Neonatal Seizures: An Update on Mechanisms and Management

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KEYWORDS

• Epilepsy • Perinatal • Synapse • Neurotransmitter receptor
• Glutamate • γ-aminobutyric acid (GABA)

Neonatal seizures are an important example of an age-specific seizure syndrome. Compared with seizures at older ages, neonatal seizures differ in etiology, semiology, and electroencephalographic signature, and can be refractory to antiepileptic drugs (AEDs) that are effective in other age populations. Their unique pathophysiology has become the subject of many research studies from a basic and clinical perspective, and is leading the way to new therapies for this often refractory disorder.

EPIDEMIOLOGY AND ETIOLOGY

The risk of seizures is highest in the neonatal period (1.8–5/1000 live births in the United States). The relative incidence is higher in premature infants less than 30 weeks's gestation,[1] occurring in 3.9% of these neonates compared with 1.5% of older infants. In the neonate, a broad range of systemic and central nervous system (CNS) disorders can increase the risk of seizures (**Box 1**). Most neonatal seizures are symptomatic; they can be extremely difficult to control with currently available AEDs, and can lead to long-term neurologic sequelae. Benign forms include benign familial neonatal seizures and transient, treatable metabolic derangements; these forms are largely without significant long-term consequences.

The most common cause of symptomatic neonatal seizures is hypoxic/ischemic encephalopathy (HIE), which affects approximately 1 to 2 of 1000 live births.[2,3] About two-thirds of cases of neonatal seizures are caused by HIE.[4] These seizures can occur in the setting of birth asphyxia, respiratory distress, or as a complication of early-life extracorporeal membrane oxygenation (ECMO) or cardiopulmonary bypass for corrective cardiac surgery.[5] In the case of HIE, these seizures usually occur within the first 1 to 2 days of birth and often remit after a few days, but carry with them

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Box 1
Diverse causes of neonatal seizures

Acute metabolic
Hypoglycemia
Hypocalcemia
Hypomagnesemia
Hypo- or hypernatremia
Withdrawal syndromes associated with maternal drug use
Iatrogenic associated with inadvertent fetal administration of local anesthetic
Rare inborn errors of metabolism (including pyridoxine responsive)
Cerebrovascular
Hypoxic/ischemic encephalopathy
Arterial and venous ischemic stroke
Intracerebral hemorrhage
Intraventricular hemorrhage
Subdural hemorrhage
Subarachnoid hemorrhage
CNS infection
Bacterial meningitis
Viral meningoencephalitis
Intrauterine ("TORCH") infections
Developmental
Multiple forms of cerebral dysgenesis
Other
Rare genetic syndromic disorders
Benign neonatal familial convulsions (sodium and potassium channel mutations identified)
Early myoclonic encephalopathy

a risk of long-term epilepsy and neurologic or cognitive deficits.[6,7] HIE is associated with a high incidence of seizures, reportedly in 40% to 60% of cases.[8,9] Other cerebrovascular disorders including arterial and venous stroke, intracerebral hemorrhage, and subarachnoid hemorrhage also frequently present clinically with seizures. Aside from HIE and cerebrovascular causes, the next most common causes of neonatal seizures are infections and malformations of cortical development. Common bacterial infectious causes include Group B streptococcus and *Escherichia coli*. Nonbacterial causes include intrauterine toxoplasmosis or cytomegalovirus infection, or neonatal encephalitis caused by toxoplasmosis, herpes simplex, coxsackie, or cytomegalovirus. Malformations of cortical development that frequently present with early-life seizures include lissencephaly, polymicrogyria, focal cortical dysplasia, and tuberous sclerosis. Metabolic disturbances responsible for neonatal seizures include hypoglycemia, hypocalcemia, hypomagnesemia, and abnormalities of other electrolytes and amino acids. Many metabolic causes are readily treatable (such as correction of glucose and electrolyte disturbances) and when such metabolic

disturbances are the primary cause of neonatal seizures, they are rarely associated with significant long-term consequences. Pyridoxine-dependent seizures can present as unremitting and refractory seizures within the first days of life, but rapidly respond to intravenous pyridoxine. Inborn errors of amino or organic acid metabolism can also present with seizures in the first days of life, such as hyperglycinemia, type II glutaric aciduria, and urea cycle disorders.

Other less common causes of neonatal seizures include benign familial neonatal convulsions, an autosomal dominant disorder that presents within the first week of life and is associated with subsequent normal development. Genetic analysis has revealed these to be related to mutations in the neuronal potassium channels KCNQ2 or KCNQ3.[10–12] Another benign syndrome possibly associated with a mutation in KCNQ2 is that of ''fifth day fits,'' which transiently occur for a day or so around the fifth or sixth postnatal day.[13]

Neonatal seizures can be refractory to AED therapy that is effective at later ages, especially when the seizures are symptomatic and a result of HIE. Conventional AEDs that are effective in older children and adults are largely inadequate, likely because seizures in the immature brain have unique mechanisms (see later discussion).

The outcome of prolonged neonatal seizures can include consequences in later life in more than 30% of survivors, with cognitive deficits ranging from learning disability (27%) to developmental delay and mental retardation (20%), and epilepsy later in life (27%).[6] The risk of mortality was reported previously as approximately 35%,[14] but recent studies of term infants with clinical seizures showed a lower neonatal mortality of less than 20% as a result of improvements in neonatal intensive care.[4,6] Despite improved survival, the long-term neurologic consequences remain high with studies reporting a range from 28%[4] to 46%.[6] Not all neonatal seizures portend the same risk, and it seems that worst prognosis is observed in those with symptomatic seizures caused by HIE or cerebral dysgenesis.[4] Better prognosis is also associated with milder electroencephalographic (EEG) abnormalities and no neuroimaging abnormalities.[15–17] As a result of advances in care, causes associated with more favorable outcome, such as hypocalcemic seizures, have decreased from accounting for approximately 30% of cases before the 1960s to less than 5% presently.[2] Currently, HIE predominates as the most common cause of refractory neonatal seizures.[4]

Although the term infant is at the highest risk for seizures, it is increasingly recognized that seizures can be a significant problem in preterm infants. According to a recent study, seizures can occur in 5.6% of very low birthweight infants; lower gestational age, male gender, and major systemic and neurologic injury, such as intraventricular hemorrhage or periventricular leukomalacia, are independent predictors of neonatal seizures.[18,19]

DIAGNOSIS

Neonatal seizures can be difficult to diagnose as there are often no clinical correlates of the electrographic seizures, a phenomenon called electroclinical dissociation. Regional interconnectivity, including interhemispheric and corticospinal, is not fully mature as a result of incomplete myelination of white matter tracts, leading to only modest behavioral manifestations of these seizures. Infants can show no signs or very subtle tonic or clonic movements, often limited to only 1 limb, making the diagnosis difficult to discern from myoclonus or other automatisms.[20] A recent study revealed that approximately 80% of EEG-documented seizures were not

accompanied by observable clinical seizures.[21] Hence, EEG is essential for diagnosis and for assessing treatment efficacy in this group. Full 20-lead EEGs are most sensitive in detecting these often multifocal seizures (**Fig. 1**). As full-lead EEGs can be difficult to obtain on an emergent basis in many neonatal intensive care units, amplitude-integrated EEG (aEEG) devices are becoming increasingly used.[22] aEEG is usually obtained from a pair or limited number of leads, and is displayed as a fast Fourier spectral transform. With aEEG, seizures are detected by acute alterations in spectral width, and a raw EEG from the single channel can be accessed by the viewer for confirmation.[23] Several reports now indicate that aEEG has relatively high specificity but compromised sensitivity, detecting approximately 75% of that of conventional full-lead montage EEG.[22,24–28]

Once neonatal seizures are confirmed, treatable metabolic and symptomatic causes need to be identified. Serologic studies include blood and serologic studies of systemic infection, and metabolic derangements such as acidosis, hypocalcemia, hypomagnesemia, and hypoglycemia. The timing of the seizures can be a helpful indicator, such as in the case of "fifth day fits," caused by hypocalcemia. Pyridoxine-dependent seizures present as refractory early neonatal seizures that uniquely respond to parenteral pyridoxine administration.[29,30] Seizures that continue to be refractory in the setting of a history consistent with HIE manifest within the first 24 to 48 hours of life, persist for several days, then seem to gradually remit.

