Published in final edited form as:

J Pediatr Neurol. 2009 January 1; 7(1): 5–12. doi:10.3233/JPN-2009-0270.

Developmental factors in the pathogenesis of neonatal seizures

Frances E. Jensen*

Department of Neurology, Children's Hospital, and Program in Neurobiology, Harvard Medical School, Boston, MA, USA

Abstract

Neonatal seizures are inherently different from seizures in the child and the adult. The phenotype, often exhibiting electroclinical dissociation, is unique: neonatal seizures can be refractory to antiepileptic drugs otherwise effective for older patients. Recent experimental and human-based research reveals that the mechanism of neonatal seizures, as well as their long-term sequelae on later brain development, appears to involve a large number of age-specific factors. These observations help explain the resistance of neonatal seizures to conventional therapy as well as identify potential areas of risk for later neurocognitive development. Emerging targets from this research may suggest new therapies for this unique population of patients.

Keywords

Epilepsy; hypoxia/ischemia; developing brain; learning disability; development; neurotransmitters

1. Introduction

The neonatal period (the first 28 days of life) has the highest incidence of seizures across the lifespan at 1.8 to 3.5/1000 live births [1–3]. The most common cause of neonatal seizures is hypoxic/ischemic encephalopathy, which is approximately 1–2/1000 live births [4–6]. Seizures occurring in this population can be very refractory to antiepileptic drugs commonly used in adults, such as phenobarbital and benzodiazepines [7–10]. Neonatal seizures are associated with a high rate of long-term disability and mortality [11]. Available data indicate that phenobarbital remains first line therapy for treatment of neonatal seizures [12]. However, a recent Cochrane Review concluded that “there is little evidence to support the use of any of the anticonvulsants currently used in the neonatal period” [13].

Neonatal seizures are most commonly symptomatic, and in response to an identifiable etiology (see Glass and Wu, this issue). As stated above, hypoxia/ischemia, either primary or secondary to another condition, is one of the most common causes of neonatal seizures. Cerebral hypoxia/ischemia occurs at term in the context of birth asphyxia, respiratory distress, congenital cardiac anomalies, sepsis, multiorgan failure, or as a complication of extracorporeal membrane oxygenation or surgical procedures for congenital cardiac disease [14]. Other causes include acute metabolic syndromes, intraventricular or intracerebral hemorrhage, central nervous system infection, congenital malformations of cortical development, and less commonly genetic epilepsy syndromes. Seizures occurring in this population can be very refractory to antiepileptic drugs commonly used in adults, such as phenobarbital and the benzodiazepines [7–9].

Increasing experimental and clinical data demonstrate that the infant's brain possesses many fundamental differences in function, cellular composition, and connectivity compared to the childhood or adult brain. New data reveal that the immature brain is differentially susceptible to various insults compared to the adult, and that diseases such as epilepsy, trauma and stroke appear to employ different cellular mechanisms than those observed in the adult brain. In addition, seizure activity may interrupt critical cortical developmental processes occurring during the neonatal period that lead to long-term neurocognitive deficits as well as later life epilepsy. Importantly, this new information offers potential age-specific targets for therapeutic intervention and specific pediatric indication. Here we will review the unique pattern of developmentally regulated factors that govern the increased susceptibility of the brain during the neonatal period. An improved understanding of the unique pathophysiological mechanisms operating in the immature brain will lead to the development of age-specific therapeutic strategies.

