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Using sex differences in the developing brain to identify nodes of influence for seizure susceptibility and epileptogenesis

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Abstract

Sexual differentiation of the developing brain organizes the neural architecture differently between males and females, and the main influence on this process is exposure to gonadal steroids during sensitive periods of prenatal and early postnatal development. Many molecular and cellular processes are influenced by steroid hormones in the developing brain, including gene expression, cell birth and death, neurite outgrowth and synaptogenesis, and synaptic activity. Perturbations in these processes can alter neuronal excitability and circuit activity, leading to increased seizure susceptibility and the promotion of pathological processes that constitute epileptogenesis. In this review, we will provide a general overview of sex differences in the early developing brain that may be relevant for altered seizure susceptibility in early life, focusing on limbic areas of the brain. Sex differences that have the potential to alter the progress of epileptogenesis are evident at molecular and cellular levels in the developing brain, and include differences in neuronal excitability, response to environmental insult, and epigenetic control of gene expression. Knowing how these processes differ between the sexes can help us understand fundamental mechanisms underlying gender differences in seizure susceptibility and epileptogenesis.

Keywords

Sexual differentiation; Brain development; Steroid hormones; Depolarizing GABA; Seizures

Introduction

Epileptogenesis is the process by which a normal neuronal network is altered to become one that is hyperexcitable and prone to unprovoked, recurrent seizures. This can occur at any point in the lifespan of an individual, although the incidence of seizures in general is higher in neonates (Kotsopoulos et al., 2002). The underlying cause of seizure induction and the eventual chronic pathology that is epilepsy may be genetic or external, but seizure vulnerability is also influenced by the neural substrates of the networks involved, and these differ between the sexes. Steroid hormones are important modulators of brain physiology in

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adults, and indeed, clinical and animal studies show that seizure vulnerability and onset are influenced by levels of circulating hormones, making this a major factor contributing to sex differences in epileptogenesis. Steroid hormones are also critically important in sexual differentiation of the brain during early development, shaping neural circuits and potentially altering seizure susceptibility in a sexually dimorphic manner. Here, we will provide an overview of how male and female brains are sculpted differently during early development, focusing on specific examples of neural physiology that differ between the sexes during the perinatal period which may be relevant for seizure induction and epileptogenesis.

Sex differences in the developing brain are shaped by steroid hormone exposure

The brain begins as a largely bipotential organ that will acquire a masculine or feminine architecture in response to external and intrinsic factors during early development. The prime mover in this process is exposure to gonadal steroid hormones, which act on the developing brain during a critical perinatal period to organize the neural architecture and delimit the sex-specific influence of gonadal steroids later in life. This is the organizational-activational hypothesis of hormone action on the developing brain, and was first articulated in a seminal study by Phoenix and colleagues which demonstrated that treatment of pregnant Guinea pigs with testosterone masculinized the sexual behavior of their female offspring as adults (Phoenix et al., 1959). Although originally codified to explain sexual differentiation of the brain in terms of reproductive behavior, the organizational/activational hypothesis still provides a framework for understanding the developmental programming of other endpoints of brain physiology that differ between males and females (Arnold, 2009). The critical period for hormonal modulation of brain development in mammals is initiated by the perinatal surge in circulating testosterone that occurs with testicular development in male fetuses. In rats, the perinatal testosterone surge begins late in gestation, around embryonic day 18, and declines a few hours after birth (Weisz and Ward, 1980). Circulating testosterone enters the brain in developing male rats and is enzymatically converted to estradiol via aromatization (McEwen et al., 1977; Naftolin et al., 1975). Expression of aromatase in the brain varies among regions and additionally, there is evidence for de novo steroidogenesis in some regions of the developing brain (Amateau et al., 2004; Tsutsui, 2012). For these reasons, androgen and estradiol content of the developing brain varies according to region and sex (Amateau et al., 2004; Konkle and McCarthy, 2011). Estradiol signaling in the developing rodent brain induces various sex differences in cellular morphology and physiology that are the hallmarks of masculinization of specific brain areas. Among these are differences in cell death and survival, neuro- and glial genesis, activity-induced synaptogenesis, dendritic branching and changes in astrocyte and microglial morphology. The precise timing of a particular process in relation to the window of steroid action in brain masculinization varies according to brain region, but in general the critical period for sexual differentiation in the rat closes during the second week of life, around postnatal day 10 (reviewed in Lenz et al., 2012; McCarthy, 2008).

