DEVELOPMENT OF THE NEONATAL CEREBRAL CORTEX

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The neonatal period is a unique time of brain development. The cortical neuron undergoes major changes in the first few weeks after birth. It is a period of rapid growth, formation of new connections and progressive maturation of electrical and synaptic activity that translates into the formation of functional modules and networks in the brain. It is also a period when genetically programmed activity patterns are influenced by early environmental influences that result in the development of cortical maps. The neonatal brain is known to be vulnerable to injury and seizures, which are likely to disrupt normal development. There is a greater potential for neuroplasticity and modification of the developing networks in response to injury. The interaction of drugs with developing neurotransmitter and neuromodulatory systems may also influence normal maturation of the cortex. Therefore, a better understanding of the maturation of neural activity and the development of cortical connections and networks in the neonatal cortex is an important prerequisite for our ability to evaluate the cause and effect of neonatal seizures and their treatment.

Embryonic development

The embryonic development of the human cerebral cortex involves a complex sequence of events that starts at the rostral end of the neural tube around embryonic day 30, at the outer surface of the embryonic cerebral vesicle (Rakic 2006, Fox et al. 2010). These events include the proliferation of neuroepithelial cells in the regions near the cerebral ventricle (ventricular zone) and the formation of early developmental layers (subventricular zone, intermediate zone, subplate, cortical plate and marginal zone) through proliferation and migration (Bystron et al. 2008, Tiberi et al. 2012). Radial migration of neurons from the dorsal pallium accumulates in the cortical plate in an inside-out sequence to create a laminar structure and generate the projection neurons (pyramidal neurons) of the neocortex (Rakic 2006). Most GABAergic interneurons originate in and migrate tangentially from a different region of the forebrain, the subpallium or basal telencephalon. Migration is nearly complete by the beginning of the third trimester, and is followed by areal, laminar and cytological differentiation. Neurogenesis in the cerebral neocortex is seen till about gestational age of 28 weeks, when all cortical neurons have arrived in the cortical plate and are densely crowded together (Huttenlocher 1990). Spontaneous electrical activity begins in the late
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embryonic period (Luhman et al. 2003, Picken Bahrey and Moody 2003). Sensory signals (resulting from spontaneous fetal movements) start to influence cortical activity at this time (Milh et al. 2007).

Morphological changes in cortical neurons in the neonatal and infant brain

There is rapid expansion of cerebral cortical volume during the first four weeks after birth, which then continues at a slower pace for the next few years (Huttenlocher 1990, Levitt 2003). This increase in volume is because of growth of axons, dendrites and glia (Figs. 1.1 and 1.2), as well as myelination of axons (Huttenlocher and Dhabolkar 1997, Levitt 2003,

![Image](image_url)

**Figure 1.1.** Morphology of layer V pyramidal neurons in the prefrontal cortex of the rat at various postnatal ages. Neurons were labelled with neurobiotin and reconstructed using the Neurolucida system. All neurons shown here were regular spiking cells. (Reproduced from *J Neurophysiol* Zhong-wei Z. Maturation of Layer V Pyramidal Neurons in the Rat Prefrontal Cortex: Intrinsic Properties and Synaptic Function 91: (3). © 2004, with kind permission of the American Physiological Society.)
Elongation of dendrites is associated with increased number and density of dendritic spines (Becker et al. 1984, Michel and Garey 1984). There is earlier growth and more elaborate branching of the dendritic tree in the deeper cortical layers. Growth of dendrites and dendritic spines occurs earlier and completes sooner in the occipital and auditory than the frontal cortex (Becker et al. 1984, Huttenlocher and Dhabolkar 1997). There is no evidence for overall regression of dendritic development; there is no reduction of the density of dendrites or dendritic spines (Becker et al. 1984). However, there is likely selective