2. Enhanced excitatory neurotransmission in the immature brain

Both seizures and stroke-induced neuronal injury are caused by excessive excitation of neuronal networks, and a relative increase in extracellular levels of the excitatory neurotransmitter glutamate. Significantly, excitation predominates in neuronal networks in the cerebral cortex and limbic structures at term and early infancy [15]. There is an overshoot of synaptic and spine density in the first 2 weeks of life in the rat and the first year of life in the human and primates [16–18]. Excitatory glutamate receptors (GluRs), ion channels and transporters are expressed at levels that promote excitation while inhibition is relatively underdeveloped compared to later in life. Extracellular glutamate accumulates in epileptic and hypoxic/ischemic tissue [19]. Glutamate is the predominant excitatory amino acid neurotransmitter in neurons and a variety of neuronal and glial cells express GluRs [15,20]. GluRs can be divided into ionotropic and metabotropic subtypes [21]. Ionotropic GluRs (iGluRs) are ligand gated ion channels, permitting influx of sodium potassium, and in some cases calcium (Ca^{2+}) depending upon their subunit make up. Glutamate can induce death in neurons, a process termed “excitotoxicity” because GluR activation results in excessive neuronal depolarization activating a signaling cascade leading to necrotic and/or apoptotic death [22]. Neurons and glia express iGluRs and metabotropic GluRs (mGluRs) [21,23]. The major subtypes of the iGluRs are N-methyl-D-aspartate receptors (NMDARs), α -amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors (AMPA), and KA receptors (KARs). iGluR function is dictated by subunit composition. NMDA receptors consist of NMDAR1 in combination with NMDAR2A, NMDAR2B, NMDAR2C, NMDAR2D and/or NMDAR-L (3A) subunits [21,23,24]. AMPARs are composed of combinations of GluR1, GluR2, GluR3 and GluR4 subunits and KARs are heteromeric combinations of GluR5, GluR6, GluR7, KA1, or KA2 subunits. While NMDARs are always permeable to Ca^{2+} , divalent cation permeability of AMPARs and KARs depends on subunit composition such that when GluR2 expression is relatively low (or absent), AMPARs are permeable to Ca^{2+} , and KARs are weakly permeable to Ca^{2+} at baseline, but become more permeable to Ca^{2+} when the GluR5 or GluR6 subunit mRNA is not edited [21,25–28].

In the immature brain, neuronal NMDARs contain high levels of the NR2B subunit that results in longer current decay times and also high levels of NR2D and NR3A subunits that are associated with low magnesium sensitivity of the NMDAR [24,29]. In both cases, these unique subunit compositions would cause increased NMDAR-mediated Ca^{2+} influx and lower the threshold for seizures and excitotoxic hypoxic/ischemic injury. In addition, this status of enhanced excitability has been implicated in the rapid synaptogenesis that is occurring in this developmental window [30]. Like-wise, the subunits of AMPAR are differentially expressed on immature cortical neurons, with a prevalence of GluR2-deficient receptors, enhancing Ca^{2+} influx [31,32]. In addition, due to an upregulation of a “flip” GluR1 isoform in early life,

AMPA receptors are less desensitizing and therefore respond with longer currents [31]. Studies of GluR expression in developing human brain confirm a similar pattern of GluR2 deficiency, specifically during the term and early postnatal period in neocortical principal neurons [33, 34]. Taken together, these unique receptor patterns are potentially age-specific therapeutic targets to attenuate excitotoxic neuronal injury and seizures in the neonatal brain. The experimental NR2B-specific antagonist ifenprodil has been shown to be effective in adult rodent stroke models [35], yet it has not been tested in models of neonatal seizures. AMPAR antagonists have been shown to be useful in neonatal stroke and seizure models [36]. 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 [37]. 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 [38,39]. In addition, topiramate in combination with hypothermia was found to be protective in a rodent neonatal stroke model [40]. 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 [41].

