesis [8], and the developmental regulation of receptor populations [9]. One well-studied receptor population includes the glutamatergic ionotropic receptors: NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionate (AMPA) and kainite [9, 10]. In the human brain, NMDA receptor activity and expression increase in the infant period, whereas AMPA and kainate receptors are elevated during mid-gestation and decrease thereafter [9]. Thus, the newborn infant brain is more susceptible to NMDA- than AMPA- or kainate-mediated injury.

This critical period of brain development is also characterized by a high rate of regulated neuronal cell death, via apoptotic mechanisms [11, 12]. This cell death affects particular brain regions during specific developmental phases, and occurs in the brainstem during the perinatal period [13], thalamus and other subcortical areas soon after birth [14, 15] and cortical areas in the first 2 postnatal weeks [15, 16]. Moreover, this cell death seems to be specifically regulated by caspase-3, a cysteine protease enzyme that initiates the apoptotic cascade, since caspase-3 mRNA is abundantly expressed in the fetal and infant cerebral cortex compared to the adult [17].

**Noxious Insults to the Developing Brain**

The clinical results of cerebral insults to the fetus or infant may be focal or global, and include mental retardation, cerebral palsy, seizures, deafness and blindness, although for many, the timing and characteristics of the insult remains unknown [18, 19]. Vulnerability of the brain to NMDA-mediated injury shows regional (table 1) and age-related differences that could result in different patterns of neurodegeneration and neurobehavioral disturbance. Age-specific patterns of vulnerability can be observed in the neonatal brain after near-total asphyxia, and vulnerabilities of particular regions are thought to be due to their shared excitatory connections and the fact that they use glutamate as their neurotransmitter [20, 21]. In the neonatal human, HI predominantly affects systems that control tone and movement [22], and magnetic resonance imaging (MRI) has revealed selective injury to the sensorimotor cortex, basal ganglia, thalamus and putamen following severe birth asphyxia in full-term infants [23].

Experimental paradigms of noxious insults that affect the developing brain can be classified as follows:

1. **Hypoxia** – lower than normal oxygen content in the lungs, blood or tissue. Hypoxemia refers to low oxygen content in arterial blood; thus, tissue can be hypoxic even though there is no hypoxemia. Hypoxia is usually achieved by reducing inspired oxygen concentrations, but hypoxic-hypoxia is achieved by administration of carbon monoxide. In animal studies, it has been shown that compensatory cerebral autoregulation increases cerebral blood flow (CBF) and protects the brain tissue from a major fall in oxygen availability [24]. Where CBF is still close to normal, glucose supply to the brain is maintained.

| Table 1. Brain regions selectively vulnerable to noxious insults |
|----------------------|--------------------------|
| Noxious insult       | Brain regions predominantly affected |
| Hypoxia              | Brainstem, hippocampus, cortical regions |
| Hypoxic-ischemia     | Cerebral cortex (white matter), thalamus, putamen, basal ganglia |
| Hypercapnic-hypoxic  | Brainstem, hippocampus |

NMDA Receptors after Noxious Insults

2. Ischemia – markedly reduced or absent circulation. The two common experimental paradigms are: (a) global ischemia, where there is a reduction of arterial blood flow to the brain caused by cardiac arrest, shock, carotid occlusion or hypotension, and (b) focal ischemia, with reduced blood flow confined to the brain, or brain regions of interest. These model the clinical situations of seizures and/or cerebrovascular accidents. In severe ischemia (with tissue hypoxia), energy production is deficient due to the insufficient delivery of oxygen to brain cells, and it is associated with a fall in glucose levels and metabolic acidosis. These factors combine to cause exhaustion of cellular oxygen stores within 30 s, and of glucose and ATP stores within 5 min of onset [25].

3. Hypoxic-ischemia (HI) – In clinical situations, severe hypoxia is often complicated by other physiological insults that counteract the homeostatic effect of cerebral vasodilation, including hypotension. This is mimicked in animals by combining vascular occlusion with hypoxia. With reduced circulation and hypoxia combined, cellular exposure includes hypoxia, hypercapnia (respiratory acidosis), hypoglycemia, and metabolic acidosis [26]. Clinical examples include cardiorespiratory failure, and perinatal asphyxia with bradycardia.

4. Asphyxia – for the purpose of this review, asphyxia is used to refer to the combination of hypercapnia and hypoxia. Whole animal exposure likely results in some accumulation of toxic metabolic products. Clinically, such asphyxial insults include sleep-related breathing disorders (e.g., obstructive sleep apnea), facial entrapment, or respiratory failure secondary to lung or neuromuscular disease. The cellular defects resulting from this insult are dependent upon the presence of hypoxia, since even at very high levels of hypercapnia without hypoxia (arterial carbon dioxide tension (PCO₂) of 90 mm Hg, with brain pH < 6.90) changed energy state or gross or irreversible brain injury are not seen [27–29]. The presence of hypercapnia in the absence of circulatory compromise is likely to exacerbate hypoxia-related injury as in studies where maintenance of other substrates (e.g., glucose) exacerbates lactic acidosis and subsequent injury [26]. Asphyxia in this setting is distinct from definitions of neonatal asphyxia, which is really hypoxia-ischemia. In neonatal asphyxia, oxygen and substrate delivery are both compromised and levels of metabolic (lactic) acidosis are used to define the severity of the condition [30].

