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Neonatal Seizures: An Update on Mechanisms and Management

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Abstract

The lifespan risk of seizures is highest in the neonatal period. Currently used therapies have limited efficacy. Although the treatment of neonatal seizures has not significantly changed in the last several decades, there has been substantial progress in understanding developmental mechanisms that influence seizure generation and responsiveness to anticonvulsants. Here we provide an overview of current approaches to the diagnosis and treatment of neonatal seizures, identifying some of the recent insights about the pathophysiology of neonatal seizures that may provide the foundation for better treatment.

INTRODUCTION

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 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 US). The relative incidence is higher in premature infants less than 30 weeks 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 CNS disorders can increase the risk of seizures (Table 1). Most neonatal seizures are symptomatic; they can be extremely difficult to control with currently available AEDs (define), 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 1–2/1000 live births. [2] [3]. In fact, about two-thirds of cases of neonatal seizures are due to 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 1–2 days of birth and often remit after a few days, but carry with them a risk of long-term epilepsy and neurological/

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cognitive deficits [6,7]. HIE is associated with a high incidence of seizures, reportedly in 40–60% 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 infectious etiologies 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 due to 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 antiepileptic drug (AED) therapy that is effective at later ages, especially when the seizures are symptomatic and due to HIE. Conventional AEDs that are effective in older children and adults are largely inadequate, likely due to the fact that seizures in the immature brain have unique mechanisms (discussed below).

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

While the term infant is at the highest risk for seizures, (this contradicts statement on page 3) 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, with lower gestational age, male gender, and major systemic and neurological injury such as intraventricular hemorrhage or periventricular leukomalacia being 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 as well as corticospinal, is not fully mature due to 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 one 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 (Figure 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 utilized.(refs) 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 [22]. Several reports now indicate that aEEG has relatively high specificity but compromised sensitivity, detecting approximately 75% of that of conventional full lead montage EEG [23–28] [29].

Once neonatal seizures are confirmed, treatable metabolic and symptomatic causes need to be identified. Serologic studies include blood and serologic studies to 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”, due to hypocalcemia.(please clarify) Pyridoxine-dependent seizures present as refractory early neonatal seizures that uniquely respond to parenteral pyridoxine administration [30,31]. Seizures that continue to be refractory in the setting of a history consistent with HIE manifest within the first 24–48 hours of life, persist over several days, then appear gradually to remit.

MR imaging provides an important assessment of risk in infants with neonatal seizures. Imaging can provide important information in terms of cerebral dysgenesis and gross structural malformations which can be associated with neonatal seizures such as tuberous sclerosis, hemimegalencephaly, or cortical dysplasia. For symptomatic seizures due to HIE, abnormal T2, FLAIR and diffusion signals can be used to pinpoint regional injury and severity [32]. Recent studies show that magnetic resonance spectroscopy can be used to predict severity and outcome in patients. Miller et al reported that in HIE cases with seizures, an increased lactate to choline ratio, as well as reduced N-acetyl-aspartate ratios, were more abnormal in patients with higher seizure burden [17]. Another study of term infants with asphyxia and/or seizures by Glass et al [16] demonstrated that after adjusting for degree of MRI abnormality, seizure severity was associated with a higher risk of neuromotor abnormalities at four 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 etiologies and direct care to correcting the primary cause. However, the most refractory seizures are those due to asphyxia (repetitive) and due to their short course (over 72–96 hrs) 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) [33], 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 (reviewed in Volpe, 2008). However, consensus is that currently used AEDs are often ineffective for treatment of neonatal seizures [34] [35]. Indeed, phenobarbital and phenytoin appear to be equally but incompletely effective, and either drug alone controls seizures in fewer than half of EEG-confirmed neonatal seizures [36]. As an alternative, second-line treatment with midazolam has variable efficacy, yet is less of a respiratory depressant (than what?) [37] [38]. Lidocaine may be effective in refractory neonatal seizures, but its use may be limited by potential cardiac toxicity [39]. Newer AEDs such as topiramate and levetiracetam have been anecdotally reported to improve acute neonatal seizures [40–42]. It is also not known how long to continue treatment following the short course of neonatal seizures [33], and how the length of treatment impacts outcome.