3. Paucity of classical inhibition and presence of depolarizing GABAergic receptors in the neonatal brain

While GluR-mediated excitation is high in the neonatal period when seizure and stroke susceptibility is enhanced, inhibitory mechanisms are relatively underdeveloped compared with later ages. Receptors for the major inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and its synthetic enzyme, glutamic acid decarboxylase, both steadily increase until the third-fourth postnatal weeks in the rat [42,43]. Furthermore, a number of studies have shown that GABA is an excitatory neurotransmitter during early post-natal life [44,45], in striking contrast to its hyperpolarizing action in later life. GABA_A receptors are permeable to chloride (Cl⁻), and hyperpolarization and inhibition occurs due to Cl⁻ influx intracellularly. GABA-mediated excitation occurs due to a maturational difference in intracellular Cl⁻ concentration, which is maintained by action of opposing Cl⁻ transporters. In immature neurons, intracellular Cl⁻ concentrations are high due to a developmental lack of expression of the Cl⁻ exporter KCC2 [46]. In the rat, KCC2 is absent to minimally expressed in cortical neurons prior to the second postnatal week, while the Cl⁻ importer NKCC2 is actually over expressed in this window compared to later in life [47]. Importantly, similar patterns are present in human brain, where NKCC1 predominates at term and KCC2 is not expressed until the middle of the first year of life [47]. Thus, the excitatory GABA_A receptor also represents a potential therapeutic target. To this end, recent studies have demonstrated that the NKCC1 inhibitor bumetanide can block seizures in a rodent model of neonatal seizures [47,48], and this agent may have potential for clinical use (see Soul, this issue).

4. Developmental status of neuronal ion channels favors depolarization in early life

In addition to ligand gated receptors, ion channels regulating neuronal excitability are also developmentally regulated. In neonatal mouse thalamic neurons, neurotransmitter release is dependent on both N- and P/Q-type channels [49]. 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 [50]. In the human, mutations Cav2.1 may be involved in absence epilepsy, suggesting a failure in the normal maturational profile [51]. Similarly, mutations in the K⁺ channels KCNQ2 and KCNQ3 are associated with benign familial neonatal convulsions [52]. These mutations interfere with the normal hyperpolarizing K⁺ current that prevents repetitive action potential firing [53]. 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 [54], 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 [55]. 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 [56], suggesting that these developmentally regulated channels may also represent a target for therapy in neonatal seizures.

5. Neuropeptides contribute to hyperexcitability of the immature brain

Neuropeptides and their receptors also contribute to age-dependent differences in susceptibility to seizures and excitotoxicity. Corticotropin releasing hormone (CRH) has potent excitatory effects on neuronal networks [57]. CRH is expressed at higher levels perinatally than in later life, and CRH receptors are also developmentally upregulated in the first 2 postnatal weeks in the rat [58]. As CRH is part of a positive feed-back loop activated by stress, seizure activity in the immature brain may be facilitated by the developmental status of CRH and its receptors. Interestingly adreno-corticotrophic hormone, which has demonstrated efficacy in infantile spasms, also is known to downregulate CRH gene expression [59], suggesting that neuropeptide modulation may be an area of future exploration in developing neonatal seizure treatments.

6. Potential for inflammation in epileptogenesis in the newborn brain

Neonatal seizures can occur in the setting of inflammation either due to an intercurrent infection or secondary to hypoxic/ischemic injury. Abundant evidence exists for early microglial activation and inflammatory cytokine production in the developing brain in both hypoxia/ischemia [60–62] and inflammation [63–65]. Importantly, microglia have been shown to be highly expressed in immature white matter in rodents and humans during cortical development [66]. 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 [67,68]. 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. Evidence from experimental models suggests that microglia activation, as seen by morphologic changes and rapid production of pro-inflammatory cytokines, occurs after acute seizures in different epilepsy animal models [69–71]. During brain development, microglia show maximal density in concomitance to most intense synaptogenesis [72]. During normal development and following injury, microglia participate in “synaptic stripping” by detaching presynaptic terminals from neurons [73,74]. Indeed, the microglial inactivators minocycline and doxycycline have been shown to be protective against seizure-induced neuronal death [75] and also protective in neonatal stroke models [76,77].

7. Selective neuronal injury in the developing brain

Subplate neurons are present in significant numbers in the deep cortical regions during the preterm and neonatal period [78]. These neurons are critical for the normal maturation of cortical networks [79,80]. Importantly, in both humans and rodents these cells possess high levels of both AMPARs and NMDARs [33,34]. 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 to overlying cortex following an hypoxic/ischemic insult

[81]. Indeed chemoconvulsant induced seizures in rats, provoked by administration of kainate in early postnatal life have produced a similar loss of subplate neurons, with consequent abnormal development of inhibitory networks [79].