5. Substances of abuse – systemic, or cellular exposure to toxins. The most commonly studied substances of abuse are ethanol (in relation to fetal alcohol syndrome [reviewed in 31, 32]), nicotine, and opioids. Their effects during pregnancy appear to include fetal hypoxia by depleting hemoglobin oxygen stores available to the fetus [33], although most clinical examples include mixed, and/or multiple exposures to these substances.

NMDA Receptors

NMDA receptors are heteromeric complexes comprising of an NR1 subunit combined with one or more NR2 or NR3 subunits. There are at least 8 splice variants of the NR1 subunit (NR1A–NR1H) [34], 4 genetically different NR2 subunits (NR2A–NR2D) [35, 36] and to date, 2 genetically different NR3 subunits (NR3A, NR3B) [37]. Although the NR1 subunit is an obligatory component of functional NMDA receptors, the NR2 subunits determine the biophysical and pharmacological activity of the receptor. Thus, the NR2 subunits determine the single-channel conductance and kinetic properties, the time course of current deactivation, and the affinity and sensitivity for agonists and antagonists such as glutamate, glycine and magnesium (Mg²⁺) [38]. The NR1 subunit is widely expressed throughout the CNS at all ages, but the expression profiles of the NR2 subunits in the brain are developmentally and regionally regulated [35]. For example, in the rat, NR2B and NR2D subunits predominate in the neonatal brain, but as development proceeds, they are supplemented with, or replaced by the NR2A and NR2C subunits in some brain regions [36, 39]. Furthermore, NR2A mRNA expression predominates in the cerebral cortex and hippocampus, while NR2B predominates in the forebrain, NR2C in the cerebellum and diencephalon and NR2D in the lower brainstem regions [35, 36, 40].

Activation of the NMDA receptor is regulated by several distinct pharmacological binding sites, which include the following: (1) neurotransmitter binding site or recognition site that binds glutamate or NMDA, (2) co-activator site that binds glycine, (3) channel site that binds Mg²⁺ site, (4) polyamine site that binds spermine and spermidine, (5) ifenprodil site, and (7) inhibitory divalent cation site that binds Zn²⁺ [41].

The NMDA receptor also has a cation-selective ion channel that gates Na⁺, K⁺ and Ca²⁺ ions. This channel is regulated by Mg²⁺, which serves to block Ca²⁺ influx in a voltage-dependent manner [42].

The influx of Ca²⁺ appears to be the initiating step for biochemical processes responsible for both NMDA receptor-induced synaptic plasticity in the developing brain [43] and NMDA receptor-mediated excitotoxicity [44].
NMDA Receptors in the Developing versus Adult Brain

Several characteristics of the NMDA neurotransmitter system make the NMDA regions of the immature brain particularly vulnerable to NMDA-mediated excitotoxicity after hypoxic insults [45]. First, during early development, brain NMDA receptor content and activity is high. Second, the functions of the NMDA system are important in the maturation and plasticity of developing neurons. Finally, changes in NMDA receptor configuration and affinity for its transmitters after exposure to noxious stimuli increase the risks for specific ‘NMDA-mediated’ sequelae (particularly excitotoxicity).

In the developing brain, NMDA receptor activity is high compared to the adult brain [46–48]. Main features of NMDA receptor activity specific to the developing brain as compared to the adult include: lower sensitivity to the channel block by Mg2+, higher sensitivity to glycine, differential modulation by polyamines, increased calcium influx through the receptor channel, longer duration of the excitatory postsynaptic potentials (EPSPs) after receptor stimulation, and enhanced ability to induce markers of synaptic plasticity such as long-term potentiation (LTP) [49–51]. All these differences have led to the proposal that the role of the NMDA receptor in regulating brain development is through activity-dependent rather than experience-dependent mechanisms [52, 53].

Receptor-binding studies and immunohistochemical localization of the subunits indicate that the expression and number of NMDA receptors are also greater in the developing than the mature brain. In the human brainstem, NMDA receptors are not expressed during the fetal period but increase to a peak during the infant period and decrease thereafter [9], with similar patterns observed in the human frontal [54], temporal [55], and prefrontal [10] cortex.

Role of the NMDA Receptor

Under Normal Conditions

NMDA receptors have a direct role in neuronal proliferation [56, 57], migration [58–60], synaptic plasticity [52, 61–63], and injury. The role of NMDA receptors in excitotoxicity and synaptic plasticity in the developing brain has been reviewed in detail elsewhere [61]. Functionally, NMDA receptors are highly expressed in brain regions that control respiration [64, 65], feeding and related physiological functions [66–68], learning and memory [66, 67, 69], and pain perception [70].