MR imaging provides an important assessment of risk in infants with neonatal seizures. Imaging can provide important information on cerebral dysgenesis and gross structural malformations, which can be associated with neonatal seizures such as tuberous sclerosis, hemimegalencephaly, or cortical dysplasia. For symptomatic seizures caused by HIE, abnormal T2, fluid attenuated inversion recovery, and diffusion signals can be used to pinpoint regional injury and severity.[31] Recent studies

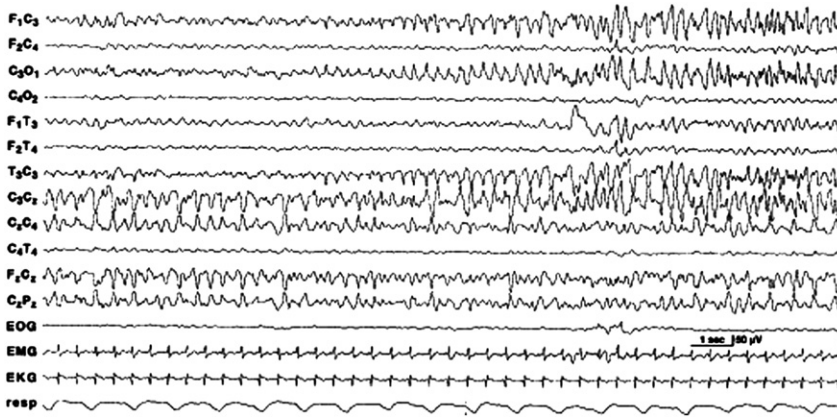

Fig. 1. Electroencephalographic appearance of neonatal seizures. Electrical seizure activity begins in the midline central region (CZ) and then shifts to the left central region (C3). Toward the end of the seizures, as the electrical activity persists in the left central region, the midline central region becomes uninvolved. This electrical seizure activity occurred in the absence of any clinical seizure activity in this 40-week gestational age female infant with hypoxic-ischemic encephalopathy. She was initially comatose and hypotonic and, at the time of EEG recording, had been treated with phenobarbital. (*Reprinted from* Mizrahi EM, Kellaway P. Characterization and classification of neonatal seizures. Neurology 1987;37(12):1837–44; with permission.)

show that magnetic resonance spectroscopy can be used to predict severity and outcome in patients. Miller and colleagues[17] reported that in HIE cases with seizures, an increased lactate to choline ratio, and reduced *N*-acetyl-aspartate levels, were more abnormal in patients with higher seizure burden. Another study of term infants with asphyxia and/or seizures by Glass and colleagues[16] showed that after adjusting for degree of MRI abnormality, seizure severity was associated with a higher risk of neuromotor abnormalities at 4 years of age than in those without seizures. These results suggest that neonatal seizures may independently worsen outcome even in the setting of documented MR lesions associated with HIE.

TREATMENT

Neonatal seizures can be extremely refractory to conventional AEDs, especially those associated with HIE. Early diagnosis should isolate metabolic or infectious causes and direct care to correcting the primary cause. However, the most refractory seizures are those caused by asphyxia and because of their short course (>72–96 hours) and poor prognosis, early treatment is essential and should be guided by EEG documentation of seizure activity. Current practices include early treatment with phenobarbital (doses ranging from 20–40 mg/kg),[32] with phenytoin (20 mg/kg), or fosphenytoin, and/or benzodiazepines such as lorazepam (0.05–0.1 mg/kg) as second-line adjuvant therapy for refractory seizures.[5] However, the consensus is that currently used AEDs are often ineffective for treatment of neonatal seizures.[33,34] Indeed, phenobarbital and phenytoin seem to be equally but incompletely effective, and either drug alone controls seizures in fewer than half of EEG-confirmed neonatal seizures.[35] As an alternative, second-line treatment with midazolam has variable efficacy, yet is less of a respiratory depressant than high dose barbiturates.[36,37] Lidocaine may be effective in refractory neonatal seizures, but its use may be limited by potential cardiac toxicity.[38] Newer AEDs such as topiramate and levetiracetam have been anecdotally reported to improve acute neonatal seizures.[39–41] It is also not known how long to continue treatment following the short course of neonatal seizures,[32] and how the length of treatment affects outcome.

In addition to pharmacologic therapy, neonates with HIE are also being increasingly treated with hypothermia. Recent clinical studies have led to a Cochrane review endorsement that early whole-body or limited cranial hypothermia improves neurologic outcome in treated neonates.[8,9,42,43] For whole-body hypothermia, current practice is to decrease core body temperature to 33.5°C for 72 hours.[8] Although aEEG is routinely employed to monitor brain activity during hypothermia, the effect of hypothermia on the incidence or severity of neonatal seizures is yet to be determined.[43]

PATHOPHYSIOLOGY

In response to the fact that neonatal seizures are refractory to conventional AEDs and can have severe consequences on long-term neurologic status, there is a growing body of active research directed at defining age-specific mechanisms of this disorder to identify new therapeutic targets and biomarkers. There have been substantial advances with regard to understanding pathophysiology, and, in particular, developmental stage-specific factors that influence mechanisms of seizure generation, responsiveness to anticonvulsants, and the impact on CNS development.[44] In addition, experimental data have raised concerns about the potential adverse effects of current treatments with barbiturates and benzodiazepines on brain development. Improved understanding of the unique age-specific mechanisms should yield new

therapeutic targets with clinical potential. Indeed, to date, no novel compounds have been developed specifically or approved by the US Food and Drug Administration (FDA) for treatment of neonatal seizures.[33]

Developmental age-specific mechanisms influence the generation and phenotype of seizures, the impact of seizures on brain structure and function, and the efficacy of anticonvulsant therapy. Factors governing neuronal excitability conspire to create a relatively hyperexcitable state in the neonatal period, as shown by the extremely low threshold to seizures in general and by the fact that this is the period of highest incidence of seizures across the life span,[45,46] similarly, in the rodent, seizure susceptibility peaks in the second postnatal week in many models.[44,47,48] In addition, the incomplete development of neurotransmitter systems results in a lack of "target" receptors for conventional AEDs. The relatively minimal status of myelination in cortical and subcortical structures results in the multifocal nature or unusual behavioral correlates of seizures at this age.[49,50]

The neonatal period is a period of intense physiologic synaptic excitability, as synaptogenesis occurring at this time point is wholly dependent on activity.[44] In the human, synapse and dendritic spine density are both peaking around term gestation and into the first months of life.[51,52] In addition, the balance between excitatory versus inhibitory synapses is tipped in the favor of excitation to permit robust activity-dependent synaptic formation, plasticity, and remodeling.[44] Glutamate is the major excitatory neurotransmitter in the CNS, and γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter. There is considerable and growing evidence from animal models and human tissue studies that neurotransmitter receptors are highly developmentally regulated (**Fig. 2**).[44,47,53] Studies of cell morphology, myelination, metabolism, and more recently neurotransmitter receptor expression suggest that the first 1 to 2 weeks of life in the rodent is a roughly analogous stage to the human neonatal brain.

Enhanced Excitability of the Neonatal Brain

Glutamate receptors are critical for plasticity and are transiently overexpressed during development compared with adulthood in animal models and human tissue studies.[44] A relative overexpression of certain glutamate receptor subtypes in rodent and human developing cortex coincides with ages of increased seizure susceptibility (see **Fig. 2**).[47,54,55] Glutamate receptors include ligand-gated ion channels, permeable to sodium, potassium, and in some cases, calcium, and metabotropic subtypes.[56] They are localized to synapses and nonsynaptic sites on neurons, and are also expressed on glia. The ionotropic receptor subtypes are classified based on selective activation by specific ligands, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate.