While many studies suggest that seizures, or status epilepticus, induce less death in the immature brain than 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 [82]. Stroke studies in neonatal rodents also suggest that there can be selective vulnerability of specific cell populations in early development [83]. A number of studies have shown that the application of clinically available antioxidants, such as erythropoietin (Epo) is protective in neonatal stroke [84,85]. Recently, Epo was shown to decrease later increases in seizure susceptibility of hippocampal neurons following hypoxia-induced neonatal seizures in rats [86].

8. Seizure-induced alteration in neuronal networks: Relationship to epileptogenesis and later neurocognitive disabilities

Given that there is minimal neuronal death in most models of neonatal seizures, the long-term outcome of neonatal seizures may be due to alterations in network modulation by the seizures. Several studies have demonstrated disordered synaptic plasticity and impaired long-term potentiation as well as learning later in life in rodents following brief neonatal seizures [87, 88]. The neonatal period represents a stage of naturally enhanced synaptic plasticity when learning occurs at a rapid pace [36,89]. 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 [90]. In addition, there is evidence that GluR-mediated molecular cascades normally with normal synaptic plasticity may be over activated by seizures, especially in the developing brain [90, 91]. Other rodent studies show that normal plasticity is reduced in neuronal networks such as hippocampus following early life seizures, raising the concern that the pathologic plasticity may have occluded normal plasticity, contributing to the impaired learning observed after early life seizures [90]. Seizures in early life also appear to affect inhibitory GABA_A receptors, inducing long-term impairments in function. Hypoxia-induced seizures in rat pups results in an immediate functional decrease in inhibitory GABAergic synapses mediated by post-translational changes in GABAA subunits [92], and flurothyl induced seizures result in a selective impairment of GABAergic inhibition within a week [93]. Importantly, there is evidence that some of these changes may be down stream 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 [90,92].

9. Conclusion

Recent cellular and molecular studies in experimental models and human tissue studies have confirmed that there are fundamental differences in neuronal and glial function in the neonatal period compared to later life. The developmental susceptibility of the brain at these stages is likely to be due to changes in the properties of multiple factors. The implication is that a better understanding of these factors will identify new targets for therapeutic intervention that will truly be age-specific in this heretofore difficult-to-treat population. Furthermore, improved understanding of the interaction between brain development and the pathologic processes

induced by seizures in the neonatal period may yield additional therapeutic approaches to reduce the high rates of long-term disability in this vulnerable group of neonates.

Acknowledgments

This work was supported by National Institutes of Health NINDS RO1 NS31718, GMS DP1 OD003347, and by a Mental Retardation Research Center Grant P30 HD18655 from the National Institute of Child Health and Human Development.

