

● PERSPECTIVE

Neonatal seizures and disruption to neurotransmitter systems

Seizure disorders and epilepsies are well documented to be associated with long-term neurological and cognitive deficits in the adult and pediatric patients, but what about seizures in the newborn? The neonatal brain is highly susceptible to seizures, with hypoxic-ischemic encephalopathy (HIE) the most common aetiology of seizures in the first 24 hours of life. Numerous neonatal rodent models have shown deficits in cognitive and behavioural tests after recurrent neonatal seizures, suggestive of persistent alterations at the cellular level. Debate exists however as to whether neonatal seizures are ‘responsible’ for neurological deficits, or whether seizures are simply a symptom of underlying neurological injury.

In developing brains, Wasterlain (1997) reviewed different models of status epilepticus (SE) concluding that chemically or electrically-induced SE on an otherwise *normal* developing brain produced significant damage (Wasterlain, 1997). Age-dependent sensitivity to pilocarpine-SE-induced apoptotic injury was reported in various regions of the developing rat hippocampus, with greater CA1 injury in younger rats. Discordant results were observed by Wirrell et al. (2001) who reported no necrotic injury in P10 rats with kainic acid (KA)-induced seizures. However, rat pups of the same age exposed to hypoxia-ischaemia (HI) with subsequent KA-induced seizures had significantly greater injury than pups exposed to HI alone. We have also documented increased neuropathology in animals with HI-induced seizures in our piglet model of neonatal HI. In human studies, neonatal seizures are often associated with worse outcomes independent of the severity of underlying pathology such as that from HI.

In neonatal animal models, seizures often develop into prolonged generalised seizures, whether chemically or electrically induced, or as a result of HI. This could be attributed to the precocious development of excitatory neurotransmission, with relatively lower activity of inhibitory systems. Excitation is essential to neural circuit development. In the early postnatal period, abundant excitatory activity is in part due to the transient over-expression of excitatory glutamatergic NMDA receptors (NMDA-R). Higher expression of the NR2B and NR2A

subunits, with longer decay times and decreased sensitivity to magnesium ions respectively, prolong excitation in the immature brain (Nardou et al., 2013). The NR1 subunit, required for all functional NMDA-Rs, has been shown to have increased expression within 1 hour of pilocarpine-SE, with increased numbers of NMDA-R at the synaptic surface, but also enhanced excitation in SE hippocampal cells (Naylor et al., 2005).

However, an additional *source* of excitation is generated from the GABA neurotransmission system, of which the GABA_A receptors (GABA_AR) are known to provide much of the excitatory drive during brain development. In the adult brain glutamate-mediated excitation is tempered by the inhibitory GABA system, largely mediated by the ligand-gated GABA_AR. However, during mammalian brain development, glutamatergic and GABAergic neurotransmission work synergistically to provide excitatory drive critical for synaptogenesis, neuronal differentiation and migration. During development, GABA-induced depolarizations along with NMDA-R depolarizing currents, facilitate synchronous neuronal firing thereby increasing susceptibility of the immature brain to seizures (Nardou et al., 2013). Depolarizing GABA_AR currents are due to the developmental differences in intracellular chloride. The immature neurone has a higher intracellular chloride (Cl⁻) concentration, such that when GABA binds to a GABA_AR there is an efflux of Cl⁻ and membrane depolarisation-*excitation* (**Figure 1**). In the mature neurone there is a shift to low intracellular Cl⁻ concentration and thus upon ligand-binding and opening of the GABA_AR channel there is an influx of extracellular Cl⁻. This influx of Cl⁻ results in membrane hyperpolarization-*inhibition*. The electrochemical gradient across the membrane is controlled by the developmentally-regulated expression of the cation-chloride cotransporters (CCC), the sodium-potassium-chloride cotransporter (NKCC1) that imports Cl⁻ and the potassium-chloride cotransporter (KCC2) that exports Cl⁻. KCC2 exists in two functional isoforms KCC2a and KCC2b, with KCC2b expression low during gestation but increasing across postnatal development. In KCC2b knockout models, mice develop generalized seizures when born and usually survive only to two weeks postnatal age. Significant reductions in parvalbumin-positive interneurons, have also been reported suggesting that GABAergic neurons play a key role in hyperexcitability and ongoing seizures (Woo et al., 2002).

In contrast, NKCC1 expression is high earlier in brain development, and has been associated with the higher susceptibility of the newborn brain to seizures. Dai et al. (2005) reported

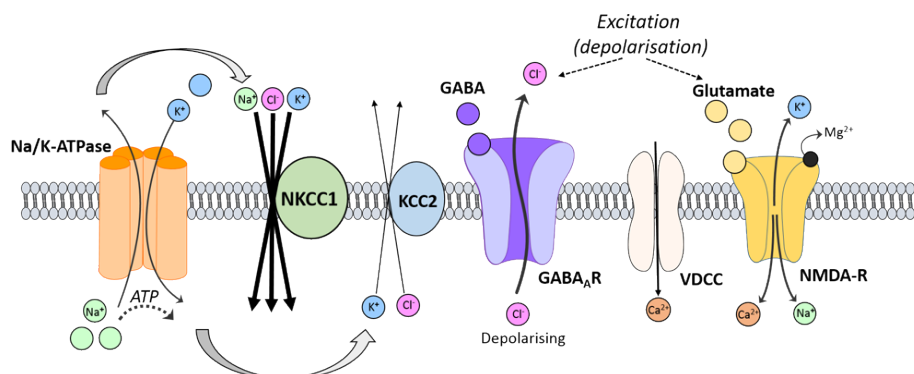


Figure 1 With higher intracellular Cl⁻, activation of the GABA_A receptor results in a net efflux of Cl⁻, leading to depolarization.