In addition to pharmacological 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 [8,9,43,44]. For whole-body hypothermia, current practice is to decrease core body temperature to 33.5 degrees C for 72 hours [8]. While 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 [44].

Pathophysiology

In response to the fact that neonatal seizures are refractory to conventional AEDs and additionally 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 in order 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 (for detailed review, see [45]). 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 FDA approved for treatment of neonatal seizures [34].

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 evidenced by the extremely low threshold to seizures in general and that this is the period of highest incidence of seizures across the life span [46] [47], and that similarly, in the rodent, seizure susceptibility peaks in the second postnatal week in many models [48] [45,49]. (please clarify) In addition the incomplete development of neurotransmitter systems results in a lack of “target” receptors for conventional AEDs. Finally 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 [50,51].

The neonatal period is a period of intense physiological synaptic excitability, as synaptogenesis occurring at this time point is wholly dependent upon activity. (REFS) In the human, synapse and dendritic spine density are both peaking around term gestation and into the first months of life [52] [53]. 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 [45]. Glutamate is the major excitatory neurotransmitter in the CNS, while γ -amino-butyric 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 [45,48] [54] (Figure 2). Studies of cell morphology, myelination, metabolism and more recently neurotransmitter receptor expression suggest that the first 1–2 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 both animal models and human tissue studies.(ref) A relative overexpression of certain glutamate receptor subtypes in both rodent and human developing cortex coincides with ages of increased seizure susceptibility (Figure 2) [48,55] [56]. Glutamate receptors include both ligand-gated ion channels, permeable to sodium, potassium, and in some cases calcium, and metabotropic subtypes [57]. They are localized both 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 [58]. 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 (Figure 2 and Figure 3) [57] [59]. NMDA receptor antagonists administered to immature rat pups have been shown to be highly effective against a variety of hypoxic/ischemic insults and seizures in the immature brain [60–62]. However, the clinical potential of NMDA antagonists may be limited due to their severe sedative effects and a potential propensity for inducing apoptotic death in the immature brain [63,64]. Importantly, memantine, an agent currently in clinical use as a neuroprotectant in Alzheimer's disease, may be an exception with fewer side effects, owing to its use-dependent mechanism of action [61,62,65].

While the NMDA receptor has been reported to be selectively activated in processes related to plasticity and learning, the AMPA subtype of glutamate receptor is thought to subserve most fast excitatory synaptic transmission. In addition, unlike the NMDA receptor, most AMPA receptors (AMPA) are not calcium permeable in the adult. AMPA receptors are heteromeric and made up of 4 subunits, including combinations of the GluR1, GluR2, GluR3 or GluR4 subunits [57]. However, in the immature rodent and human brain, AMPA receptors are calcium permeable because they lack the GluR2 subunit (Figure 2 and Figure 3) [56] [66]. AMPA receptor 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 [55] [67]. Hence AMPA receptors in the immature brain, owing to 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. Both NMDA and AMPA receptors 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 (Figure 2).

Rodent studies show that AMPA receptor antagonists appear to be potently effective against neonatal seizures, even superior to NMDA receptor antagonists or conventional AEDs and GABA agonists. Topiramate, which is FDA approved for seizure control in children and adults, has been shown to be an AMPAR antagonist, in addition to several other potential anticonvulsant mechanisms [68]. Topiramate has been demonstrated to be effective in

suppressing seizures and long-term neurobehavioral deficits in a rodent seizure model, even when administered following seizures [69,70]. In addition, topiramate in combination with hypothermia was found to be protective in a rodent neonatal stroke model [71]. Finally, the specific AMPAR antagonist talampanel, currently in Phase II trials for epilepsy in children and adults as well as amyotrophic lateral sclerosis, was recently shown to protect against neonatal seizures in a rodent model [72].

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 [48] [73]. 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 (Figure 3) [74]. Notably, when the $\alpha 4$ subunit is expressed the receptor is less sensitive to benzodiazepines compared with receptors containing $\alpha 1$ [75], and as is often the case clinically, seizures in the immature rat respond poorly to benzodiazepines [76] [77].