References

1. Cowan LD. The epidemiology of the epilepsies in children. *Ment Retard Dev Disabil Res Rev* 2002;8:171–181. [PubMed: 12216061]
2. Cowan F, Rutherford M, Groenendaal F, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet* 2003;361:736–742. [PubMed: 12620738]
3. Hauser WA, Kurland LT. The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. *Epilepsia* 1975;16:1–66. [PubMed: 804401]
4. Ronen GM, Penney S, Andrews W. The epidemiology of clinical neonatal seizures in Newfoundland: a population-based study. *J Pediatr* 1999;134:71–75. [PubMed: 9880452]
5. Lanska MJ, Lanska DJ, Baumann RJ, Kryscio RJ. A population-based study of neonatal seizures in Fayette County, Kentucky. *Neurology* 1995;45:724–732. [PubMed: 7723962]
6. Saliba RM, Annegers JF, Waller DK, Tyson JE, Mizrahi EM. Incidence of neonatal seizures in Harris County, Texas, 1992–1994. *Am J Epidemiol* 1999;150:763–769. [PubMed: 10512430]
7. Sankar R, Painter MJ. Neonatal seizures: after all these years we still love what doesn't work. *Neurology* 2005;64:776–777. [PubMed: 15753407]
8. Painter MJ, Scher MS, Stein AD, et al. Phenobarbital compared with phenytoin for the treatment of neonatal seizures. *N Engl J Med* 1999;341:485–489. [PubMed: 10441604]
9. Painter MJ, Alvin J. Neonatal seizures. *Curr Treat Options Neurol* 2001;3:237–248. [PubMed: 11282039]
10. Tekgul H, Gauvreau K, Soul J, et al. The current etiologic profile and neurodevelopmental outcome of seizures in term newborn infants. *Pediatrics* 2006;117:1270–1280. [PubMed: 16585324]
11. Ronen GM, Buckley D, Penney S, Streiner DL. Long-term prognosis in children with neonatal seizures: a population-based study. *Neurology* 2007;69:1816–1822. [PubMed: 17984448]
12. Barth A, Shen J, Katz KH, et al. Neonatal seizures: multi-center variability in current treatment practices. *Pediatric Neurol* 2007;37:85–90.
13. Booth D, Evans DJ. Anticonvulsants for neonates with seizures. *Cochrane Database Syst Rev*. 2004 CD004218.
14. Volpe, JJ. *Neurology of the Newborn*. 4th ed.. Philadelphia: WB Saunders; 2001.
15. Sanchez RM, Jensen FE. Maturation aspects of epilepsy mechanisms and consequences for the immature brain. *Epilepsia* 2001;42:577–585. [PubMed: 11380563]
16. Huttenlocher PR, deCourten C, Garey LJ, Van der Loos H. Synaptogenesis in human visual cortex – evidence for synapse elimination during normal development. *Neurosci Lett* 1982;33:247–252. [PubMed: 7162689]
17. Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of primate cortex. *Science* 1989;232:232–235. [PubMed: 3952506]
18. Harris KM, Jensen FE, Tsao BS. Comparison of dendritic spines and synapses in area CA1 of the rat hippocampus at postnatal day 15 and adults, with implications for the maturation of long term potentiation. *J Neurosci* 1992;12:2685–2705. [PubMed: 1613552]
19. Silverstein FS, Naik B, Simpson J. Hypoxia-ischemia stimulates hippocampal glutamate efflux in perinatal rat brain: an *in vivo* microdialysis study. *Pediatr Res* 1991;30:587–590. [PubMed: 1687160]
20. Jensen FE. Role of glutamate receptors in periventricular leukomalacia. *J Child Neurol* 2005;20:950–959. [PubMed: 16417841]
21. Hollmann M, Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci* 1994;17:31–108. [PubMed: 8210177]