NMDA receptors are heteromeric, including an obligate NR1 subunit, and their make-up is developmentally regulated. In the immature brain, the NR2 subunits are predominantly those of the NR2B subunit, with the functional correlate of longer current decay time compared with the NR2A subunit, which is the form expressed in later life on mature neurons.[57] Other developmentally regulated subunits with functional relevance include the NR2C, NR2D, and NR3A subunits. Rodent studies show that these are all increased in the first 2 postnatal weeks, that this period is associated with lower sensitivity to magnesium, the endogenous receptor channel blocker; these features in turn result in increased neuronal excitability (**Figs. 2** and **3**).[56,58] NMDA receptor antagonists administered to immature rat pups have been shown to be highly effective against various hypoxic/ischemic insults and seizures in the immature brain.[59–61] However, the clinical potential of NMDA antagonists may be limited because of their severe sedative effects and a potential propensity for inducing

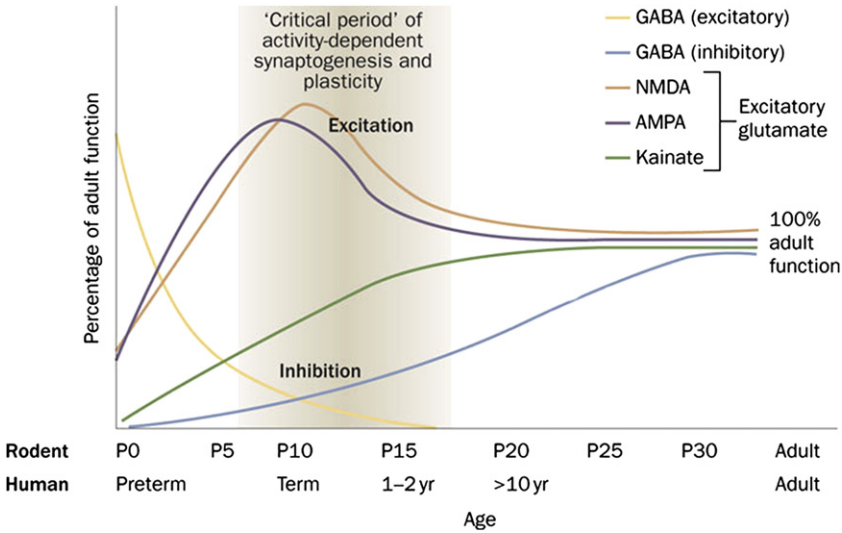

Fig. 2. Developmental profile of glutamate and GABA receptor expression and function. Equivalent developmental periods are displayed for rats and humans on the top and bottom x-axes, respectively. Activation of GABA receptors is depolarizing in rats early in the first postnatal week and in humans up to and including the neonatal period. Functional inhibition, however, is gradually reached over development in rats and humans. Before full maturation of GABA-mediated inhibition, the NMDA and AMPA subtypes of glutamate receptors peak between the first and second postnatal weeks in rats and in the neonatal period in humans. Kainate receptor binding is initially low and gradually rises to adult levels by the fourth postnatal week. Neonatal seizures emerge within the critical period of synaptogenesis and cerebral development. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate; P, postnatal day. (*Reprinted from* Rakhade SN, Jensen FE. Epileptogenesis in the immature brain: emerging mechanisms. Nat Rev Neurol 2009;5(7):380–91; with permission.)

apoptotic death in the immature brain.[62,63] Memantine, an agent currently in clinical use as a neuroprotectant in Alzheimer disease, may be an exception with fewer side effects, as a result of its use-dependent mechanism of action.[60,61,64]

Although the NMDA receptor has been reported to be selectively activated in processes related to plasticity and learning, the AMPA subtype of glutamate receptor is believed to subserve most fast excitatory synaptic transmission. In addition, unlike the NMDA receptor, most AMPA receptors (AMPAR) are not calcium permeable in the adult. AMPARs are heteromeric and made up of 4 subunits, including combinations of the GluR1, GluR2, GluR3, or GluR4 subunits.[56] However, in the immature rodent and human brain, AMPARs are calcium permeable because they lack the GluR2 subunit (see **Figs. 2** and **3**).[55,65] AMPAR subunits are developmentally regulated, with GluR2 expressed only at low levels until the third postnatal week in rodents and later in the first year of life in the human cortex.[54,66] Hence, AMPARs in the immature brain, because of their enhanced calcium permeability, may play an important role in contributing not only to excitability but also to activity-dependent signaling down stream of the receptor. NMDA and AMPARs are expressed at levels and with subunit composition that enhance excitability of neuronal networks around term in the human and in the first 2 postnatal weeks in the rodent (see **Fig. 2**).

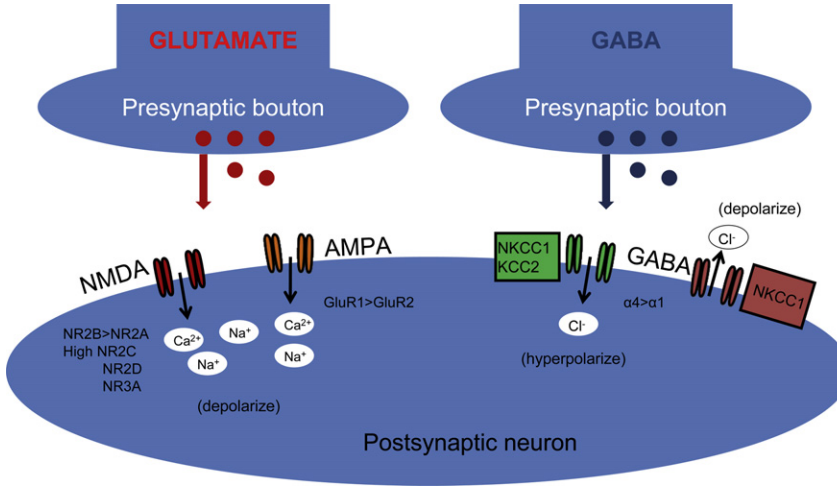

Fig. 3. Dynamics of synaptic transmission at cortical synapses in the neonatal period. Depicted are an excitatory glutamatergic synapse (*left panel*) and a GABAergic inhibitory synapse (*right panel*). Presynaptic release of glutamate results in depolarization (excitation) of the postsynaptic neuron (*left panel*) by activation of NMDA and AMPARs. In contrast, release of GABA (*right panel*) results in hyperpolarization (inhibition) when the postsynaptic neuron expresses sufficient quantities of the Cl^- transporter KCC2, but depolarization (excitation) when intracellular Cl^- accumulates as a result unopposed action of the Cl^- importer NKCC1. The immature glutamatergic receptors (*left panel*) are comprised of higher levels of NR2B, NR2C, NR2D, and NR3A subunits of the NMDA receptor, enhancing influx of Ca^{2+} and Na^+ compared with mature synapses. In addition, AMPARs are relatively deficient in GluR2 subunits, resulting in increased Ca^{2+} permeability compared with mature synapses. Hence, specific NMDA receptor antagonists and AMPAR antagonists may prove to be age-specific therapeutic targets for treatment development. In addition, although $GABA_A$ receptor activation normally results in hyperpolarization and inhibition at mature synapses, because of the coexpression of NKCC1 and KCC2, the expression of KCC2 is low in the neonatal period compared with later in life and thus Cl^- levels accumulate intracellularly; opening of $GABA_A$ receptors allows the passive efflux of Cl^- out of the cell, resulting in paradoxic depolarization. $GABA_A$ receptor subunit expression in the immature brain is typified by higher levels of the α4 subunit, which is functionally associated with diminished benzodiazepine sensitivity. Both these attributes of the $GABA_A$ receptor make classic GABA agonists such as barbiturates and benzodiazepines less effective in the neonatal brain. The NKCC1 channel blocker bumetanide has anticonvulsant efficacy when administered with phenobarbital, suggesting a synergistic effect.