The role of the NMDA receptor in neuronal proliferation (neurogenesis) was determined pharmacologically in some brain regions. Blocking NMDA receptors with daily injections of the antagonist MK-801 for 3 days in the 2-day-old rat resulted in a significant increase of cell birth in the dentate gyrus [56, 57], while activation of NMDA receptors resulted in decreased proliferation of the granule cells of the dentate gyrus [71].

Recent studies have identified a role for the NMDA receptor in neuronal migration. Migration (maturation) of postmitotic neurons from where they were generated to their final destination, before differentiation and synaptogenesis, is a central event in brain development [59, 60]. Migrating cortical neurons possess functional NMDA receptors before they undergo synaptogenesis [72], and pharmacological studies have shown that blocking of NMDA receptors in cerebellar slices, or increasing magnesium concentrations in the medium, slows the rate of granule cell migration. Confirming that NMDA is integral to this process, the rate of migration was increased if magnesium was removed, glutamate increased, or exogenous glycine was added to the medium [58]. Patch clamp studies show that the migrating granular cells have higher functional NMDA receptor content compared to premigratory neurons [73]. Similarly, in cortical slices from the rat and mouse, NMDA receptor activation stimulates neuronal migration [74, 75].

Synaptic plasticity (synaptogenesis) is the key factor in shaping the wiring pattern of the brain, and is responsible for the mechanism of learning and memory [62, 63, 76]. Direct injections of NMDA into the occipital cortex of 8-day-old rats (age at which there is a peak vulnerability to both NMDA injections and HI [61]) resulted in an increase in synaptic density (number of synapses) [62]. Conversely, NMDA receptor blockade resulted in a decrease in the total number of synapses [63], and was associated with deficits in learning and memory [77], thus confirming an important role of the NMDA receptor in synaptogenesis. Currently, the two most studied examples of synaptic plasticity are LTP (a long-lasting increase of synaptic efficacy consequent to a short stimulation with high frequency bursts [78]) and long-term depression (LTD; the opposite of LTP and refers to a long-lasting decrease of synaptic efficacy following a high frequency stimulation [79]).
**Under Pathological Conditions**

Much attention has been paid to changes in the NMDA receptor under noxious conditions since the identification of the process known as ‘excitotoxicity’, an excessive and abnormal activation of glutamate receptors leading to cell death [6]. While this process is not specific to the NMDA-glutamatergic system, the NMDA receptor is the predominant excitatory neurotransmitter system involved in this process. The final outcomes of hypoxia induced and NMDA-mediated brain injury may take a few hours and days to present (fig. 1) and are often determined by the events that arise during the period following the insult. For example, brain injury in infants who suffer from birth asphyxia, is associated with a period of encephalopathy with seizures, reduced level of consciousness and poor feeding within hours and days of the insult [80–82].

**Cell Death**

During and/or after hypoxic exposures, a cascade of events is triggered (schematically represented in fig. 2) including an increase in extracellular glutamate that results in the overactivation of the NMDA receptors, and calcium entry into cells causing increased intracellular Ca$^{2+}$, and activation of proteins (e.g., caspase-3) that lead to cell death (apoptosis or necrosis).

Whether the resulting cell death is apoptotic or necrotic in type depends on the duration and intensity of the initiating insult and the age of the neuron. In vitro and in vivo studies in the CNS suggest that a mild excitotoxic insult leads to transient mitochondrial depolarization and reversible energy compromise with cellular apoptosis. More intense injuries produce irreversible mitochondrial depolarization and permanent energy collapse with cellular necrosis [44, 83]. There are also age-associated influences on these processes, since apoptotic death predominates amongst immature neurons whilst necrotic death predominates amongst mature neurons. Although these are usually represented as completely distinct phenomena, recent studies suggest that some cells undergo a hybrid form of cell death with features of both apoptosis and necrosis [84]. In addition, the cell death process is transient, commencing approximately 30 min after NMDA
Table 2. Animal models of hypoxic, asphyxic (hypercapnic-hypoxic) and hypoxic-ischemic (HI) brain injury