Although estrogen is required for sexual differentiation of many phenotypic endpoints in the rodent brain, this appears not to be the case for humans. Direct evidence testing the

aromatization hypothesis is obviously precluded in humans, but correlations between circulating fetal testosterone and sex-specific volumes of brain nuclei, as well as studies in non-human primates, indicate that testosterone is the main organizing hormone driving sexual differentiation in the fetal brain (Lombardo et al., 2012; Wallen, 2005), although the presence of aromatase activity during late gestation and early postnatal life indicates there may be a role for estrogen in sex-specific development in some brain areas (MacLusky et al., 1987; Naftolin et al., 1971). Masculinization occurs prenatally, and fetal testosterone production begins late in the first trimester and peaks around gestational week 16 (Abramovich and Rowe, 1973; Reyes et al., 1974). Circulating testosterone then falls to levels equivalent to what is found in female fetuses until it rises again at birth, declining around 24 h later (Corbier et al., 1990; Forest and Cathiard, 1975; Tapanainen, 1983). The magnitude of the perinatal testosterone surge in males is highly variable, but in general circulating testosterone in males at this time is 2–3 times higher than in females (Corbier et al., 1990). A second postnatal surge in testosterone occurs in male infants at 1 to 3 months of age (Forest and Cathiard, 1975; Winter et al., 1976) (Fig. 1).

One of the difficulties in modeling sex differences in the developing brain arises when trying to extrapolate the protracted gestational time-line of humans to that of experimental animals such as rodents, and a number of studies have sought to do this by correlating developmental milestones such as formation of neuroanatomical structures, region-specific neurogenesis and myelination, and synaptic pruning. From such studies it is generally considered that the perinatal period in rodents is roughly equivalent to the third trimester in terms of human brain development, and at about 12–13 days of life the rat brain is developmentally similar to that of a full term infant on the day of birth (Clancy et al., 2007; Semple et al., 2013).

Sex differences in neuronal excitability during early development

Epilepsy is commonly characterized as a chronic imbalance in excitatory and inhibitory transmission within neural circuits. This is largely based on the anticonvulsant effects of GABA receptor agonists as well as alterations in inhibitory interneuron function associated with epilepsy in adults (Fritschy, 2008), but evidence also comes from altered content of amino acid neurotransmitters in biopsies of epileptogenic brain tissue (Perry and Hansen, 1981). Although studies that directly determine levels of neurotransmitters in the developing brain are few, Davis and colleagues employed HPLC to quantitate amino acid transmitters in specific regions of the rat brain across the first 20 days of postnatal development (Davis et al., 1999). They found that males have more of the neurotransmitters glutamate, aspartate, and GABA in the arcuate and ventromedial nuclei of the hypothalamus, and also the CA1 region of the hippocampus on the first postnatal day, but not later. These data point to an increased excitatory tone in specific brain areas of neonatal males. More striking, however, are subsequent studies that focus on cellular aspects of GABA signaling and provide a compelling picture of early postnatal sex differences in neuronal excitability.