Figure 1.2. Camera-lucida reconstruction of a pyramidal neuron located in the motor cortex of the adult monkey. (a) Soma, dendrites and axon and (b) soma, axon and axon collaterals. The antidromic response to pyramidal tract (Pyr) stimulation is shown alongside. (Reproduced from J Physiol Ghosh S, Porter R. Morphology of Pyramidal Neurones in Monkey Motor Cortex and the Synaptic Actions of Their Intracortical Axon Collaterals. 400: 593–615. © 1988, with kind permission of John Wiley and Sons.)
pruning and remodelling of dendrites, spines and synapses. Synaptic density (calculated as average number of synapses per neuron or per unit length of dendrite) increases greatly during late fetal life and early infancy to reach a maximum in the first year; it then declines to reach adult values by about 10–15 years. Synaptic density increases and declines later in the frontal than in the occipital and auditory cortices (Huttenlocher 1979, Huttenlocher et al. 1982, 1987, Huttenlocher and Dhabolkar 1997, Fox et al. 2010). Based on calculations of neuronal, dendritic and synaptic density and estimates of cortical volume, it appears likely that there is a loss of synapses after the first year (Huttenlocher 1990). Comparative studies in animals suggest that elimination of synapses is a more prominent developmental event as the complexity of the brain increases (Aghajanian and Bloom 1967, Changeux and Danchin 1976, Rakic et al. 1989). The large increase in the formation of synapses seen in the neonatal and infant brain and their later elimination during childhood is associated with greater plasticity in the brain. However, the activity in the exuberant synapses and their associated metabolic demands likely make the developing brain more vulnerable to metabolic and hypoxic insults, as well as to seizures (Holmes 2009).

**Synaptic plasticity in the developing cortex**

Development of the cortex is dependant on the synaptic plasticity driven by patterned physiological activity resulting from early experience (Fox et al. 2010). For example, refinement and maintenance of detailed sensory maps are dependant on early and continuing sensory experience (Khazipov et al. 2004, Milh et al. 2007, Fox et al. 2010). Nearly all activity-driven developmental events that involve gene expression are triggered initially by Ca++ influx into cells, and the effects are determined by the amplitude, frequency and spatial location of this influx (Greer and Greenberg 2008). This in turn is influenced by the excitability of neurons and the action of neurotransmitters and neuromodulators.

**Excitability of cortical neurons**

Experimental studies in animals suggest that many factors are developmentally regulated during the neonatal period to increase the excitability of neurons and neural networks. These include voltage-gated ion channels, neurotransmitter receptors and neuromodulators. This excitatory drive regulates spontaneous activity of neurons, dendritic growth and the formation and refinement of synaptic connections. These activity-dependant changes also result in transition to the mature physiological state.

Spontaneous electrical activity is required for the maturation of neuronal excitability and synaptic connectivity. For this reason configuration of ion channels and receptors in the neonatal cortex are optimized to mediate spontaneous activity, synchronize it among cells and allow Ca++ influx that transduces activity into development programs (Moody and Bosma 2005). During this time there is an increase in density of Na+ currents, increase in Ca++ activated K+ channels and the appearance of hyperpolarization-dependant cation channels (Picken Bahry and Moody 2003). There is an increase in the resting membrane potential and a reduction in input resistance and membrane time constant (Zhang 2004). As a result of these changes action potentials become larger in amplitude and shorter in duration (Fig. 1.3), and neurons gain the ability to fire repetitive action potentials.
Figure 1.3. Developmental changes in action potential of pyramidal neurons in the rat prefrontal cortex. (A) Examples of action potential recorded at P3, P9, P13 and P21. Thin line indicates 0mV level, and spike threshold is shown by dashed lines; filtered at 2kHz and digitized at 8kHz. Please note increase in spike amplitude and reduction in spike duration during development; (B1) scatter plot of spike threshold at various ages; (B2) mean ± SE from cells recorded at a given age. Data can be fit to a single exponential function with a time constant of 6.2 d ($R^2 \pm 0.98$); (C1) difference between spike threshold ($V_{\text{thres}}$) and resting potential ($V_r$), measured at various ages; (C2) mean ± SE. A linear fit gives a slope of ±0.006 mV/d, suggesting that the difference between $V_{\text{thres}}$ and $V_r$ remains stable during the first 5 weeks after birth. (Reproduced from J Neurophysiol Zhong-wei Z. Maturation of Layer V Pyramidal Neurons in the Rat Prefrontal Cortex: Intrinsic Properties and Synaptic Function 91: (3). © 2004, with kind permission of the American Physiological Society.)
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Neurotransmitters in the neonatal cortex

Gap junctions between dendrites appear early and transiently in the neonatal cortex, and increase synchronized activity between adjacent neurons to create local ensembles (Peinado et al. 1993, Dupont et al. 2006). This is followed by progressive increase in synaptic activity involving N-methyl D-aspartate (NMDA) and other glutaminergic receptors (Dupont et al. 2006).