<table>
<thead>
<tr>
<th>Insult</th>
<th>Prenatal reference</th>
<th>characteristics</th>
<th>Postnatal reference</th>
<th>characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxic</td>
<td>92, 93</td>
<td>Inspired hypoxia</td>
<td>2, 95–99</td>
<td>Inspired hypoxia (FiO₂ = 0.07–0.15)</td>
</tr>
<tr>
<td></td>
<td>Duration: 60 min</td>
<td>(anesthesia and mechanical ventilation)</td>
<td></td>
<td>Duration: 20–60 min</td>
</tr>
<tr>
<td></td>
<td>Animal: guinea pig</td>
<td>Confirmation: ↓ ATP and PCr (~ 90%)</td>
<td></td>
<td>Animal: newborn piglet, 2–4 days old</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Confirmation: PaO₂ on average &lt; 25 mm Hg,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ ATP (~ 50%) and PCr (~ 80%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Hypoxic chamber (6.5% O₂)</td>
<td></td>
<td>Hypoxic chamber (6.5% O₂)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Duration: 70 min</td>
<td></td>
<td>Duration: 70 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal: infant rat, 4 days old</td>
<td></td>
<td>Animal: infant rat, 4 days old</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Confirmation: no data</td>
<td></td>
<td>Confirmation: no data</td>
</tr>
<tr>
<td>Asphyxic</td>
<td>5, 100</td>
<td>Inspired hypercapnia (7% CO₂) and</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>hypoxia (8% O₂) (HH)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Duration: intermittent for total 24 min of HH</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal: infant piglet, 9–12 days old</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Confirmation: PaO₂ 40.9 ± 1.9 mm Hg, PaCO₂ 61.2 ± 4.2 mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxic-ischemia</td>
<td>101</td>
<td>Uterine vessel clamp</td>
<td>102</td>
<td>Unilateral carotid artery ligation and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Duration: 30 min with reperfusion</td>
<td></td>
<td>hypoxia (8% O₂)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal: rat pups studied on postnatal</td>
<td></td>
<td>Duration: 90–120 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>days 1, 4, 8 and 30</td>
<td></td>
<td>Animal: infant and adult rat (7 and 21 days)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Confirmation: no data</td>
<td></td>
<td>Confirmation: no data</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>Hypoxia (mechanically ventilated; FiO₂ = 0.1) then airway occlusion</td>
<td></td>
<td>Hypoxia (mechanically ventilated; FiO₂ = 0.1) then airway occlusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Duration: 30 and 70 min</td>
<td></td>
<td>Duration: 30 and 70 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Animal: newborn piglet, 7 days old</td>
<td></td>
<td>Animal: newborn piglet, 7 days old</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Confirmation: hypoxia SaO₂ (30%)</td>
<td></td>
<td>Confirmation: hypoxia SaO₂ (30%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemia SaO₂ (5%)</td>
<td></td>
<td>Ischemia SaO₂ (5%)</td>
</tr>
</tbody>
</table>

‘Confirmation’ = Confirmation of tissue hypoxia. ATP = Adenosine tri-phosphate; FiO₂ = fractional inspired oxygen concentration; mm Hg = millimeters of mercury; PaO₂ = arterial oxygen tension; PaCO₂ = arterial carbon dioxide tension; PCr = phosphocreatinine; SaO₂ = arterial oxygen saturation.

NMDA Receptors after Noxious Insults

Animal Models Used to Study Changes in NMDA Receptors

The majority of studies that focus on delineating the mechanisms of brain damage induced in the human infant by noxious insults are undertaken in isolated cellular preparations, or in animal models. Various animal models of hypoxic, asphyxic, and hypoxic-ischemic (HI) brain injury have been developed and used for the study of the NMDA system. Those of relevance to this review are summarized in table 2.

Experimental techniques commonly employed to study the expression of NMDA receptors focus on the localization and distribution of subunit mRNAs and/or proteins, and include immunohistochemistry, in-situ hybridization, polymerase chain reaction, or receptor binding. Expression of NMDA subunits, or function of the NMDA receptors, varies in response to noxious stimuli (increase, decrease or no change), but these differences may be explained by the fact that subunit expression does not always guarantee the presence of functional NMDA...
receptors, so a change in expression cannot be taken to infer a change in function. Nonetheless, changes in expression after a noxious insult provide information regarding alterations in activity and disturbances in regulatory processes (translational regulation), particularly when mRNA and protein are studied simultaneously. Expressional changes of the NMDA receptor after noxious insults during development are reviewed below and summarized in table 3.

**Table 3.** Changes in NMDA subunit expression after noxious insults during development

<table>
<thead>
<tr>
<th>Evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR1</td>
<td></td>
</tr>
<tr>
<td>↑ in 3 brainstem nuclei (mRNA; HH), caudate and putamen (protein; HI)</td>
<td>5, 86</td>
</tr>
<tr>
<td>↓ in 1 brainstem nucleus (protein; HH), cortex and hippocampus (mRNA; HI)</td>
<td>5, 101</td>
</tr>
<tr>
<td>No change in 9 brain regions (protein; hypoxia) and forebrain (protein; HI)</td>
<td>91, 102</td>
</tr>
<tr>
<td>NR2A</td>
<td></td>
</tr>
<tr>
<td>↑ in cortex (mRNA; nicotine)</td>
<td>104</td>
</tr>
<tr>
<td>↓ (delayed) in forebrain (protein; HI)</td>
<td>102</td>
</tr>
<tr>
<td>No change in 9 brain regions (protein; hypoxia), in cortex, hippocampus, caudate and putamen (protein; HI)</td>
<td>86, 91, 101</td>
</tr>
<tr>
<td>NR2B</td>
<td></td>
</tr>
<tr>
<td>↑ in caudate and putamen (protein; HI)</td>
<td>86</td>
</tr>
<tr>
<td>↓ in forebrain (protein; HI), thalamus (mRNA; nicotine)</td>
<td>102, 104</td>
</tr>
<tr>
<td>No change in 9 brain regions (protein; hypoxia), cortex and hippocampus (protein; HI)</td>
<td>91, 101</td>
</tr>
<tr>
<td>NR2C and NR2D</td>
<td>No change in cortex and hippocampus (protein; HI)</td>
</tr>
</tbody>
</table>

HH = Hypercapnic-hypoxia; HI = hypoxic-ischemia. Refer to table 2 for details of exposure and animal model.