Depolarizing GABA

Of the three known receptor subtypes that mediate GABA transmission, the GABA_A receptor is commonly associated with inhibitory imbalance in seizures and epilepsy (Briggs

and Galanopoulou, 2011; Nardou et al., 2013). In mature neurons, the GABA_A receptor mediates fast synaptic inhibition by gating the influx of chloride ions, thereby hyperpolarizing the cell and reducing action potential firing. Thus in the adult central nervous system, GABA is the main inhibitory neurotransmitter. During early development, however, the chloride gradient in immature neural cells is reversed, and activation of GABA_A receptors causes efflux of intracellular chloride ions and membrane depolarization. The timing of the developmental switch when GABA signaling moves from depolarizing to hyperpolarizing varies among brain regions and is dictated by a shift in the driving force for chloride, and this in turn is determined by the relative expression of chloride transporters on the cell membrane. The transporters most relevant for the developmental shift in chloride gradient are the sodium–potassium–chloride cotransporter 1 (NKCC1), which facilitates electroneutral transport of chloride into the cell, and the potassium–chloride cotransporter 2 (KCC2), which pumps chloride ions out of the cell. Expression of these transporters is developmentally regulated within the cell. NKCC1 expression is high and KCC2 expression is essentially absent in immature neurons, but as the cell matures KCC2 expression is upregulated and the relative shift in chloride transport reverses the chloride gradient (Fig. 2). This process is a hallmark of neuronal maturation, and KCC2 expression is downregulated in adult neurons in epilepsy (Rivera et al., 1999, 2005; Wang et al., 2002). The preferential expression of the NKCC1 transporter renders GABA depolarizing in immature neurons and is associated with increased seizure susceptibility during early development (Ben-Ari and Holmes, 2006; Dzhalal et al., 2005).

A fundamental sex difference exists in the timing of the shift from depolarizing to hyperpolarizing GABA, wherein males make this shift later in development. In the rat substantia nigra, GABA_A receptor activation becomes hyperpolarizing in females around postnatal 10, but in males this does not occur until postnatal day 17 (Galanopoulou et al., 2003; Kyrozis et al., 2006). Similarly in the CA1 region of the hippocampus, females respond to GABA agonists with hyperpolarizing currents before the end of the first postnatal week, but in males the response remains depolarizing until the second week of life (Galanopoulou, 2008; Nuñez and McCarthy, 2007). This corresponds with sex differences in the developmentally-regulated chloride transporters, where an earlier rise in KCC2 expression in females has been demonstrated (Galanopoulou, 2008; Kyrozis et al., 2006; Nuñez and McCarthy, 2007; Perrot-Sinal et al., 2007). Sex differences are also evident in the magnitude of the response to depolarizing GABA. In vitro calcium influx in response to the GABA_A receptor agonist muscimol demonstrates a greater initial rise in calcium transients in hippocampal neurons derived from neonatal females, but a shorter decay time compared to male-derived neurons. Repeated muscimol administration attenuates this response in females, but not in males. The desensitization of GABA_A receptor activation is blocked by androgen administration in females and correlates with increased expression of the $\gamma 2$ subunit of the GABA_A receptor, indicating that perinatal hormone exposure modulates the response to GABA by regulating the pharmacological properties of the GABA_A receptor (Nuñez and McCarthy, 2008). Estradiol signaling during the perinatal period also potentiates the response to depolarizing GABA in cultured hippocampal and hypothalamic neurons (Nuñez and McCarthy, 2009; Nuñez et al., 2005, 2008; Perrot-Sinal et al., 2001). This may not only be due to upregulated NKCC1 expression in the presence of estradiol (Nuñez et al.,

2005), but is also due to indirect enhancement of NKCC1 activity by estradiol via the kinases OSR1 and SPAK (Nugent et al., 2012).