Rodent studies show that AMPAR antagonists seem to be potently effective against neonatal seizures, even superior to NMDA receptor antagonists or conventional AEDs and GABA agonists. Topiramate, which is approved by the FDA for seizure control in children and adults, has been shown to be an AMPAR antagonist, in addition to several other potential anticonvulsant mechanisms.[67] Topiramate has been shown to be effective in suppressing seizures and long-term neurobehavioral deficits in a rodent seizure model, even when administered following seizures.[68,69] In addition, topiramate in combination with hypothermia was found to be protective in a rodent neonatal stroke model.[70] The specific AMPAR antagonist, talampanel, currently in phase 2 trials for epilepsy and amyotrophic lateral sclerosis in children and adults, was recently shown to protect against neonatal seizures in a rodent model.[71]

Decreased Efficacy of Inhibitory Neurotransmission in the Immature Brain

Expression and function of the inhibitory $GABA_A$ receptors are also developmentally regulated. Rodent studies show that GABA receptor binding, synthetic enzymes, and overall receptor expression are lower in early life compared with later.[47,72] GABA receptor function is regulated by subunit composition, and the $\alpha 4$ and $\alpha 2$ subunits are relatively overexpressed in the immature brain compared with the $\alpha 1$ subunit (see **Fig. 3**).[73] When the $\alpha 4$ subunit is expressed the receptor is less sensitive to benzodiazepines compared with receptors containing $\alpha 1$,[74] and as is often the case clinically, seizures in the immature rat respond poorly to benzodiazepines.[75,76]

Receptor expression and subunit composition can partially explain the resistance of seizures in the immature brain to conventional AEDs that act as GABA agonists. However, in the mature brain, inhibition of neuronal excitability via GABA agonists relies on the ability of $GABA_A$ receptors to cause a net influx of chloride (Cl^-) from the neuron, resulting in hyperpolarization.[77] In the immature forebrain, GABA receptor activation can cause depolarization rather than hyperpolarization[78–80] because the Cl^- gradient is reversed in the immature brain: intracellular Cl^- levels are high in the immature brain because of a relative underexpression of the Cl^- exporter KCC2 compared with mature brain (see **Figs. 2** and **3**).[81] Recent studies in human brain have shown that KCC2 is virtually absent in cortical neurons until late in the first year of life, and gradually increases thereafter, although the Cl^- importer NKCC1 is overexpressed in the neonatal human brain and during early life in the rat when seizures are resistant to GABA agonists.[81] The NKCC1 inhibitor, bumetanide, shows efficacy against kainate-induced seizures in the immature brain[82]; this agent, already approved by the FDA as a diuretic, is currently under evaluation in a phase 1/2 trial as an add-on agent in the treatment of neonatal seizures (http://www.clinicaltrials.gov trial ID: NCT00830531).

Ion Channel Configuration Favors Depolarization in Early Life

Ion channels also regulate neuronal excitability and, like neurotransmitter receptors, are developmentally regulated. As stated earlier, mutations in the K^+ channels KCNQ2 and KCNQ3 are associated with benign familial neonatal convulsions.[83] These mutations interfere with the normal hyperpolarizing K^+ current that prevents repetitive action potential firing.[84] Hence, at the time when there is an overexpression of GluRs and incomplete network inhibition, a compensatory mechanism is not available in these mutations. Another K^+-channel superfamily member, the HCN (or h) channels, is also developmentally regulated. The h currents are important for maintenance of resting membrane potential and dendritic excitability,[85] and function is regulated by isoform expression. The immature brain has a relatively low expression of the HCN1 isoform, which serves to reduce dendritic excitability in the adult brain.[86] Hence, ion channel maturation can also contribute to the hyperexcitability of the immature brain, and can have a cumulative effect when occurring in combination with the aforementioned differences in ligand-gated channels. Recently, selective blockers of HCN channels have been shown to disrupt synchronous epileptiform activity in the neonatal rat hippocampus,[87] suggesting that these developmentally regulated channels may also represent a target for therapy in neonatal seizures. N- and P/Q-type voltage sensitive calcium channels regulate neurotransmitter release.[88] With maturation, this function is taken over exclusively by the P/Q-type channels, formed by Cav2.1 subunits, a member of the Ca^{2+} channel superfamily.[89] Mutations in Cav2.1 may be involved in absence epilepsy, suggesting a failure in the normal maturational profile.[90]

A Role for Neuropeptides in the Hyperexcitability of the Immature Brain

Neuropeptide systems are also dynamically fluctuating in the perinatal period. An important example is corticotrophin-releasing hormone (CRH), which elicits potent neuronal excitation.[91,92] Compared with later life, in the perinatal period CRH and its receptors are expressed at higher levels, specifically in the first 2 postnatal weeks in the rat.[93] CRH levels increase during stress, and thus seizure activity in the immature brain may exacerbate subsequent seizure activity. Adrenocorticotropic hormone, which has shown efficacy in infantile spasms, is also known to down-regulate CRH gene expression.[94] Hence, neuropeptide modulation may be an area of future clinical importance in developing novel neonatal seizure treatments.

Enhanced Potential for Inflammatory Response to Seizures in the Immature Brain

Neonatal seizures can occur in the setting of inflammation either because of an intercurrent infection or secondary to hypoxic/ischemic injury. Experimental and clinical evidence exists for early microglial activation and inflammatory cytokine production in the developing brain in hypoxia/ischemia[95,96] and inflammation.[97,98] Microglia have been shown to be highly expressed in immature white matter in rodents and humans during cortical development.[99] Antiinflammatory compounds or agents that inhibit microglial activation, such as minocycline, have been reported to attenuate neuronal injury in models of excitotoxicity and hypoxia/ischemia.[100] During the term period, microglia density in deep gray matter is higher than at later ages, likely because of a migration of the population of cells en route to more distal cortical locations. Experimental models show microglia activation, as seen by morphologic changes and rapid production of proinflammatory cytokines occurring after acute seizures in different epilepsy animal models.[101,102] During brain development, microglia show maximal density simultaneous with the period of peak synaptogenesis.[103] During normal development and in response to injury, microglia participate in ''synaptic stripping'' by detaching presynaptic terminals from neurons.[104,105] The microglial inactivators minocycline and doxycycline have been shown to be protective against seizure-induced neuronal death[106] and in neonatal stroke models.[107,108]

Selective Neuronal Injury in the Developing Brain

Although many studies suggest that seizures, or status epilepticus, induce less death in the immature brain than in the adult, there is evidence that some neuronal populations are vulnerable. Similar to the sensitivity of subplate neurons, hippocampal neurons in the perinatal rodent have been shown to undergo selective cell death and oxidative stress following chemoconvulsant-induced cell death.[109] Stroke studies in neonatal rodents also suggest that there can be selective vulnerability of specific cell populations in early development.[110] Subplate neurons are present in significant numbers in the deep cortical regions during the preterm and neonatal period.[111] These neurons are critical for the normal maturation of cortical networks.[112,113] In humans and rodents these cells possess high levels of AMPARs and NMDARs.[49,54] These cells may also lack oxidative stress defenses present in mature neurons. Animal models have revealed that these neurons are selectively vulnerable compared with overlying cortex following a hypoxic/ischemic insult.[114] Indeed chemoconvulsant-induced seizures in rats, provoked by the convulsant kainate in early postnatal life, have produced a similar loss of subplate neurons, with consequent abnormal development of inhibitory networks.[112]

Several studies have shown that the application of clinically available antioxidants, such as eythropoetin (Epo), is protective against neuronal injury in neonatal

stroke.[115,116] Recently, Epo was shown to decrease later increases in seizure susceptibility of hippocampal neurons following hypoxia-induced neonatal seizures in rats.[117]