Glutamate is the major excitatory neurotransmitter in the brain, and there are many types of glutamate receptors. Depending on their mechanisms of action, they are grouped into ionotropic and metabotropic receptors; the former include the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kynurenic acid and NMDA receptors (Blanke and Van Dongen 2009). The metabotropic glutamate receptors are members of the G-protein receptor coupled superfamily (Niswender and Conn 2010). Because of their slow kinetics, voltage dependence and high permeability to Ca++ ions, NMDA receptors play a dominant role in activity-dependant synaptic plasticity and are critical for the development of the brain and the processes underlying learning and neuroplasticity. Studies in rats show that the density of NMDA receptors in the neocortex peaks in the first postnatal week (Haberny et al. 2002). The molecular subunit composition of the NMDA and other glutamate receptors also contributes to increased excitability in the neonatal period (Johnston 2005, Rakhade and Jensen 2009). AMPA receptors in the neonate are more permeable to Ca++ and may contribute to activity-dependant Ca++ influx into neurons (Rakhade and Jensen 2009). Enhanced excitability and activity of cortical neurons is important for development; experimental studies in rats show that blocking NMDA receptors, even transiently, in the early postnatal period is associated with apoptotic neurodegeneration and abnormal axonal arborization (Fox et al. 1996, Haberny et al. 2002).

GABA (gamma-Aminobutyric acid) is the predominant inhibitory neurotransmitter in the cortex. However, unlike in the adult brain, activation of GABA-A receptors during early postnatal development produces membrane depolarization (which may reach spike threshold), activation of voltage-gated Ca++ channels and removal of the voltage-dependant Mg++ block of NMDA channels, leading to Ca++ influx (Gaiarsa et al. 1995, Ben Ari et al. 2012). The developmental expression of cation-chloride importer NKCC1 and exporter KCC2 determines the depolarizing action of GABA in immature neurons; increased functional expression of NKCC1 and reduced expression of KCC2 result in a higher intracellular concentration of chloride and GABA-A receptor mediated depolarization. The excitatory action of GABA and its subsequent shift to inhibitory action are important for the maturation of neuronal morphology and cortical networks (Cancedda et al. 2007). In contrast, GABA-B mediated presynaptic inhibition is well developed at birth (Gaiarsa et al. 1995). Animal studies suggest that the density and molecular composition of GABA-A and GABA-B receptors vary during the early postnatal period, resulting in changes in receptor kinetics and function as well as their response to drugs such as benzodiazepines (Laurie et al. 1992, Gaiarsa et al. 1995).
Neurotrophic factors and peptides play an important role in neural activity and survival (Moody and Bosma 2005). These include brain-derived neurotrophic factor, parathyroid hormone-related peptide and pituitary adenylate cyclase activating polypeptide. Increased excitability of cortical neurons in the neonatal period may make them more vulnerable to stress and the influence of stress-related excitatory neuropeptides (e.g. corticotrophin-releasing hormone; Baram and Hatalski 1998).

Neonatal cortical development and seizures
It is most likely that the increased susceptibility of the neonate to seizures is caused by the intrinsic properties of cortical neurons and networks that are associated with increased excitability and their propensity to generate spontaneous and synchronized activity. The majority of neonatal seizures arise in the neocortex (Mizrahi 1999). Depending on their severity, neonatal seizures may disrupt normal activity-driven developmental processes and result in varying degrees of long-term neurological impairment (Glass et al. 2009, Nagarajan et al. 2010). Neonatal seizures are often symptomatic and associated with developmental or perinatal injury to the brain. However, seizures worsen outcomes, independent of the underlying injury (Glass et al. 2009, Payne et al. 2014).