The depolarizing action of GABA in immature neurons can generate action potentials by activating voltage-gated sodium channels, promote glutamate signaling by removing the magnesium block of NMDA receptors, and increase intracellular Ca^{2+} by activating voltage-gated calcium channels. Through these mechanisms depolarizing GABA promotes synchronous network oscillations and modulates activity-dependent processes such as neuronal maturation and synaptogenesis (Ben-Ari et al., 2007). Tonic activation of GABA_A receptors on immature neuronal cells also modulates their proliferation and migration (Behar et al., 1996; Manent et al., 2005). Depolarizing GABA thus plays a critical role in shaping the architecture of the developing brain, and likely does so in a sexually dimorphic manner. Evidence for this comes in at least two forms in the neonatal rat hippocampus. First, phosphorylation of the transcription factor CREB in response to GABA_A receptor activation with muscimol increases in the CA1 region of the hippocampus in males, but is decreased in females (Auger et al., 2001), and this is also demonstrated in the developing substantia nigra (Galanopoulou, 2006). Because CREB activation promotes expression of immediate early genes, this indicates that downstream activation of gene expression in response to depolarizing GABA differs in neonatal males and females, and divergent neuronal physiology may result. Second, excitotoxic damage induced by muscimol in neonates results in greater cell death in all areas of the hippocampus for males compared to females, and muscimol-induced cell death is exacerbated in females by pretreatment with a masculinizing dose of estradiol (Núñez and McCarthy, 2003; Núñez et al., 2003a, 2003b). Depolarizing GABA is involved not only in acute changes in neuronal excitability during neonatal seizures, but is also perfectly poised to translate developmental sex differences into altered seizure susceptibility and progression.

Perinatal sex differences modulating sub-acute changes in the epileptogenic pathway

Epileptogenic seizures that are not caused by genetic mutation or developmental malformation of the cortex are precipitated by environmental insult, and during the perinatal and early life period these are typically sustained fever, trauma, or hypoxia–ischemia. In addition to acute changes in network excitability, early-life epileptogenic insults initiate subacute changes in neuronal physiology that occur over a period of hours to weeks, mostly during a period of seizure latency, and further promote the development of epilepsy (Rakhade and Jensen, 2009). The manifestation of, or response to many of these subacute changes exhibits sex differences in the developing brain which may alter the progression of epileptogenesis between males and females.

Neuroimmune activation

Many clinical and animal studies have established the involvement of infection and inflammatory mediators in the development of epilepsy and modulation of seizure frequency (Vezzani et al., 2011; Xu et al., 2013). Networks of cytokines and chemokines are upregulated in hippocampal tissue from adults with mesial temporal lobe epilepsy (Kan et

al., 2012), and rats exposed developmentally to maternal infection exhibit increased susceptibility to adult seizure induction, which is mediated by upregulation of the inflammatory cytokines IL-6 and IL-1 β in the hippocampus (Pineda et al., 2013). Reactive gliosis, which is often associated with limbic epilepsy and occurs in response to neuroinflammation and trauma, begins with recruitment of macrophages and microglia to the site of injury and culminates in proliferation of surrounding astrocytes to form a glial scar. There is increasing evidence supporting a critical role for glial activation in promoting epileptogenesis (Cusick et al., 2013; Devinsky et al., 2013). Data concerning sex differences in inflammatory mediators and other neuroimmune factors in the developing brain are still relatively scarce, but emerging evidence suggests male and female brains respond differently to immune challenge during the perinatal period. For example, bacterial infection in neonatal mice profoundly alters patterns of gene expression in the adult hippocampus in a sexually dimorphic manner (Wynne et al., 2011). Similarly, expression of IL-6, IL-1 β and TNF α in response to bacterial lipopolysaccharide is much greater in cortical astrocytes and microglia from neonatal males versus females, in spite of equivalent levels of these inflammatory cytokines in untreated astrocytes. This sex difference in response to challenge is apparently due to the effects of prenatal steroids, as it can be reversed in female-derived glia by treatment with testosterone or estradiol (Loram et al., 2012; Santos-Galindo et al., 2011). There are baseline sex differences in cytokine expression in the rat hippocampus, amygdala and cortex (Schwarz et al., 2012), and in microglia isolated from developing murine cortex (Crain et al., 2013). These sex differences are evident in the first postnatal week but not before birth, further suggesting a role for perinatal gonadal steroids. In addition to neuroimmune signaling molecules, the early postnatal brain of rodents exhibits sex differences in the profile of resident immunocompetent cells, particularly microglia, which both secrete and respond to proinflammatory molecules. Microglia are particularly abundant in the developing brain, where they not only survey and scavenge in response to pathogenic stimuli, but also play a critical role in developmental synaptic pruning (Paolicelli and Gross, 2011; Schafer and Stevens, 2013). We found that male rats have twice as many activated microglia in the preoptic area during the early postnatal period compared to females (Lenz et al., 2013), and that these activated microglia are essential for mediating the effects of prostaglandin E2 in establishing a male pattern of neuronal spine density and astrocyte morphology in this area of the brain, thereby programming network connectivity and subsequent male sexual behavior (Amateau and McCarthy, 2002, 2004; Lenz et al., 2013). Schwarz et al. (2012) report sex differences in the numbers and activation state of microglia in several other brain areas across development, including the cortex, amygdala and hippocampal formation. Consistent across studies is the observation of elevated levels of factors mediating proinflammatory reactivity in males, particularly in brain areas associated with seizure induction.