Seizure-induced Neuronal Network Dysfunction: Potential Interaction Between Epileptogenesis and Development of Neurocognitive Disability

Given that there is minimal neuronal death in most models of neonatal seizures, the long-term outcome of neonatal seizures is believed to be caused by seizure-induced alterations in surviving networks of neurons. Evidence for this theory comes from several studies that reveal disordered synaptic plasticity and impaired long-term potentiation and impaired learning later in life in rodents following brief neonatal seizures.[118,119] The neonatal period represents a stage of naturally enhanced synaptic plasticity when learning occurs at a rapid pace.[120,121] A major factor in this enhanced synaptic plasticity is the predominance of excitation over inhibition, which also increases susceptibility to seizures, as mentioned earlier. However, seizures that occur during this highly responsive developmental window seem to access signaling events that have been found to be central to normal synaptic plasticity. There are rapid increases in synaptic potency that seem to mimic long-term potentiation, and this pathologic activation may contribute to enhanced epileptogenesis.[122] In addition, GluR-mediated molecular cascades associated with physiologic synaptic plasticity may be overactivated by seizures, especially in the developing brain.[122,123] Rodent studies show a reduction in synaptic plasticity in neuronal networks such as hippocampus following early-life seizures, suggesting that the pathologic plasticity may have occluded normal plasticity, contributing to the impaired learning observed after early-life seizures.[122] Many models reveal that neonatal seizures alter synaptic plasticity,[124] and recent studies are delineating the molecular signaling cascades that are altered following early-life seizures.[125,126] In addition to glutamate receptors, inhibitory $GABA_A$ receptors can also be affected by seizures in early life, resulting in long-term impairments in function. Early and immediate functional decreases in inhibitory GABAergic synapses mediated by post-translational changes in $GABA_A$ subunits are seen following hypoxia-induced seizures in rat pups.[125] Flurothyl-induced seizures result in a selective impairment of GABAergic inhibition within a week.[127] There is evidence that some of these changes may be downstream of Ca^{2+} permeable glutamate receptors and Ca^{2+} signaling cascades, and that early postseizure treatment with GluR antagonists or phosphatase inhibitors may interrupt these pathologic changes that underlie the long-term disabilities and epilepsy.[122,125]

ANTICONVULSANTS AND THE DEVELOPING BRAIN

Emerging identification of age-specific mechanisms for neonatal seizures is pointing to the use of novel therapeutic targets. Caution must be exercised when devising new therapies, as the target may indeed be essential for normal brain development, albeit a contributor to neuronal hyperexcitability. More than 2 decades ago, experimental data emerged showing that phenobarbital exposure had adverse effects on survival and morphology of cultured neurons, derived from fetal mouse tissue, and these observations raised concerns about risks of this drug for treatment of neonatal seizures.[128,129] Subsequent studies in neonatal rats showed that daily treatment with phenobarbital or diazepam in the first postnatal month resulted in measurable changes in regional cerebral metabolism and behavior.[130,131]

More recently, evidence emerged that brief systemic treatment with conventional AEDs such as phenobarbital, diazepam, phenytoin, and valproate all increase

apoptotic neuronal death in normal immature rodents.[132] Similarly, NMDAR antagonists also induce an increase in constitutive apoptosis in the developing rodent brain.[62] Yet, the AMPAR antagonists NBQX and topiramate do not cause such adverse effects,[62,133] although the mechanism for this relative safety over the other agents is not understood. The novel AED levetiracetam also has no effect on apoptosis in the developing brain.[134]

Despite these data on adverse effects or lack thereof in rodents, no evidence of similar phenomena exists for other species, and it remains unknown if these toxicity mechanisms are relevant for human neonates. Moreover, interpretation of AED toxicity studies must be tempered by the consideration that these experiments are typically performed in normal animals, and that the impact of AED administration may well differ in normal animals and in those with seizures.

Table 1
Candidate potential targets and therapies from emerging experimental and clinical literature

Temporal Profile	Mechanism Targeted	Potential Therapeutic Options
Acute changes	Immediate early genes	Chromatin acetylation modifiers/ histone deacetylation inhibitors (valproate)
	NMDA receptors	NMDA receptor inhibitors (memantine, felbamate)
		NR2B-specific inhibitors (Ifenprodil)
	AMPARs	AMPAR antagonists (topiramate, talampanel, GYKI compounds)
	NKCC1 chloride transporters	NKCC1 inhibitor (bumetanide in combination with GABA agonists phenobarbital, benzodiazepines)
	GABA receptors	GABA receptor agonists (phenobarbital, benzodiazepines)
	Phosphatases (eg, calcineurin)	Phosphatase inhibitors (FK-506)
	Kinases (activation of PKA, PKC, CaMKII, Src kinases, and so forth)	Kinase inhibitors (CaMKII inhibitor KN-62, PKA inhibitor KT5720, PKC inhibitor chelerythrine)
Subacute changes	Inflammation	Antiinflammatory compounds (ACTH), microglial inactivators (minocycline, doxycycline)
	Neuronal injury	Erythropoietin, antioxidants, NO inhibitors, NMDAR antagonists (memantine)
	HCN channels	I(h)-blocker ZD7288
	CB1 receptor	CB1 receptor antagonists (SR 14176A, rimonabant)
Chronic changes	Sprouting	Protein synthesis inhibitors (rapamycin, cycloheximide)
	Gliosis	Antiinflammatory agents,(Cox-2 inhibitors, minocycline, doxycycline)

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FUTURE DIRECTIONS AND NEW THERAPEUTIC TARGETS

Refractory neonatal seizures remain a significant clinical problem, and no new treatments for this condition have been introduced for decades. Many new mechanisms and components of neonatal seizures have been uncovered. These present important new possibilities for novel therapeutic strategies in the population of neonates at risk for acute and long-term neurologic damage from neonatal seizures. Several major classes of agents with possible age-specific effects have emerged and are summarized in **Table 1**. These include modulators of neurotransmitter receptors and ion channels and transporters, antiinflammatory compounds, neuroprotectants, and antioxidants. Interdisciplinary collaboration between neonatologists and neonatal neurologists is essential for the success of such studies. As basic research reveals new age-specific therapeutic targets, these targets can be validated with analysis of cell-specific gene and protein expression in human autopsy samples. Experimental data regarding the potential efficacy of agents such as bumetanide, topiramate, and levetiracetam are encouraging, but the duration of use of these agents may be limited by safety concerns related to their effects on long-term brain development. Animal model trials and human studies must be aligned to understand how safety and efficacy data from rodent and nonhuman primates predict human responses. Several early-life seizure models exist in which there are indeed long-term effects on learning, and these could also be employed to address the effects of treatment on brain and cognitive development. Clinical therapeutic trials in neonates would be greatly improved if there were accurate biomarkers of acute and chronic therapeutic efficacy, yet none exist other than the EEG. Measures of brain metabolic integrity such as magnetic resonance spectroscopy or near infrared spectroscopy, when combined with EEG data, may provide surrogate measures of treatment efficacy. Incorporation of continuous EEG monitoring into clinical studies of neonatal seizure therapy will be essential. Seizure cessation is an important therapeutic goal, yet improved neurodevelopmental outcome is clearly of critical importance.