22. Siesjo BK, Siesjo P. Mechanisms of secondary brain injury. *Eur J Anaesthesiol* 1996;13:247–268. [PubMed: 8737117]
23. Bettler B, Mülle C. Review: neurotransmitter receptors II: AMPA and kainate receptors. *Neuropharmacology* 1995;34:123–139. [PubMed: 7542368]
24. Sucher NJ, Akbarian S, Chi CL, et al. Developmental and regional expression pattern of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. *J Neurosci* 1995;15:6509–6520. [PubMed: 7472413]
25. Alberdi E, Sánchez-Gómez MV, Marino A, Matute C. Ca(2+) influx through AMPA or kainate receptors alone is sufficient to initiate excitotoxicity in cultured oligodendrocytes. *Neurobiol Dis* 2002;9:234–243. [PubMed: 11895374]
26. Sanchez-Gomez MV, Alberdi E, Ibarretxe G, Torre I, Matute C. Caspase-dependent and caspase-independent oligo-dendrocyte death mediated by AMPA and kainate receptors. *J Neurosci* 2003;23:9519–9528. [PubMed: 14573531]
27. Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. *Pharmacol Rev* 1999;51:7–61. [PubMed: 10049997]
28. Ozawa S, Kamiya H, Tsuzuki K. Glutamate receptors in the mammalian central nervous system. *Prog Neurobiol* 1998;54:581–618. [PubMed: 9550192]
29. Murphy KM, Beston BR, Boley PM, Jones DG. Development of human visual cortex: a balance between excitatory and inhibitory plasticity mechanisms. *Dev Psychobiol* 2005;46:209–221. [PubMed: 15772972]
30. Li B, Chen N, Luo T, Otsu Y, Murphy TH, Raymond LA. Differential regulation of synaptic and extra-synaptic NMDA receptors. *Nat Neurosci* 2002;5:833–834. [PubMed: 12195433]
31. 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;21:8154–8163. [PubMed: 11588188]
32. Kumar SS, Bacci A, Kharazia V, Huguenard JR. A developmental switch of AMPA receptor subunits in neocortical pyramidal neurons. *J Neurosci* 2002;22:3005–3015. [PubMed: 11943803]
33. 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;497:42–60. [PubMed: 16680782]
34. 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;497:61–77. [PubMed: 16680761]
35. Chen M, Lu TJ, Chen XJ, et al. Differential roles of NMDA receptor subtypes in ischemic neuronal cell death and ischemic tolerance. *Stroke* 2008;39:3042–3048. [PubMed: 18688011]
36. Silverstein FS, Jensen FE. Neonatal seizures. *Ann Neurol* 2007;62:112–120. [PubMed: 17683087]
37. Shank RP, Gardocki JF, Streeter AJ, Maryanoff BE. An overview of the preclinical aspects of topiramate: pharmacology, pharmacokinetics, and mechanism of action. *Epilepsia* 2000;41:S3–S9. [PubMed: 10768292]
38. Koh S, Tibayan FD, Simpson J, Jensen FE. NBQX or topiramate treatment following perinatal hypoxia-induced seizures prevents later increases in seizure-induced neuronal injury. *Epilepsia* 2004;45:569–575. [PubMed: 15144420]
39. Koh S, Jensen FE. Topiramate blocks perinatal hypoxia-induced seizures in rat pups. *Ann Neurol* 2001;50:366–372. [PubMed: 11558793]
40. Liu Y, Barks JD, Xu G, Silverstein FS. Topiramate extends the therapeutic window for hypothermia-mediated neuroprotection after stroke in neonatal rats. *Stroke* 2004;35:1460–1465. [PubMed: 15105511]
41. Aujla AK, Fetell M, Jensen F. Talampanel suppresses the acute and chronic effects of seizures in a rodent neonatal seizure model. *Epilepsia*. 2009 (in press).
42. Swann JW, Brady RJ, Martin DL. Postnatal development of GABA-mediated synaptic inhibition in rat hippocampus. *Neuroscience* 1989;28:551–561. [PubMed: 2710330]

43. Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Kelly ME, Coulter DA. Gamma-aminobutyric acid (A) receptor subunit expression predicts functional changes in hippocampal dentate granule cells during postnatal development. *J Neurochem* 2001;77:1266–1278. [PubMed: 11389177]
44. Cherubini E, Gaiarsa JL, Ben-Ari Y. GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci* 1991;14:515–519. [PubMed: 1726341]
45. Loturco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* 1995;15:1287–1298. [PubMed: 8845153]
46. Rivera C, Voipio J, Kaila K. Two developmental switches in GABAergic signalling: the K⁺-Cl⁻ cotransporter KCC2 and carbonic anhydrase CAVII. *J Physiol* 2005;562:27–36. [PubMed: 15528236]
47. Dzhalal VI, Talos DM, Sdrulla DA, et al. NKCC1 transporter facilitates seizures in the developing brain. *Nat Med* 2005;11:1205–1213. [PubMed: 16227993]
48. Dzhalal VI, Brumback AC, Staley KJ. Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. *Ann Neurol* 2008;63:222–235. [PubMed: 17918265]
49. Iwasaki S, Momiyama A, Uchitel OD, Takahashi T. Developmental changes in calcium channel types mediating central synaptic transmission. *J Neurosci* 2000;20:59–65. [PubMed: 10627581]
50. Noebels JL. The biology of epilepsy genes. *Annu Rev Neurosci* 2003;26:599–625. [PubMed: 14527270]
51. Chen Y, Lu J, Pan H, et al. Association between genetic variation of CACNA1H and childhood absence epilepsy. *Ann Neurol* 2003;54:239–243. [PubMed: 12891677]
52. Cooper EC, Jan LY. M-channels: neurological diseases, neuromodulation, and drug development. *Arch Neurol* 2003;60:496–500. [PubMed: 12707061]
53. Yue C, Yaari Y. KCNQ/M channels control spike after-depolarization and burst generation in hippocampal neurons. *J Neurosci* 2004;24:4614–4624. [PubMed: 15140933]
54. Pape HC. Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annu Rev Physiol* 1996;58:299–327. [PubMed: 8815797]
55. 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;106:689–698. [PubMed: 11682156]
56. Bender RA, Galindo R, Mameli M, Gonzalez-Vega R, Valenzuela CF, Baram TZ. Synchronized network activity in developing rat hippocampus involves regional hyperpolarization-activated cyclic nucleotide-gated (HCN) channel function. *Eur J Neurosci* 2005;22:2669–2674. [PubMed: 16307610]
57. Baram TZ, Hatalski CG. Neuropeptide-mediated excitability: a key triggering mechanism for seizure generation in the developing brain. *Trends Neurosci* 1998;21:471–476. [PubMed: 9829688]
58. Brunson KL, Eghbal-Ahmadi M, Bender R, Chen Y, Baram TZ. 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 USA* 2001;98:8856–8861. [PubMed: 11447269]
59. Brunson KL, Khan N, Eghbal-Ahmadi M, Baram TZ. Corticotropin (ACTH) acts directly on amygdala neurons to down-regulate corticotropin-releasing hormone gene expression. *Ann Neurol* 2001;49:304–312. [PubMed: 11261504]
60. Ivacko JA, Sun R, Silverstein FS. Hypoxic-ischemic brain injury induces an acute microglial reaction in perinatal rats. *Pediatr Res* 1996;39:39–47. [PubMed: 8825384]
61. Silverstein FS, Barks JD, Hagan P, Liu XH, Ivacko J, Szaflarski J. Cytokines and perinatal brain injury. *Neurochem Int* 1997;30:375–383. [PubMed: 9106251]
62. Dommergues MA, Plaisant F, Verney C, Gressens P. Early microglial activation following neonatal excitotoxic brain damage in mice: a potential target for neuroprotection. *Neuroscience* 2003;121:619–628. [PubMed: 14568022]
63. Debillon T, Gras-Leguen C, Leroy S, Caillon J, Roze JC, Gressens P. Patterns of cerebral inflammatory response in a rabbit model of intrauterine infection-mediated brain lesion. *Brain Res Dev Brain Res* 2003;145:39–48.
64. Lehnardt S, Ahnert-Hilger G, Bigalke H, Jons T. Acid secretion of parietal cells is paralleled by a redistribution of NSF and alpha, beta-SNAPs and inhibited by tetanus toxin. *Histochem Cell Biol* 2000;114:387–391. [PubMed: 11151408]