Neuronal cell death

The most common cause of neonatal seizures, and thus a significant risk factor for epilepsy, is hypoxia–ischemia (HI) (Ronen et al., 1999). Both clinical and animal studies indicate that males incur greater damage in terms of brain injury and long-term outcomes of cognitive impairment after perinatal asphyxia, compared to females (reviewed in Hill and Fitch, 2012). Hypoxia–ischemia promotes excitotoxicity in neurons and glia, increasing sodium and

calcium influx and resulting in acute necrosis and subsequent activation of apoptosis (Northington et al., 2001). An important divergence between the sexes occurs in the activation of intrinsic apoptotic pathways (Du et al., 2004; Lang and McCullough, 2008; Nijboer et al., 2007; Renolleau et al., 2008; Zhu et al., 2006). In males, cell death associated with hypoxia-ischemia in neonates (and adults) is effected through a caspase-independent pathway, in which activation of poly(ADP-ribose) polymerase-1 (PARP-1) effects the release of apoptosis-inducing factor (AIF) from mitochondria, resulting in cell death. Pharmacological inhibition or genetic deletion of PARP-1 significantly reduces HI-induced cell death in neonatal males, but not in females, indicating that a distinct effector pathway for programmed cell death is utilized in females (Hurn et al., 2005; Nijboer et al., 2007). PARP-1 activation in response to ischemia additionally upregulates activity of the calcium-permeable transient receptor potential M2 ion channel (TRPM2), which mediates cell death in response to oxidative stress preferentially in males. PARP-1 regulation of TRPM2 activity is dependent upon androgen receptor signaling (Vagnerova et al., 2010), and PARP-1 expression is also upregulated by androgen (Shimizu et al., 2013), suggesting that the caspase-independent apoptotic pathway utilized in male neurons is programmed during the perinatal testosterone surge. In contrast, cell death following neonatal HI or trauma in females is executed via the caspase-dependent pathway of apoptosis, in which cytochrome-c release from mitochondria promotes formation of the nuclear apoptosome and subsequent activation of caspase cascades. An important regulator of the caspase-dependent pathway is the X-linked inhibitor of apoptosis (XIAP), which inhibits effector caspase activity (Wang et al., 2004). Inhibition of XIAP following neonatal HI worsens the outcome in females but not in males (Hill et al., 2011), furthering the evidence for preferential activation of caspase-dependent apoptosis in neonatal females, and suggesting that XIAP may be a protective factor in females. Although sex differences in XIAP expression in the developing brain are not known, adult females have higher baseline gene expression of XIAP regardless of whether they are gonadally intact or gonadectomized, suggesting intrinsic sex-specific regulation of XIAP (Siegel et al., 2011).