REFERENCES

1. Scher MS, Aso K, Beggarly ME, et al. Electrographic seizures in preterm and full-term neonates: clinical correlates, associated brain lesions, and risk for neurologic sequelae. Pediatrics 1993;91:128–34.
2. Ronen GM, Penney S, Andrews W. The epidemiology of clinical neonatal seizures in Newfoundland: a population-based study. J Pediatr 1999;134(1): 71–5.
3. Saliba RM, Annegers JF, Waller DK, et al. Incidence of neonatal seizures in Harris County, Texas, 1992–1994. Am J Epidemiol 1999;150(7):763–9.
4. Tekgul H, Gauvreau K, Soul J, et al. The current etiologic profile and neurodevelopmental outcome of seizures in term newborn infants. Pediatrics 2006;117: 1270–80.
5. Volpe JJ. Neurology of the newborn. 5th edition. Philadelphia: Saunders/Elsevier; 2008.
6. Ronen GM, Buckley D, Penney S, et al. Long-term prognosis in children with neonatal seizures: a population-based study. Neurology 2007;69(19):1816–22.
7. Bergamasco B, Penna P, Ferrero P, et al. Neonatal hypoxia and epileptic risk: a clinical prospective study. Epilepsia 1984;25:131–46.
8. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. N Engl J Med 2005;353(15): 1574–84.

9. Gluckman PD, Wyatt JS, Azzopardi D, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. Lancet 2005;365(9460):663–70.
10. Coppola G, Castaldo P, Miraglia del Giudice E, et al. A novel KCNQ2 K^+ channel mutation in benign neonatal convulsions and centrotemporal spikes. Neurology 2003;61(1):131–4.
11. Singh NA, Westenskow P, Charlier C, et al. KCNQ2 and KCNQ3 potassium channel genes in benign familial neonatal convulsions: expansion of the functional and mutation spectrum. Brain 2003;126(Pt 12):2726–37.
12. Coppola G, Veggiotti P, Del Giudice EM, et al. Mutational scanning of potassium, sodium and chloride ion channels in malignant migrating partial seizures in infancy. Brain Dev 2006;28(2):76–9.
13. Claes LR, Ceulemans B, Audenaert D, et al. De novo KCNQ2 mutations in patients with benign neonatal seizures. Neurology 2004;63(11):2155–8.
14. Holden KR, Mellits ED, Freeman JM. Neonatal seizures. I. Correlation of prenatal and perinatal events with outcomes. Pediatrics 1982;70(2):165–76.
15. McBride MC, Laroia N, Guillet R. Electrographic seizures in neonates correlate with poor neurodevelopmental outcome. Neurology 2000;55(4):506–13.
16. Glass HC, Glidden D, Jeremy RJ, et al. Clinical neonatal seizures are independently associated with outcome in infants at risk for hypoxic-ischemic brain injury. J Pediatr 2009;155(3):318–23.
17. Miller SP, Ramaswamy V, Michelson D, et al. Patterns of brain injury in term neonatal encephalopathy. J Pediatr 2005;146(4):453–60.
18. Kohelet D, Shochat R, Lusky A, et al. Risk factors for neonatal seizures in very low birthweight infants: population-based survey. J Child Neurol 2004;19(2): 123–8.
19. Kohelet D, Shochat R, Lusky A, et al. Risk factors for seizures in very low birthweight infants with periventricular leukomalacia. J Child Neurol 2006;21(11): 965–70.
20. Mizrahi EM, Kellaway P. Diagnosis and management of neonatal seizures. Philadelphia: Lippincott-Raven; 1998.
21. Clancy RR. Prolonged electroencephalogram monitoring for seizures and their treatment. Clin Perinatol 2006;33(3):649–65, vi.
22. Shellhaas RA, Soaita AI, Clancy RR. Sensitivity of amplitude-integrated electroencephalography for neonatal seizure detection. Pediatrics 2007;120(4): 770–7.
23. Lawrence R, Mathur A, Nguyen The Tich S, et al. A pilot study of continuous limited-channel aEEG in term infants with encephalopathy. J Pediatr 2009; 154(6):835–41 e1.
24. Tekgul H, Bourgeois BF, Gauvreau K, et al. Electroencephalography in neonatal seizures: comparison of a reduced and a full 10/20 montage. Pediatr Neurol 2005;32(3):155–61.
25. Navakatikyan MA, Colditz PB, Burke CJ, et al. Seizure detection algorithm for neonates based on wave-sequence analysis. Clin Neurophysiol 2006;117(6): 1190–203.
26. Shellhaas RA, Clancy RR. Characterization of neonatal seizures by conventional EEG and single-channel EEG. Clin Neurophysiol 2007;118(10):2156–61.
27. Clancy RR. Summary proceedings from the neurology group on neonatal seizures. Pediatrics 2006;117(3 Pt 2):S23–7.
28. de Vries LS, Toet MC. Amplitude integrated electroencephalography in the full-term newborn. Clin Perinatol 2006;33(3):619–32, vi.

29. Grillo E, da Silva RJ, Barbato JH Jr. Pyridoxine-dependent seizures responding to extremely low-dose pyridoxine. Dev Med Child Neurol 2001;43(6):413–5.
30. Baxter P. Pyridoxine-dependent and pyridoxine-responsive seizures. Dev Med Child Neurol 2001;43(6):416–20.
31. Grant PE, Yu D. Acute injury to the immature brain with hypoxia with or without hypoperfusion. Radiol Clin North Am 2006;44(1):63–77, viii.
32. Bartha A, Shen J, Katz KH, et al. Neonatal seizures: multi-center variability in current treatment practices. Pediatr Neurol Res 2007;37(2):85–90.
33. Sankar R, Painter MJ. Neonatal seizures: after all these years we still love what doesn't work. Neurology 2005;64(5):776–7.
34. Booth D, Evans DJ. Anticonvulsants for neonates with seizures. Cochrane Database Syst Rev 2004;(4):CD004218.
35. Painter MJ, Scher MS, Stein AD, et al. Phenobarbital compared with phenytoin for the treatment of neonatal seizures. N Engl J Med 1999;341(7):485–9.
36. Boylan GB, Young K, Panerai RB, et al. Dynamic cerebral autoregulation in sick newborn infants. Pediatr Res 2000;48(1):12–7.
37. Carmo KB, Barr P. Drug treatment of neonatal seizures by neonatologists and paediatric neurologists. J Paediatr Child Health 2005;41(7):313–6.
38. Malingre MM, Van Rooij LG, Rademaker CM, et al. Development of an optimal lidocaine infusion strategy for neonatal seizures. Eur J Pediatr 2006;165(9): 598–604.
39. Silverstein FS, Ferriero DM. Off-label use of antiepileptic drugs for the treatment of neonatal seizures. Pediatr Neurol 2008;39(2):77–9.
40. Pellock J. Antiepileptic drugs trials: neonates and infants. Epilepsy Res 2006; 68(1):42–5.
41. Hmaimess G, Kadhim H, Nassogne MC, et al. Levetiracetam in a neonate with malignant migrating partial seizures. Pediatr Neurol 2006;34(1):55–9.
42. Jacobs S, Hunt R, Tarnow-Mordi W, et al. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev 2007;(4):CD003311.
43. Azzopardi D, Brocklehurst P, Edwards D, et al. The TOBY Study. Whole body hypothermia for the treatment of perinatal asphyxial encephalopathy: a randomised controlled trial. BMC Pediatr 2008;8:17.
44. Rakhade SN, Jensen FE. Epileptogenesis in the immature brain: emerging mechanisms. Nat Rev Neurol 2009;5(7):380–91.
45. Hauser WA, Annegers JF, Kurland LT. Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935–1984. Epilepsia 1993;34:453–68.
46. Aicardi J, Chevrie JJ. Convulsive status epilepticus in infants and children. A study of 239 cases. Epilepsia 1970;11(2):187–97.
47. Sanchez RM, Jensen FE. Maturational aspects of epilepsy mechanisms and consequences for the immature brain. Epilepsia 2001;42:577–85.
48. Sanchez RM, Jensen FE, Pitanken A, et al. Modeling hypoxia-induced seizures and hypoxic encephalopathy in the neonatal period. Models of seizures and epilepsy. San Diego (CA): Elsevier; 2005.
49. Talos DM, Follett PL, Folkerth RD, et al. Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. II. Human cerebral white matter and cortex. J Comp Neurol 2006; 497(1):61–77.
50. Haynes RL, Borenstein NS, DeSilva TM, et al. Axonal development in the cerebral white matter of the human fetus and infant. J Comp Neurol 2005;484(2): 156–67.