**Table 4.** Changes in NMDA receptor function after noxious insults during development

<table>
<thead>
<tr>
<th>Evidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding affinity for MK-801</td>
<td>2</td>
</tr>
<tr>
<td>↑ in cerebral cortex (hypoxia)</td>
<td>2, 101</td>
</tr>
<tr>
<td>↓ in hypothalamus, amygdaloid nuclei, cortex (hypoxia), and hippocampus (hypoxia, HI)</td>
<td>2, 101</td>
</tr>
<tr>
<td>Binding affinity for glutamate, Mg^{2+}, CPP</td>
<td>2, 94–96</td>
</tr>
<tr>
<td>↑ in cerebral cortex (hypoxia)</td>
<td>2, 94–96</td>
</tr>
<tr>
<td>Number of NMDA receptors</td>
<td>92, 96, 98</td>
</tr>
<tr>
<td>↓ in cerebral cortex (hypoxia)</td>
<td>92, 96, 98</td>
</tr>
<tr>
<td>Not changed in hippocampus (HI)</td>
<td>101</td>
</tr>
<tr>
<td>Activation of NMDA receptors</td>
<td>93</td>
</tr>
<tr>
<td>↑ in spermine-dependent activation in the cortex (hypoxia)</td>
<td>93</td>
</tr>
<tr>
<td>↓ in glutamate- and glycine-dependent activation in the cortex (hypoxia)</td>
<td>93</td>
</tr>
<tr>
<td>Phosphorylation and nitration of subunits</td>
<td>86, 102</td>
</tr>
<tr>
<td>↑ phosphorylation of NR1 in caudate and putamen, and of NR2B in forebrain (HI)</td>
<td>86, 102</td>
</tr>
<tr>
<td>↑ nitration of NR1, NR2A and NR2B in cerebral cortex (hypoxia)</td>
<td>99</td>
</tr>
</tbody>
</table>

HH = Hypercapnic-hypoxia; HI = hypoxic-ischemia. Refer to table 2 for details of exposure and animal model.
Experimental techniques for studying receptor function include receptor binding, receptor blockade, and receptor activation. Binding characteristics include the number of receptors ($B_{max}$) and the binding affinity of the receptors ($K_d$). The phosphorylation state of receptor proteins also provides information of receptor function since functional activity of the NMDA receptor is regulated by phosphorylation [87–90]. Functional changes of the NMDA receptor after noxious insults are reviewed below and summarized in table 4.

**Hypoxia**

Studies to date have predominantly investigated functional changes in the NMDA receptor, and no changes have been found in receptor expression, after hypoxia. However, NMDA receptor function is clearly affected by hypoxia. In the newborn, hypoxia induces a reduction in the total number of NMDA receptors, but more variable changes in receptor binding. The regional and age-dependent variability in receptor binding can be explained by differences in the response of the NMDA receptor subunits. Some findings can be explained by either increased NMDA ion-channel-binding sites, or decreased numbers of NMDA receptors.

*Expression:* In the newborn piglet model of postnatal hypoxia, 1 h of hypoxia has no effect on NR1, NR2A and NR2B protein levels in nine brain regions, including the frontal, parietal and temporal cortices, thalamus, hypothalamus, hippocampus, white matter, basal ganglia and cerebellum [91].

*Function:* Prenatal hypoxia resulted in a decrease in the number of NMDA receptors [90], decreased glutamate- and glycine-dependent activation of the NMDA receptor, and increased spermine-dependent receptor activation [93]. Postnatal hypoxia increases NMDA receptor affinity for the antagonist MK-801 in the piglet cortex [2], but decreases it in several brain regions of the infant rat, including the hypothalamus, amygdaloid nuclei, entorhinal cortex, perirhinal cortex and hippocampus [3]. Postnatal hypoxia also induced an increased affinity for glutamate [2], $\text{Mg}^{2+}$ [94, 95], and for the antagonist, CPP (3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid) [96, 97], and reduced the numbers of glutamate [96, 98] and CPP-binding sites [96, 97]. Subunit-specific increases in nitration of the NR1, NR2A and NR2B have also been reported [99].