Transport of Cl⁻, Na⁺ and K⁺ ions is driven by chemical gradients generated by Na/K ATPase, which has effects on secondary active transporters of Cl⁻ NKCC1 and KCC2. After neonatal seizure, there is a loss of ATP, functional upregulation of NKCC1 and downregulation of KCC2 leading to enhanced excitation in the immature neurone through both NMDA-R and GABA_AR. Additional alterations to GABA_AR and NMDA-R expression following neonatal seizure will have significant functional and developmental consequences in the newborn brain. ATP: Adenosine triphosphate; Cl⁻: chloride; GABA_AR: type A gamma-aminobutyric acid receptor; KCC2: potassium-chloride cotransporter; NKCC1: sodium-potassium-chloride cotransporter; NMDA-R: N-methyl-D-aspartate receptor; VDCC: voltage-dependent calcium channel.



upregulation of NKCC1 mRNA expression in the neonatal P7 rat at 48 hours post-HI, although NKCC1 expression had returned to levels similar to that of controls by 72 hours post-HI (Dai et al., 2005). Upregulation of NKCC1 has also been observed after pilocarpine-induced SE in adult mice (Li et al., 2008) supporting the theory that altered GABAergic neurotransmission is involved in increased seizure susceptibility. In neonatal seizure models (rat and *in vitro*) blocking NKCC1 activity has been shown to result in a hyperpolarizing shift in the equilibrium potential of GABA (E_{GABA}), thereby enhancing inhibition (Dzhala et al., 2005). This in turn reduces seizure susceptibility, providing evidence that enhancing GABAergic inhibition is crucial to adequate seizure control. Indeed, Dzhala et al. (2005) reported an age-dependent response in the anticonvulsant efficacy of the NKCC1 blocker bumetanide in a neonatal rodent seizure model. In the immature hippocampus (P7–9) bumetanide suppressed seizure-like discharges while phenobarbital (a GABA agonist) had no effect. At older ages (P15–21) however bumetanide afforded no anticonvulsant effect whereas phenobarbital reduced ictal activity (Dzhala et al., 2005). The promise of bumetanide as a neonatal anticonvulsant was realized in 2011 with the start of a clinical trial using bumetanide in conjunction with phenobarbital to treat HI-induced neonatal seizures (NEMO, trial number NCT01434225). Sadly however the primary outcome of > 80% seizure reduction was not achieved and 3/11 neonates completing the study suffered hearing loss. Although not specifically linked to the use of bumetanide, the trial was prematurely ceased. The utilization of therapies such as bumetanide, to shift GABAergic signalling towards inhibition, has the potential to deliver vastly more efficacious seizure treatments. Failures of such promising clinical trials highlight the value of appropriate and extensive preclinical investigations, particularly in larger animal models.

Transition from excitatory GABAergic neurotransmission to inhibitory is critical to normal cortical development. While there is substantial evidence of CCC involvement in seizure activity and susceptibility, alterations to the GABA_AR itself and that of its functional state will have long-term consequences on normal brain development. The GABA_AR has been shown to be highly sensitive to short- and long-term alterations in seizure-models of epilepsy. Naylor et al. (2005) showed internalisation of the $\beta_{2/3}$ and γ_2 subunits of the GABA_AR after 1 hour in an SE rodent model suggesting alterations to GABAergic neurotransmission (Naylor et al., 2005). We have recently published evidence of reduced expression of GABA_A α_1 and α_3 subunit expression after HI-induced neonatal seizures in our preclinical piglet model of birth asphyxia (Miller et al., 2016).

It is a necessity to treat neonatal seizures, but current treatments are ineffective and the use of AEDs poses significant unwanted effects on the developing brain. Painter et al. (1999) have reported less than 50% of neonatal seizures are effectively controlled with current AEDs phenobarbital and phenytoin. Furthermore, use of AEDs has been reported to increase apoptotic cell death, and may influence the progression of normal brain development through alterations in neurogenesis, synapse formation and cell proliferation and migration (Ikonomidou and Turski, 2010). Effective treatment strategies need to use a multi-targeted approach to treat neonatal seizure activity, balanced with consideration of the normal brain maturation processes.

In conclusion, Naylor and colleagues have conclusively shown that in rodent models of SE, there is a significant upregulation of synaptic NMDA-R expression, while there is significant internalization of the GABA_AR within 1 hour of seizure onset. In combination these two findings suggest an imbalance in the expression of excitatory NMDA-R and inhibitory GABA_AR systems that contribute to seizure activity. In the developing brain

however, there is much less evidence of the mechanisms that drive neonatal seizures. Along with various published reports showing alterations to the CCCs expression after seizures (Dzhala et al., 2005; Li et al., 2008), there is strong evidence to suggest that neonatal seizures result in dysfunctional alterations to GABA neurotransmission. Disruption to excitatory and inhibitory neurotransmission in the neonatal brain has much broader implications than the lack of efficacy of anticonvulsants. Not only do these changes alter seizure susceptibility, disruption to neurotransmitter systems in the developing brain can cause significant disturbance to normal synaptogenesis, neurogenesis and neuronal differentiation which can profoundly impact long-term neurodevelopmental outcomes. Together these findings highlight the need for correct management of neonatal seizures.

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Accepted: 2017-02-06

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doi: 10.4103/1673-5374.200803

How to cite this article: Miller SM, Goasdoue K, Björkman ST (2017) Neonatal seizures and disruption to neurotransmitter systems. *Neural Regen Res* 12(2):216-217.

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