65. Saliba E, Henrot A. Inflammatory mediators and neonatal brain damage. *Biol Neonate* 2001;79:224–227. [PubMed: 11275656]
66. 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;497:199–208. [PubMed: 16705680]
67. Tikka T, Fiebich BL, Goldsteins G, Keinänen R, Koistinaho J. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J Neurosci* 2001;21:2580–2588. [PubMed: 11306611]
68. 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;77:137–145. [PubMed: 12873443]
69. Shapiro LA, Wang L, Ribak CE. Rapid astrocyte and microglial activation following pilocarpine-induced seizures in rats. *Epilepsia* 2008;49:33–41. [PubMed: 18226170]
70. Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. *Epilepsia* 2005;46:1724–1743. [PubMed: 16302852]
71. Vezzani A, Balosso S, Ravizza T. The role of cytokines in the pathophysiology of epilepsy. *Brain Behav Immun* 2008;22:797–803. [PubMed: 18495419]
72. Dalmau I, Vela JM, Gonzalez B, Finsen B, Castellano B. Dynamics of microglia in the developing rat brain. *J Comp Neurol* 2003;458:144–157. [PubMed: 12596255]
73. Pfriede FW, Barres BA. Synaptic efficacy enhanced by glial cells *in vitro*. *Science* 1997;277:1684–1687. [PubMed: 9287225]
74. Stevens B, Allen NJ, Vazquez LE, et al. The classical complement cascade mediates CNS synapse elimination. *Cell* 2007;131:1164–1178. [PubMed: 18083105]
75. 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;398:195–200. [PubMed: 16469440]
76. 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;34:379–393. [PubMed: 18221261]
77. 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;25:314–324. [PubMed: 15647741]
78. Kinney, HC.; Haynes, RL.; Folkerth, RD. White matter lesions in the perinatal period (invited). In: Golden, JA.; Harding, B., editors. *Pathology and Genetics: Acquired and Inherited Diseases of the Developing Nervous System*. Basel: ISN Neuropathology Press; 2004. p. 156–170.
79. Lein ES, Finney EM, McQuillen PS, Shatz CJ. Subplate neuron ablation alters neurotrophin expression and ocular dominance column formation. *Proc Natl Acad Sci USA* 1999;96:13491–13495. [PubMed: 10557348]
80. Kanold PO, Kara P, Reid RC, Shatz CJ. Role of subplate neurons in functional maturation of visual cortical columns. *Science* 2003;301:521–525. [PubMed: 12881571]
81. McQuillen PS, Sheldon RA, Shatz CJ, Ferriero DM. Selective vulnerability of subplate neurons after early neonatal hypoxia-ischemia. *J Neurosci* 2003;23:3308–3315. [PubMed: 12716938]
82. Wasterlain CG, Niquet J, Thompson KW, et al. Seizure-induced neuronal death in the immature brain. *Prog Brain Res* 2002;135:335–353. [PubMed: 12143353]
83. Stone BS, Zhang J, Mack DW, Mori S, Martin LJ, Northington FJ. Delayed neural network degeneration after neonatal hypoxia-ischemia. *Ann Neurol* 2008;64:535–546. [PubMed: 19067347]
84. Chang YS, Mu D, Wendland M, et al. Erythropoietin improves functional and histological outcome in neonatal stroke. *Pediatr Res* 2005;58:106–111. [PubMed: 15879287]
85. Gonzalez FF, McQuillen P, Mu D, et al. Erythropoietin enhances long-term neuroprotection and neurogenesis in neonatal stroke. *Dev Neurosci* 2007;29:321–330. [PubMed: 17762200]
86. 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;48:175–181. [PubMed: 17241225]

87. Ben Ari Y, Holmes GL. Effects of seizures on developmental processes in the immature brain. *Lancet Neurol* 2006;5:1055–1063. [PubMed: 17110286]
88. Sayin U, Sutula TP, Stafstrom CE. Seizures in the developing brain cause adverse long-term effects on spatial learning and anxiety. *Epilepsia* 2004;45:1539–1548. [PubMed: 15571512]
89. Maffei A, Turrigiano G. The age of plasticity: developmental regulation of synaptic plasticity in neocortical micro-circuits. *Prog Brain Res* 2008;169:211–223. [PubMed: 18394476]
90. Rakhade SN, Zhou C, Aujla PK, Fishman R, Sucher NJ, Jensen FE. Early alterations of AMPA receptors mediate synaptic potentiation induced by neonatal seizures. *J Neurosci* 2008;28:7979–7990. [PubMed: 18685023]
91. Cornejo BJ, Mesches MH, Coultrap S, Browning MD, Benke TA. A single episode of neonatal seizures permanently alters glutamatergic synapses. *Ann Neurol* 2007;61:411–426. [PubMed: 17323345]
92. Sanchez RM, Dai W, Levada RE, Lippman JJ, Jensen FE. AMPA/kainate receptor-mediated downregulation of GABAergic synaptic transmission by calcineurin after seizures in the developing rat brain. *J Neurosci* 2005;25:3442–3451. [PubMed: 15800199]
93. Isaeva E, Isaev D, Khazipov R, Holmes GL. Selective impairment of GABAergic synaptic transmission in the flurothyl model of neonatal seizures. *Eur J Neurosci* 2006;23:1559–1566. [PubMed: 16553619]