Epigenetics

Long-term alterations in gene expression in response to external factors can be achieved through chromatin modifications which don't involve changes in gene sequence, and are thus collectively termed epigenetic, or "above genetics". Such changes can be heritable, are effected by enzymatic processes and are therefore reversible for some mechanisms, and often underlie aberrant gene expression in disease pathology. Altered patterns of several types of epigenetic "marks" that contribute to permanent changes in gene expression and network excitability are associated with epilepsy and chronic seizures in human postmortem tissues and animal models, indicating that epigenetic modification occurs along the pathway of epileptogenesis (reviewed in Qureshi and Mehler, 2010; see also Qureshi and Mehler, in this issue).

During perinatal development, sex differences in epigenetic mechanisms are involved in the normal differentiation of the male and female brain, both intrinsically and in response to external factors such as maternal care, stress or trauma (McCarthy et al., 2009; Murray and Sweatt, 2010; Nugent and McCarthy, 2011; Roth, 2012). In many cases these mechanisms

are known to be regulated by the hormonal milieu of the developing brain. A particularly well-studied mechanism of epigenetic control is DNA methylation, which involves covalent attachment of methyl groups to cytosine residues within islands of CpG dinucleotides. Methylation of upstream regulatory regions is typically correlated with reduced gene expression. An example of hormonally modulated DNA methylation that fundamentally impacts brain development is seen in the estrogen receptor α gene, in which males and females exhibit different patterns of DNA methylation in the promoter of this gene within the preoptic area and hypothalamus that are altered by perinatal exposure to estrogens (Kundakovic et al., 2013; Kurian et al., 2010; Schwarz et al., 2010).

DNA methylation is catalyzed by DNA methyltransferases, and steroid-dependent sex differences in their expression are found. Kolodkin and Auger (2011) examined the expression of the DNA methyltransferases DNMT1 and DNMT3a and found higher levels of DNMT3a in the amygdala of females on the day of birth, but not during the second postnatal week. Treatment of females with estradiol or the non-aromatizable androgen DHT on the day of birth lowered DNMT3a expression to that of males, indicating that gene expression in the amygdala of males is regulated by hormonal influences on DNA methylation activity during the perinatal period. Prenatal exposure to the estrogenic compound BPA also alters DNMT expression in a sexually-dimorphic manner in various regions of the mouse brain (Kundakovic et al., 2013).

Histone modifications, which influence gene expression by impacting how tightly DNA is bound within the nucleosome, are also critical to the organizing effects of steroids during brain development. In the principal nucleus of the basal ganglia (BNSTp), a sex difference in cell death during the first postnatal week results in larger BNSTp volume in males and testosterone treated females. A single injection of the histone deacetylase inhibitor valproic acid on the day of birth increases acetylation of histone H3 in the BNSTp, and decreases BNSTp volume in males and testosterone treated females (Murray et al., 2009). Although not directly shown in this study, sex differences in histone modifications can be determined by hormone exposure. For example, in combined cortex and hippocampus of mice, males have more acetylated histone H3 and trimethylated histone H3 compared to females on embryonic day 18, day of birth, and postnatal day 6 (Tsai et al., 2009). No sex differences in these histone modifications are seen in the amygdala or hypothalamus. Gestational administration of testosterone increases H3 acetylation in the cortex and hippocampus of female neonates, but does not alter H3 methylation. Thus, histone modifications are differentially regulated by hormones in the perinatal brain, but other factors must contribute to their sex-specific regulation. One candidate factor is UTX, a lysine demethylase that specifically targets histone H3. On the day of birth, *Utx* gene expression is higher in female mouse brains compared to males, which may account for the lower levels of methylated H3 in females (Xu et al., 2008). What is more intriguing about UTX, however, is that it is encoded on the X chromosome, yet it escapes X-inactivation. Balanced expression between males and females of the genes encoded on the X chromosome is generally achieved by X-inactivation, although 15 to 25% of X-linked genes in a given cell escape this process (Carrel and Willard, 2005). X-inactivation escape of *Utx* illustrates that sexual dimorphism in the brain can be epigenetically programmed

independently of hormones, allowing for sexually divergent responses to extrinsic factors throughout pre- and postnatal development.