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 (efflux?) of chloride (Cl⁻) from the neuron, resulting in hyperpolarization [78]. In the immature forebrain, GABA receptor activation can cause depolarization rather than hyperpolarization [79] [80] [81] because the Cl⁻ gradient is reversed in the immature brain: intracellular ? Cl⁻ levels are high in the immature brain due to a relative underexpression of the Cl⁻ exporter KCC2 compared to mature brain (Figure 2 and Figure 3) [82]. 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, while the Cl⁻ importer NKCC1 is overexpressed both in the neonatal human brain and during early life in the rat when seizures are resistant to GABA agonists [82]. The NKCC1 inhibitor, bumetanide, shows efficacy against kainate-induced seizures in the immature brain [83]; this agent, already FDA approved as a diuretic, is currently under evaluation in a Phase I/II 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 above, mutations in the K⁺ channels KCNQ2 and KCNQ3 are associated with benign familial neonatal convulsions [84]. These mutations interfere with the normal hyperpolarizing K⁺ current that prevents repetitive action potential firing [85]. 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 super-family member, the HCN (or h) channels, is also developmentally regulated. The h currents are important for maintenance of resting membrane potential and dendritic excitability [86], 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 [87]. Hence, ion channel maturation can also contribute to the hyperexcitability of the immature brain, and can also 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 [88], suggesting that these developmentally regulated channels may also represent a target for therapy in neonatal seizures. Both N- and P/Q-type voltage sensitive calcium channels regulate neurotransmitter release [89]. With maturation,

this function is taken over exclusively by the P/Q-type channels, formed by Cav2.1 subunits, a member of the Ca²⁺ channel super family [90]. Mutations in Cav2.1 may be involved in absence epilepsy, suggesting a failure in the normal maturational profile [91].

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 corticotropin releasing hormone (CRH), which elicits potent neuronal excitation [92,93]. 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 [94]. CRH levels increase during stress, and thus seizure activity in the immature brain may exacerbate subsequent seizure activity. Notably, adrenocorticotrophic hormone, which has demonstrated efficacy in infantile spasms, also is known to downregulate CRH gene expression [95]. Hence, neuropeptide modulation may be an area of future clinical import 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 due to 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 both hypoxia/ischemia [96,97] and inflammation [98,99]. Importantly, microglia have been shown to be highly expressed in immature white matter in rodents and humans during cortical development [100]. Anti-inflammatory compounds or agents that inhibit microglial activation, such as minocycline, have been reported to attenuate neuronal injury in models of excitotoxicity and hypoxia/ischemia [101]. During the term period, microglia density in deep grey matter is higher than at later ages, likely due to a migration of the population of cells en route to more distal cortical locations. Experimental models demonstrate microglia activation, as seen by morphologic changes and rapid production of pro-inflammatory cytokines, occurring after acute seizures in different epilepsy animal models [102,103]. During brain development, microglia show maximal density simultaneous with the period of peak synaptogenesis [104]. During normal development as well as in response to injury, microglia participate in “synaptic stripping” by detaching presynaptic terminals from neurons [105,106]. Importantly, the microglial inactivators minocycline and doxycycline have been shown to be protective against seizure-induced neuronal death [107] and also protective in neonatal stroke models [108, 109].

Selective neuronal injury in the developing brain

While 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 as well as oxidative stress following chemoconvulsant-induced cell death [110]. Stroke studies in neonatal rodents also suggest that there can be selective vulnerability of specific cell populations in early development [111]. Subplate neurons are present in significant numbers in the deep cortical regions during the preterm and neonatal period [112]. These neurons are critical for the normal maturation of cortical networks [113,114]. Importantly, in both humans and rodents these cells possess high levels of both AMPARs and NMDARs [50,55]. In addition, 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 an hypoxic/ischemic insult [115]. 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 [113].

A number of studies have shown that the application of clinically available antioxidants, such as erythropoietin (Epo), is protective against neuronal injury in neonatal stroke [116,117]. Recently, Epo was shown to decrease later increases in seizure susceptibility of hippocampal neurons following hypoxia-induced neonatal seizures in rats [118].