51. Takashima S, Chan F, Becker LE, et al. Morphology of the developing visual cortex of the human infant: a quantitative and qualitative Golgi study. J Neuropathol Exp Neurol 1980;39(4):487–501.
52. Huttenlocher PR, deCourten C, Garey LJ, et al. Synaptogenesis in human visual cortex – evidence for synapse elimination during normal development. Neurosci Lett 1982;33:247–52.
53. Johnston MV. Neurotransmitters and vulnerability of the developing brain. Brain Dev 1995;17(5):301–6.
54. Talos DM, Fishman RE, Park H, et al. Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. I. Rodent cerebral white matter and cortex. J Comp Neurol 2006;497(1):42–60.
55. Sanchez RM, Koh S, Rio C, et al. Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. J Neurosci 2001;21(20):8154–63.
56. Hollmann M, Heinemann S. Cloned glutamate receptors. Annu Rev Neurosci 1994;17:31–108.
57. Jiang Q, Wang J, Wu X, et al. Alterations of NR2B and PSD-95 expression after early-life epileptiform discharges in developing neurons. Int J Dev Neurosci 2007;25(3):165–70.
58. Wong HK, Liu XB, Matos MF, et al. Temporal and regional expression of NMDA receptor subunit NR3A in the mammalian brain. J Comp Neurol 2002;450(4):303–17.
59. Stafstrom CE, Tandon P, Hori A, et al. Acute effects of MK801 on kainic acid-induced seizures in neonatal rats. Epilepsy Res 1997;26(2):335–44.
60. Mares P, Mikulecka A. Different effects of two *N*-methyl-D-aspartate receptor antagonists on seizures, spontaneous behavior, and motor performance in immature rats. Epilepsy Behav 2009;14(1):32–9.
61. Chen HS, Wang YF, Rayudu PV, et al. Neuroprotective concentrations of the *N*-methyl-D-aspartate open-channel blocker memantine are effective without cytoplasmic vacuolation following post-ischemic administration and do not block maze learning or long-term potentiation. Neuroscience 1998;86(4):1121–32.
62. Ikonomidou C, Bosch F, Miksa M, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283:70–4.
63. Bittigau P, Sifringer M, Ikonomidou C. Antiepileptic drugs and apoptosis in the developing brain. Ann N Y Acad Sci 2003;993:103–14 [discussion: 123–4].
64. Manning SM, Talos DM, Zhou C, et al. NMDA receptor blockade with memantine attenuates white matter injury in a rat model of periventricular leukomalacia. J Neurosci 2008;28(26):6670–8.
65. Kumar SS, Bacci A, Kharazia V, et al. A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. J Neurosci 2002;22(8):3005–15.
66. Talos DM, Follett PL, Folkerth RD, et al. Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. II. Human cerebral white matter and cortex. J Comp Neurol 2006;497(1):61–77.
67. Shank RP, Gardocki JF, Streeter AJ, et al. An overview of the preclinical aspects of topiramate: pharmacology, pharmacokinetics, and mechanism of action. Epilepsia 2000;41(Suppl 1):S3–9.
68. Koh S, Tibayan FD, Simpson J, et al. NBQX or topiramate treatment following perinatal hypoxia-induced seizures prevents later increases in seizure-induced neuronal injury. Epilepsia 2004;45(6):569–75.

69. Koh S, Jensen F. Topiramate blocks perinatal hypoxia- induced seizures in rat pups. Ann Neurol 2001;50(3):366–72.
70. Liu Y, Barks JD, Xu G, et al. Topiramate extends the therapeutic window for hypothermia-mediated neuroprotection after stroke in neonatal rats. Stroke 2004;35(6):1460–5.
71. Aujla PK, Fetell M, Jensen FE. Talampanel suppresses the acute and chronic effects of seizures in a rodent neonatal seizure model. Epilepsia 2009;50(4):694–701.
72. Swann JW, Brady RJ, Martin DL. Postnatal development of GABA-mediated synaptic inhibition in rat hippocampus. Neuroscience 1989;28(3):551–61.
73. Brooks-Kayal A, Jin H, Price M, et al. Developmental expression of GABA(A) receptor subunit mRNAs in individual hippocampal neurons in vitro and in vivo. J Neurochem 1998;70(3):1017–28.
74. Kapur J, Macdonald RL. Postnatal development of hippocampal dentate granule cell γ-aminobutyric acid A receptor pharmacological properties. Mol Pharmacol 1999;55:444–52.
75. Jensen FE, Alvarado S, Firkusny IR, et al. NBQX blocks the acute and late epileptogenic effects of perinatal hypoxia. Epilepsia 1995;36(10):966–72.
76. Swann J, Moshe SL, Engel J Jr, et al. Developmental issues in animal models epilepsy: a comprehensive textbook. Philadelphia: Lippincott-Raven Publishers; 1997. p. 467–80.
77. Dzhala VI, Staley KJ. Excitatory actions of endogenously released GABA contribute to initiation of ictal epileptiform activity in the developing hippocampus. J Neurosci 2003;23(5):1840–6.
78. Khazipov R, Khalilov I, Tyzio R, et al. Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus. Eur J Neurosci 2004;19(3):590–600.
79. Loturco JJ, Owens DF, Heath MJ, et al. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron 1995;15(6):1287–98.
80. Owens DF, Boyce LH, Davis MB, et al. Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. J Neurosci 1996;16(20):6414–23.
81. Dzhala VI, Talos DM, Sdrulla DA, et al. NKCC1 transporter facilitates seizures in the developing brain. Nat Med 2005;11(11):1205–13.
82. Dzhala VI, Brumback AC, Staley KJ. Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. Ann Neurol 2008;63(2):222–35.
83. Cooper EC, Jan LY. M-channels: neurological diseases, neuromodulation, and drug development. Arch Neurol 2003;60(4):496–500.
84. Yue C, Yaari Y. KCNQ/M channels control spike afterdepolarization and burst generation in hippocampal neurons. J Neurosci 2004;24(19):4614–24.
85. Pape HC. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. Annu Rev Physiol 1996;58:299–327.
86. Bender RA, Brewster A, Santoro B, et al. Differential and age-dependent expression of hyperpolarization-activated, cyclic nucleotide-gated cation channel isoforms 1–4 suggests evolving roles in the developing rat hippocampus. Neuroscience 2001;106(4):689–98.
87. Bender RA, Galindo R, Mameli M, et al. Synchronized network activity in developing rat hippocampus involves regional hyperpolarization-activated cyclic nucleotide-gated (HCN) channel function. Eur J Neurosci 2005;22(10):2669–74.
88. Iwasaki S, Momiyama A, Uchitel OD, et al. Developmental changes in calcium channel types mediating central synaptic transmission. J Neurosci 2000;20(1):59–65.