Another example of developmental sex differences in epigenetic programming that has particular relevance for epileptogenesis is found in MeCP2, a methyl-CpG binding domain protein that is encoded on the X chromosome. Mutations in the MeCP2 gene cause the pervasive neurological disorder Rett syndrome, and even mildly altering expression of MeCP2 promotes seizures in animal models (Amir et al., 1999; Bodda et al., 2013; Collins et al., 2004). MeCP2 binds to methylated regions of the DNA and recruits corepressors, histone deacetylases and other chromatin modifiers to alter gene expression, and thereby plays a critical role in neuronal maturation and synaptogenesis (Smrt et al., 2007). Sex differences in MeCP2 expression are evident in the early postnatal brain of rats, where females have higher levels of the transcript and protein in the amygdala on postnatal day 1, but not on postnatal day 10 (Kurian et al., 2007). Transient knockdown of MeCP2 expression in the amygdala during the first 2 days postnatally disrupts social play behavior in juvenile males but not in females (Kurian et al., 2008). This suggests that during the perinatal period even brief alterations in MeCP2 activity, as is known to occur during membrane depolarization and seizures (Chen et al., 2003; Zhou et al., 2006), can have long-term consequences for network organization that differs between the sexes.

Perinatal sex differences in mechanisms that promote long-term network alterations

The acute and sub-acute alterations in cellular physiology induced by seizure activity can promote long-term changes in neuronal activity that ultimately leads to increased seizure susceptibility, thus completing the process of epileptogenesis. One way in which this can occur is through altered neuron morphology and synaptogenesis, both of which are seen after neonatal seizures in the rat (Holmes et al., 2002; Pugh et al., 2011). As a fundamental developmental process, synaptogenesis is regulated in a sexually dimorphic manner under the influence of steroid hormones, as has been demonstrated in several areas of the brain in the neonatal rat. Estradiol induces a male pattern of increased spine density in the preoptic area by increasing glutamate release from astrocytes and stabilizing AMPA receptors on developing neurons, and both of these processes are dependent on biosynthesis of prostaglandin E2 (Amateau et al., 2004; Lenz et al., 2011). In the developing hypothalamus of males, estradiol promotes dendritic branching via increased glutamate signaling (Mong et al., 1999, 2001; Schwarz et al., 2008), and increases GABA synthesis to alter astrocyte morphology (Mong et al., 2002). Neonatal males also have more synapses in the CA1 region of the hippocampus, although this is apparently regulated by androgens, since androgen receptor antagonism decreases spine density (Zhang et al., 2010).

Another fundamental process in the developing brain for which alterations have been strongly linked to seizures and epilepsy is neurogenesis. In adults, seizure activity induces increased production of granule cells in the dentate gyrus, and is associated with ectopic localization of newborn granule cells and mossy fiber sprouting, which potentially contribute to persistent changes in hippocampal circuitry (Scharfman and Gray, 2007;

Scharfman and McCloskey, 2009). In the neonate, recurrent seizures result in a short-term decrease in cell proliferation in the dentate gyrus (McCabe et al., 2001; Xiu-Yu et al., 2007), but an overall increase in survival of newborn neurons is seen in this region of the brain long-term (Holmes et al., 1998; Xiu-Yu et al., 2007). The long-term increase in neurogenesis resulting from recurrent neonatal seizures is also associated with mossy fiber sprouting, altered network excitability and increased seizure susceptibility (Holmes et al., 1998), although studies which examine whether this occurs to the same degree in both sexes are scarce.