**Hypoxic-Ischemia (HI)**

*Expression:* Intrauterine HI induces sustained effects on NR1 mRNA levels during infancy. After intrauterine HI in rats, mRNA for NR1 was decreased in the cortex at 1 day, and in the hippocampus at 4, 8, and 30 days of age [101]. NR1 protein was also decreased in the hippocampus, with no changes in the NR2A–D subunit. Postnatal HI induced an increase in NR1 protein in the caudate and putamen in the newborn piglet as well as increased NR2B expression, with no change in NR2A [86]. In the infant rat, immediately following an HI insult, NR2B decreased in the forebrain while tyrosine phosphorylation of NR2B increased. After 1–24 h recovery, NR2A decreased, whereas NR1 was unchanged (immediately and after recovery) [102].

*Function:* After intrauterine HI, binding affinity of the NMDA receptor to MK-801 was reduced in the hippocampus of infant rats, although the number of binding sites was not changed [101]. HI also increased phosphorylation of the NR1 protein in the caudate and putamen of newborn piglets [86] and of NR2B in the forebrain of the infant rat [102].

**Substances of Abuse – Nicotine**

*Expression:* Nicotine produces a selective increase in the NMDA receptor portion of the EPSP by over 100% with no change in the non-NMDA portion [103]. Only one study has examined the effects of chronic postnatal nicotine exposure on the NMDA receptor [104]. NR2A and NR2B mRNA expressions were studied in the auditory forebrain before and after nicotine exposure in 8- to 12-day-old rats. Two days of exposure produced no effects, but after 5 days, NR2A mRNA was increased in the cortex and NR2B mRNA was decreased in the thalamus [104].
**Fig. 3.** NR1 mRNA visualized by non-radioactive in situ hybridization in a normal developing piglet brainstem. (A) Transverse section of the piglet caudal medulla, (B) motor neurons of the XII nucleus, and (C) sensory neurons of the NTS. XII = Hypoglossal nucleus; DMNV = dorsal motor nucleus of the vagus; LRt = nucleus of the lateral reticular formation; ION = principle inferior olivary nucleus; NTS = nucleus of the solitary tract; Gr = gracile; Cu = cuneate; NSTT = nucleus of the spinal trigeminal tract.

**Fig. 4.** NR1 protein visualized by immunohistochemistry in a normal developing piglet brainstem. A Transverse section of the piglet caudal medulla, and B neurons of the ION. NR1-positive neuron (black filled arrow) and NR1-negative neuron (white filled arrowhead). See figure 3 for abbreviations.
NMDA Receptors after Noxious Insults

SIDS is the leading cause of death among infants less than 1 year of age in developed countries and occurs in approximately 1–2 infants per 1,000. Victims of SIDS die suddenly during a sleep period, the cause of which remains unknown, although many hypotheses implicating hypoxic mechanisms exist [105]. The majority of infants dying in this manner have autopsies, and so brain tissue is available for study. Compared to infants dying from a known cause, infants dying from SIDS showed increased NR1 mRNA in 6 of 8 brainstem nuclei [106]. However, this increased NR1 mRNA expression only translated to increased protein expression in one of the six nuclei. These results suggest that the NMDA receptor is altered in SIDS infants, but further studies will be required to determine whether there are indications of associated functional changes in the NMDA receptor.

Acute perinatal asphyxia resulting in hypoxic-ischemic encephalopathy (HIE) occurs in approximately 2–4 per 1,000 live term newborns and leads to disabling neurological disorders in 20–30% of affected neonates [107]. MK-801 binding was studied and compared between newborn infants that had died with HIE after birth asphyxia and a control group of newborns who had died from causes unrelated to brain injury [108]. Of the four cortical regions studied (prefrontal, motor, occipital and temporal) asphyxiated infants showed an increase in response to glutamate only in the temporal cortex. The severity of hypoxia did not correlate with the level of change in MK-801 binding.

Therapeutic Interventions

Regarding the responses to the noxious stimuli reviewed above, the two main options available for therapeutic intervention, and of likely potential, are NMDA receptor blockade and inhibition of caspase-3 activation, or a combination of both [109]. NMDA receptor blockade has been extensively explored as a neuroprotective mechanism against noxious insults to the brain, but it is important to note that NMDA receptor blockade under normal conditions can exacerbate neuronal cell death [44].

Limitations exist on the use of NMDA antagonists during early development, because of their documented or potential side effects. The use of NMDA receptor blockade as therapeutic intervention, requires knowledge of the regulational factors listed above (primarily age, timing and duration of the insult, resulting damage, and brain region affected), with ongoing observation and research to determine how interactions with other neurotransmitter systems affect the response.