89. Noebels JL. The biology of epilepsy genes. Annu Rev Neurosci 2003;26: 599–625.
90. Chen Y, Lu J, Pan H, et al. Association between genetic variation of CACNA1H and childhood absence epilepsy. Ann Neurol 2003;54(2):239–43.
91. Baram TZ, Hatalski CG. Neuropeptide-mediated excitability: a key triggering mechanism for seizure generation in the developing brain. Trends Neurosci 1998;21(11):471–6.
92. Ju WK, Kim KY, Neufeld AH. Increased activity of cyclooxygenase-2 signals early neurodegenerative events in the rat retina following transient ischemia. Exp Eye Res 2003;77(2):137–45.
93. Brunson KL, Eghbal-Ahmadi M, Bender R, et al. Long-term, progressive hippocampal cell loss and dysfunction induced by early-life administration of corticotropin-releasing hormone reproduce the effects of early-life stress. Proc Natl Acad Sci U S A 2001;98(15):8856–61.
94. Brunson KL, Khan N, Eghbal-Ahmadi M, et al. Corticotropin (ACTH) acts directly on amygdala neurons to down-regulate corticotropin-releasing hormone gene expression. Ann Neurol 2001;49(3):304–12.
95. Ivacko JA, Sun R, Silverstein FS. Hypoxic-ischemic brain injury induces an acute microglial reaction in perinatal rats. Pediatr Res 1996;39(1):39–47.
96. Dommergues MA, Plaisant F, Verney C, et al. Early microglial activation following neonatal excitotoxic brain damage in mice: a potential target for neuroprotection. Neuroscience 2003;121(3):619–28.
97. Debillon T, Gras-Leguen C, Leroy S, et al. Patterns of cerebral inflammatory response in a rabbit model of intrauterine infection-mediated brain lesion. Brain Res Dev Brain Res 2003;145(1):39–48.
98. Saliba E, Henrot A. Inflammatory mediators and neonatal brain damage. Biol Neonate 2001;79(3–4):224–7.
99. Billiards SS, Haynes RL, Folkerth RD, et al. Development of microglia in the cerebral white matter of the human fetus and infant. J Comp Neurol 2006; 497(2):199–208.
100. Tikka T, Fiebich BL, Goldsteins G, et al. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. J Neurosci 2001;21(8):2580–8.
101. Shapiro LA, Wang L, Ribak CE. Rapid astrocyte and microglial activation following pilocarpine-induced seizures in rats. Epilepsia 2008;49(Suppl 2):33–41.
102. Vezzani A, Balosso S, Ravizza T. The role of cytokines in the pathophysiology of epilepsy. Brain Behav Immun 2008;22(6):797–803.
103. Dalmau I, Vela JM, Gonzalez B, et al. Dynamics of microglia in the developing rat brain. J Comp Neurol 2003;458(2):144–57.
104. Pfrieger FW, Barres BA. Synaptic efficacy enhanced by glial cells in vitro. Science 1997;277(5332):1684–7.
105. Stevens B, Allen NJ, Vazquez LE, et al. The classical complement cascade mediates CNS synapse elimination. Cell 2007;131(6):1164–78.
106. Heo K, Cho YJ, Cho KJ, et al. Minocycline inhibits caspase-dependent and -independent cell death pathways and is neuroprotective against hippocampal damage after treatment with kainic acid in mice. Neurosci Lett 2006; 398(3):195–200.
107. Lechpammer M, Manning SM, Samonte F, et al. Minocycline treatment following hypoxic/ischaemic injury attenuates white matter injury in a rodent model of periventricular leucomalacia. Neuropathol Appl Neurobiol 2008;34(4): 379–93.

108. Jantzie LL, Cheung PY, Todd KG. Doxycycline reduces cleaved caspase-3 and microglial activation in an animal model of neonatal hypoxia-ischemia. J Cereb Blood Flow Metab 2005;25(3):314–24.
109. Wasterlain CG, Niquet J, Thompson KW, et al. Seizure-induced neuronal death in the immature brain. Prog Brain Res 2002;135:335–53.
110. Stone BS, Zhang J, Mack DW, et al. Delayed neural network degeneration after neonatal hypoxia-ischemia. Ann Neurol 2008;64(5):535–46.
111. Kinney HC, Haynes RL, Folkerth RD, et al. White matter lesions in the perinatal period. In: Golden JA, Harding B, editors. Pathology and genetics: acquired and inherited diseases of the developing nervous system. Basel (Switzerland): ISN Neuropathology Press; 2004.
112. Lein ES, Finney EM, McQuillen PS, et al. Subplate neuron ablation alters neurotrophin expression and ocular dominance column formation. Proc Natl Acad Sci U S A 1999;96(23):13491–5.
113. Kanold PO, Kara P, Reid RC, et al. Role of subplate neurons in functional maturation of visual cortical columns. Science 2003;301(5632):521–5.
114. McQuillen PS, Sheldon RA, Shatz CJ, et al. Selective vulnerability of subplate neurons after early neonatal hypoxia-ischemia. J Neurosci 2003;23(8):3308–15.
115. Chang YS, Mu D, Wendland M, et al. Erythropoietin improves functional and histological outcome in neonatal stroke. Pediatr Res 2005;58(1):106–11.
116. Gonzalez FF, McQuillen P, Mu D, et al. Erythropoietin enhances long-term neuroprotection and neurogenesis in neonatal stroke. Dev Neurosci 2007;29(4–5):321–30.
117. Mikati MA, El Hokayem JA, El Sabban ME. Effects of a single dose of erythropoietin on subsequent seizure susceptibility in rats exposed to acute hypoxia at P10. Epilepsia 2007;48(1):175–81.
118. Ben Ari Y, Holmes GL. Effects of seizures on developmental processes in the immature brain. Lancet Neurol 2006;5(12):1055–63.
119. Sayin U, Sutula TP, Stafstrom CE. Seizures in the developing brain cause adverse long-term effects on spatial learning and anxiety. Epilepsia 2004; 45(12):1539–48.
120. Silverstein FS, Jensen FE. Neonatal seizures. Ann Neurol 2007;62(2):112–20.
121. Maffei A, Turrigiano G. The age of plasticity: developmental regulation of synaptic plasticity in neocortical microcircuits. Prog Brain Res 2008;169:211–23.
122. Rakhade SN, Zhou C, Aujla PK, et al. Early alterations of AMPA receptors mediate synaptic potentiation induced by neonatal seizures. J Neurosci 2008; 28(32):7979–90.
123. Cornejo BJ, Mesches MH, Coultrap S, et al. A single episode of neonatal seizures permanently alters glutamatergic synapses. Ann Neurol 2007;61(5): 411–26.
124. Stafstrom CE, Moshe SL, Swann JW, et al. Models of pediatric epilepsies: strategies and opportunities. Epilepsia 2006;47(8):1407–14.
125. Sanchez RM, Dai W, Levada RE, et al. AMPA/kainate receptor-mediated downregulation of GABAergic synaptic transmission by calcineurin after seizures in the developing rat brain. J Neurosci 2005;25(13):3442–51.
126. Raol YH, Lund IV, Bandyopadhyay S, et al. Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. J Neurosci 2006;26(44): 11342–6.
127. Isaeva E, Isaev D, Khazipov R, et al. Selective impairment of GABAergic synaptic transmission in the flurothyl model of neonatal seizures. Eur J Neurosci 2006;23(6):1559–66.

128. Bergey GK, Swaiman KF, Schrier BK, et al. Adverse effects of phenobarbital on morphological and biochemical development of fetal mouse spinal cord neurons in culture. Ann Neurol 1981;9(6):584–9.
129. Serrano EE, Kunis DM, Ransom BR. Effects of chronic phenobarbital exposure on cultured mouse spinal cord neurons. Ann Neurol 1988;24(3):429–38.
130. Pereira de Vasconcelos A, Colin C, Desor D, et al. Influence of early neonatal phenobarbital exposure on cerebral energy metabolism and behavior. Exp Neurol 1990;108(2):176–87.
131. Schroeder H, Humbert AC, Koziel V, et al. Behavioral and metabolic consequences of neonatal exposure to diazepam in rat pups. Exp Neurol 1995; 131(1):53–63.
132. Bittigau P, Sifringer M, Genz K, et al. Antiepileptic drugs and apoptotic neurodegeneration in the developing brain. Proc Natl Acad Sci U S A 2002;99(23): 15089–94.
133. Glier C, Dzietko M, Bittigau P, et al. Therapeutic doses of topiramate are not toxic to the developing rat brain. Exp Neurol 2004;187(2):403–9.
134. Manthey D, Asimiadou S, Stefovska V, et al. Sulthiame but not levetiracetam exerts neurotoxic effect in the developing rat brain. Exp Neurol 2005;193(2): 497–503.