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 thought to be due to 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 as well as impaired learning later in life in rodents following brief neonatal seizures [119,120]. The neonatal period represents a stage of naturally enhanced synaptic plasticity when learning occurs at a rapid pace [121, 122]. A major factor in this enhanced synaptic plasticity is the predominance of excitation over inhibition, which also increases susceptibility to seizures, as mentioned above. However, seizures that occur during this highly responsive developmental window appear to access signaling events that have been found to be central to normal synaptic plasticity. There are rapid increases in synaptic potency that appear to mimic long-term potentiation, and this pathologic activation may contribute to enhanced epileptogenesis [123]. In addition, GluR-mediated molecular cascades associated with physiological synaptic plasticity may be overactivated by seizures, especially in the developing brain [123,124]. Rodent studies demonstrate 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 [123]. Many models reveal that neonatal seizures alter synaptic plasticity [125], and recent studies are delineating the molecular signaling cascades that are altered following early life seizures [126] [127]. 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 [126]. Flurothyl-induced seizures result in a selective impairment of GABAergic inhibition within a week [128]. Importantly, there is evidence that some of these changes may be downstream of Ca²⁺ permeable glutamate receptors and Ca²⁺ signaling cascades, and that early post-seizure treatment with GluR antagonists or phosphatase inhibitors may interrupt these pathologic changes that underlie the long-term disabilities and epilepsy [123,126].

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. Over two decades ago, experimental data emerged demonstrating 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 [129] [130]. Subsequent studies in neonatal rats demonstrated that daily treatment with phenobarbital or diazepam in the first post-natal month resulted in measurable changes in regional cerebral metabolism and behavior [131] [132].

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 [133]. Similarly, NMDAR antagonists also induce an increase in constitutive apoptosis in the developing rodent brain [63]. Yet, the AMPAR antagonists NBQX and topiramate do not cause such adverse effects [63] [134], 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 [135].

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.

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. As above, 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 2. These include modulators of neurotransmitter receptors and ion channels and transporters, anti-inflammatory 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 non-human primates predict human responses. A number of 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.

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Figure 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 with permission from [136])

Figure 2. Schematic depiction of 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. Prior to 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. Abbreviations: AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole propionate; GABA, γ -aminobutyric acid; NMDA, N-methyl-D-aspartate; P, postnatal day. Reprinted from [45]. Permission obtained from Nature Reviews Neurology **.

Figure 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 AMPA receptors. In contrast, release of GABA (right panel) results in hyperpolarization (inhibition) when the post synaptic neuron expresses sufficient quantities of the Cl^- transporter KCC2, but depolarization (excitation) when intracellular Cl^- accumulates due to 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 to mature synapses. In addition, AMPA receptors are relatively deficient in GluR2 subunits, resulting in relatively increased Ca^{2+} permeability compared to mature synapses. Hence specific NMDA receptor antagonists and AMPA receptor antagonists may prove to be age-specific therapeutic targets for treatment development. In addition, while GABA_A receptor activation normally results in hyperpolarization and inhibition at mature synapses, due to 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 and opening of GABA_A receptors allows the passive efflux of Cl^- out of the cell, resulting in paradoxical depolarization. In addition, GABA_A receptor subunit expression in the immature brain is typified by higher levels of the $\alpha 4$ subunit, which is functionally associated with diminished benzodiazepine sensitivity. Both these attributes of the GABA_A receptor make classical 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.

Table 1

Diverse etiologies 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
Intra-cerebral hemorrhage
Intra-ventricular hemorrhage
Sub-dural hemorrhage
Sub-arachnoid 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

Table 2

Candidate potential targets and therapies from emerging experimental and clinical literature (reprinted with permission from [45])

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),
	AMPA receptors	AMPA 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, etc)	Kinase inhibitors (CaMKII inhibitor KN-62, PKA inhibitor KT5720, PKC inhibitor chelerythrine)
Sub-Acute Changes	Inflammation	Anti-inflammatory 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	Anti-inflammatory agents, (Cox-2 inhibitors, minocycline, doxycycline)