Clinical studies in early development are thus far limited to NMDA receptor blockade via magnesium compounds. In human neonates, MgSO4 is seen as a promising therapeutic agent, with neuroprotection greatest after 26 weeks’ gestation and up to early infancy. Higher doses are required during early development than in older animals [10]. Epidemiological studies suggest that MgSO4 taken by mothers with pre-eclampsia during pregnancy reduces the incidence of cerebral palsy in low-birthweight infants [110, 111]. A clinical trial in 15 full-term infants with severe, acute asphyxial injury evaluated two doses of MgSO4 (250 vs. 400 mg/kg) and found dose-dependent respiratory depression, with an unacceptable risk of hypotension at the higher dose [112]. Results in animal models have been equivocal. For example, MgSO4 in three doses (400 mg/kg 1 h after resuscitation and 200 mg/kg at 12 and 24 h) was not neuroprotective in piglets after HI, as indicated by the still present damage (apoptosis and necrosis) in the cerebral cortex [113] and the continuing cerebral energy failure [114]. In contrast, neuroprotection was observed when piglets receiving MgSO4 before and during hypoxia (600 mg/kg over 30 min followed by 300 mg/kg during 60 min of hypoxia), whereby NMDA receptor number and affinity in the cerebral cortex was preserved [115], and the 2-fold increase in Bax:Bcl-2 ratio was prevented [116].

Animal studies have also shown that the high-affinity antagonist MK-801 has the potential for neuroprotection, but in contrast to MgSO4, it is associated with increased mortality, induces seizures, and may interfere with learning [117]. For example, MK-801 offered effective neuronal protection in rats when given within 2 h after the insult. Injury associated with HI was reduced by 53%, but there was an associated 5-fold increase in mortality [118]. There is also evidence of direct toxicity from NMDA antagonists including MK-801, ketamine and CPP. Administered in the absence of any other noxious insult these agents can induce neuronal injury, with evidence of massive apoptotic neurodegeneration in several brain regions of 7-day-old rats [44]. Neurodegeneration induced by NMDA receptor blockade has also been shown to induce deficits in hippocampal synaptic function, and persistent memory/learning impairments [119]. This may have other clinically important implications, because the routine use of sedatives, anticonvulsants and anesthetics in obstetric and pediatric medicine can have the effect of NMDA receptor blockade. Pharmacological studies are therefore being directed towards newer, low-affinity...
NMDA receptor channel blockers that have wider therapeu-
tic windows than MK-801, and clinical studies have
been undertaken in adults, for example with memantine in Alzheimer’s disease [120].

Conclusion

The glutamatergic system, predominantly the NMDA
receptor, has important functions in the perinatal period
regarding neurodevelopment. However, the characteris-
tics of the transmitter system at this critical develop-
mental period also mean that there is enhanced vulnera-
bility to excitotoxic damage after exposure to noxious
insults. Experimental evidence shows that hypoxia, hyp-
oxia-ischemia, or intermittent asphyxia during early de-
velopment induces expression and functional changes
in the NMDA receptor and for some, also neuronal cell
death. Further evidence of the effects of re-oxygenation or
re-perfusion will be required to evaluate potential therapeu-
tic targets within these paradigms of neuronal injury.
Therapeutic options are currently limited in the clinical
setting because any protective effects of the NMDA
antagonists are coupled with currently unacceptable risks
for damage to neurodevelopmental processes.

Acknowledgements

Research funded by NH&MRC #980504, National SIDS Austra-
lia, The Ramaciotti Foundation, Financial Markets Trust for Chil-
dren & CHATA, NSW. Ms Rita Machaalani is a scholarship recipi-
ent from Community Health and Tuberculosis Association in Aus-
tralia (CHATA). Dr. Waters is supported by an NH&MRC Practi-
tioner Fellowship #206507.