Because of differences in neural and network properties, it is likely that consequences of seizures will be different in the neonatal period. Experimental studies in animals have found that prolonged seizures cause less neuronal loss and synaptic rearrangements in neonates than in adults (Sperber et al. 1991, Cataltepe et al. 1995, Haas et al. 2001). However, there is abnormal neurogenesis and axonal sprouting in neonatal animals subjected to seizures, and resultant impairment in memory and learning (Holmes and Ben-Ari, 1998, McCabe et al. 2001, Sayin et al. 2004). Recurrent neonatal seizures result in long-term increases in neocortical excitability and likelihood of seizures (Isaeva et al. 2010). Other studies have shown that hypoxic injury to the neonatal brain lowering seizure threshold and makes it more susceptible to recurrent seizures (Jensen 1999).

Neonatal seizures are hard to diagnose, may have variable or no motor features and are often refractory to pharmacotherapy (Misrahi 1999, Scher et al. 2003, Nagarajan et al. 2011, 2012). There is limited evidence regarding the best pharmacological treatment of neonatal seizures (Slaughter et al. 2013, Glass 2014, Thoresen and Sabir 2015). Since GABA is an excitatory neurotransmitter in the neonatal brain, effects of GABA-A receptor modulators are likely to be different than in adults (Dzhala et al. 2005, Rakhade and Jensen 2009, Glass 2014). In addition, GABA plays an important role in the development of patterns of activity, synaptogenesis and formation of functional circuits (Ben Ari 2006). Animal studies have shown that commonly used antiepileptic drugs (AEDs) that block voltage-gated sodium channels, enhance GABAergic inhibition or block glutamate mediated excitation cause apoptosis of neurons in the developing forebrain (Bittigau et al. 2002). NMDAr antagonists can impair motor and cognitive functions (Mares and Mikulecka 2009). There is less data from the clinic about the adverse effects of AEDs on brain development (Silverstein and Ferriero 2008). Phenobarbitone and other AEDs have been shown to depress cognitive performance in children (Farwell et al. 1990, Loring and Meador 2004, Loring et al. 2007). Thus, seizures as well as their treatment can impair normal brain development.
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in the neonate, and there is a need for basic and clinical studies to assess the effects of seizures and pharmacotherapy on developmental outcomes.

Neonatal seizures and the associated aetiopathology (e.g. hypoxic-ischaemic encephalopathy) increase the risk of subsequent epilepsy (Rakhade and Jensen 2009, Nagarajan et al. 2010, Chapman et al. 2012). A better understanding of underlying mechanisms should aid in the development of AEDs.

The molecular, cellular and network changes underlying epileptogenesis are not completely understood and less so in the developing brain. Most animal studies of epileptogenesis, in both neonatal and adult brains, have focused on the hippocampus because of the region’s well-characterized network architecture and cellular interactions. Such studies suggest that seizures (and associated Ca++ influx) activate and upregulate immediate early genes, alter ion channel and neurotransmitter function and activate inflammatory cascades. In neonatal animal models of epilepsy, observed changes include internalization of Kv2.1 channels, decreased expression of KCC2 cotransporters, GABA-A receptor endocytosis, modification of AMPA receptors, increase in neurotrophic factor expression, activation of inflammatory mediators and microglia and aberrant sprouting and network connectivity (Sanchez et al. 2005, Epzstein et al. 2008, Rakhade and Jensen 2009). These changes cause impaired inhibition, increased permeability of excitatory channels and increased network excitability and are thought to contribute to epileptogenesis. Based on these studies, inhibitors of cation-chloride cotransporters (e.g. bumetanide), AMPA receptor antagonists, antioxidants such as erythropoetin, and microglial inactivators have been explored as treatment to prevent seizures and epileptogenesis in such models (Koh et al. 2004, Rakhade and Jensen 2009, Chapman et al. 2012, Pressler and Mangum 2013).

Conclusion

The neonatal period is a unique time of brain development where there is rapid growth and maturation of cortical neurons, and formation of exuberant synapses and connections. It is a period when genetic programmes for development are influenced by early experiences. As a result, the brain reacts differently to injury and the consequences of injury include the interruption or modification of the proper sequence of development. Research studies in animals continue to provide important insights into cortical development and the dynamic changes in cellular and molecular processes that occur in the neonatal period. Animal and clinical studies have revealed significant immediate and long-term effects of injury to the brain during this vulnerable period. However, minimizing injury and its consequences continues to remain a challenge, and is an important focus for future research.

REFERENCES


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