References

1 Waters KA, Gozal D: Responses to hypoxia
during early development. Respir Physiol Neuro-
obiol 2003;136:115–129.
2 Hoffman DJ, McGowan JE, Marro PJ, Mishra
OP, Delivoria-Papadopoulos M: Hypoxia-in-
duced modification of the N-methyl-D-aspar-
tate receptor in the brain of the newborn piglet.
3 Otoya RE, Seltzer AM, Donoso AO: Acute and
long-lasting effects of neonatal hypoxia on (+)-3-
[125I]MK-801 binding to NMDA brain recep-
4 Delivoria-Papadopoulos M, Mishra OP: Mech-
anisms of cerebral injury in perinatal asphyxia
132:S30–S34.
5 Machaalani R, Waters KA: Distribution and
quantification of NMDA R1 mRNA and pro-
tein in the piglet brainstem and effects of inter-
mittent hypercapnic hypoxia. Brain Res 2002;
951:293–300.
6 Choi DW: The role of glutamate neurotoxicity
in hypoxic-ischemic neuronal death. Annu Rev
7 Dobbing J, Sands J: Comparative aspects of the
brain growth spurt. Early Hum Dev 1979;3:
79–83.
8 Huttonlocher PR: Synapse elimination and
plasticity in developing human cerebral cortex.
9 Panigyrahy A, Rosenberg PA, Assmann S, Foley
EC, Kinney HC: Differential expression of glu-
tamate receptor subtypes in human brainstem
10 Dikranian K, Ishimaru MJ, Tenkova T, La-
man PD: Pathophysiology of perinatal asphyxia.
11 Rabinowicz T, de Courton-Myres GM, Petetot
JM, Xi G, de los Reyes E: Human cortex devel-
oper: Estimates of neuronal numbers indi-
cate major loss late during gestation. J Neuro-
12 Dikranian K, Ishimaru MJ, Tenkova T, La-
bruyère J, Qin YQ, Ikonomidou C, et al: Apop-
tosis in the in vivo mammalian forebrain. Neu-
robiol Dis 2001;8:359–379.
13 Miller MW, al-Ghoul WM: Numbers of neu-
rons in the developing principal sensory nu-
cleus of the trigeminal nerve: Enhanced surviv-
als of early-generated neurons over late-gen-
501.
14 Wams PM, Li L, Ashwell KW: Developmental
and lesion induced cell death in the rat ventro-
15 Spreafico R, Frassoni C, Arcelli P, Selvaggio
M, De Biasi S: In situ labeling of apoptotic cell
dead in the cerebral cortex and thalamus of rats
16 Ferrer I, Serrato T, Soriano E: Naturally occur-
ing cell death in the subicular complex and
hippocampus in the rat during development.
17 Namura S, Zhu J, Fink K, Endres M, Sriniva-
san A, Tomasselli KJ, et al: Activation and
cleavage of caspase-3 in apoptosis induced by
experimental cerebral ischemia. J Neurosci
18 Hill A: Current concepts of hypoxic-ischemic
cerebral injury in the term newborn. Pediatr
19 McDonald JV: The surgical management of
severe open brain injuries with consideration of
the long-term results. J Trauma 1980;20:
842–847.
20 Alexander GE, Crutcher MD: Functional ar-
chitecture of basal ganglia circuits. Neural sub-
strates of parallel processing. Trends Neurosci
21 Johnston MV, Trescher WH, Isida A, Nakaji-
ma W: Novel treatments after experimental brain
22 Johnston MV, Nakajima W, Hagberg H: Mech-
anisms of hypoxic neurodegeneration in the
developing brain. Neuroscientist 2002;8:212–
220.
23 Menkes JH, Curran J: Clinical and MR corre-
lates in children with extrapyramidal cerebral
24 Salford LG, Siejo BK: The influence of arterial
hypoxia and unilateral carotid artery occlusion
upon regional blood flow and metabolism in the
141.
25 Siejo BK: Cell damage in the brain: A specula-
1:155–185.
27 Siejo BK, Folbergrova J, MacMillan V: The
effect of hypcapnia upon intracellular pH in
the brain, evaluated by the bicarbonate-car-
boxyl acid method and from the creatine phos-
phokinase equilibrium. J Neurochem 1972;19:
2483–2495.
28 Folbergrova J, MacMillan V, Siejo BK: The
effect of moderate and marked hypcapnia
upon the energy state and upon the cytoplasmic
NADH–NAD+ ratio of the rat brain. J Neuro-
29 Paljarvi L, Soderfeldt B, Kalimo H, Olsson Y,
Siejo BK: The brain in extreme respiratory
acidosis. A light- and electron-microscopic
study in the rat. Acta Neuropathol (Berl) 1982;
58:87–94.
30 Williams CE, Mallard C, William T, Gluck-
man PD: Pathophysiology of perinatal asphyx-


57. Gould E, Cameron HA: Early NMDA receptor blockade impairs defensive behavior and in- creases cell proliferation in the dentate gyrus of developing rats. Behav Neurosci 1997;111:49–56.


NMDA Receptors after Noxious Insults

173


85 Machaalani R, Waters KA: Correlations between brainstem NMDA receptor changes and active neuronal cell death after intermittent hypercapnic hypoxia in the developing piglet. Brain Res 2003;975:141–148.


88 Swayne SL, Moss SJ, Raymond LA, Huganir RL: Regulation of ligand-gated ion channels by protein phosphorylation. Adv Second Messen-

89 Tingley WG, Roche KW, Thompson AK, Huganir RL: Regulation of NMDA receptor phosphorylation by alternative splicing of the C-terminal domain. Nature 1993;364:70–73.


91 Cai Z, Rhodes PG: Intraretinal hypoxia-isch-


93 Zanello SA, Ashraf QM, Mishra OP, Nitration is a mechanism of regulation of the NMDA receptor function during hypoxia. Neurosci-ence 2002;112:869–877.


98 Arakakis VB, Hsich CY, Leslie FM, Meth- rate R: A critical period for nicotine-induced disruption of synaptic development in rat au-


101 Cai Z, Rhodes PG: Intraretinal hypoxia-isch-


103 Arakakis VB, Hsich CY, Leslie FM, Meth- rate R: A critical period for nicotine-induced disruption of synaptic development in rat au-


105 Kinney HC, Filiano JJ, Harper RM: The neu- ropathology of the sudden infant death syn-

106 Machaalani R, Waters KA: NMDA receptor 1 expression in the brainstem of human in-

107 Finer NN, Robertson CM, Richards RT, Pin- nell LE, Peters KL: Hypoxic-ischemic en-

108 Waters KA: NMDA receptor 1 expression in the brainstem of human in-