Under normal physiological conditions, differences in cell proliferation and survival between males and females are seen in two limbic areas which are involved in seizure induction. In the amygdala, female rats have a higher rate of cell proliferation during the first postnatal week compared to males, which translates into increased numbers of glial cells in females during later life (Krebs-Kraft et al., 2010). This sex difference in glial genesis is due to a dampening of cell proliferation in males by elevated levels of endocannabinoids, and is correlated with sexually differentiated play behavior in juveniles. Conversely, in the neonatal hippocampus a robust sex difference in cell genesis is seen during the first week of life, wherein males have higher rates of proliferation in the dentate gyrus and CA1, and the majority of proliferating cells in these regions differentiate into neurons. This male-biased difference in neurogenesis is regulated by steroid hormones during the perinatal period (Bowers et al., 2010; Waddel et al., 2013; Zhang et al., 2008; 2010). Because the vast majority of studies using rat models of neonatal seizures are done using males, the basal sex difference in hippocampal neurogenesis may actually be responsible for the altered neurogenesis seen following early life seizures. Support for this is seen in a study by Lemmens et al. (2005) which examined sex-specific effects of neonatal seizures on long-term cell genesis in the dentate gyrus. In rats experiencing hyperthermic seizures on postnatal day 10 and labeled with BrdU for 5 days thereafter, males have higher numbers of granule cells born after seizure induction compared to non-seizure controls, while females showed no difference in cell genesis after hyperthermic seizures (Lemmens et al., 2005.)

Conclusion

A number of sex differences are seen in the limbic regions of the brain that are associated with seizure induction, and may contribute to the progression of epileptogenesis in ways that differ between the sexes. Heightened baseline neuronal excitability and a more reactive neuroimmune response may confer a greater susceptibility to seizures in neonatal males, and may also account for the greater damage and poorer outcome males suffer after early life seizures. Sex-specific mechanisms that sculpt the neuronal architecture during normal development have the potential to contribute to long-term alterations in circuit function that result in epilepsy. Many of these, such as depolarizing GABA, modulation of neurogenesis, and epigenetic regulation of gene expression, are critical interfaces between environmental insult and persistent alterations in brain physiology and as such are mechanistically important in the development of epilepsy as a result of perinatal injury. Studies in animal models which reveal how sexual differentiation of the brain is achieved have great potential to inform our understanding of gender differences in seizure susceptibility and epileptogenesis.

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Fig. 1.

Levels of circulating testosterone in male rat and human, aligned with equivalent periods of brain development. Serum testosterone derived from the fetal testis peaks in male rats shortly before birth around embryonic day 18 (E18), and again on the day of birth. The aromatization of testosterone (T) to estradiol (E2) in the brain during the perinatal period is critical for the development of a masculine brain phenotype. In humans, prenatal testosterone production from the fetal testis is elevated in males from gestational weeks 9 (GW9) to 24 (GW24), with a peak around gestational week 16. Circulating testosterone is elevated on the day of birth, and again peaks in newborns between 1 and 3 months old (MO) (Corbier et al., 1990; Forest and Cathiard, 1975; Reyes et al., 1974; Tapanainen, 1983; Winter et al., 1976). Unlike in rats, evidence indicates that androgen is responsible for brain masculinization in humans, although the potential for an undiscovered role for estrogens remains. During the third trimester, human brain development resembles that of the rat during the period of brain masculinization in the perinatal period and first week of life. The brain of a term infant is developmentally equivalent to that of a 12 to 13-day-old rat (dashed vertical line).

Fig. 2.

The developmental shift in GABA activity. In immature neuronal cells in the developing brain, high intracellular concentration of chloride relative to the outside of the cell dictates that the driving force for chloride ($df Cl^-$) is outward and the reversal potential for chloride ($E_{chloride}$) is positive relative to the resting membrane potential (V_M). Activation of $GABA_A$ receptors moves the membrane potential in a positive direction toward the threshold potential ($V_{threshold}$), depolarizing the membrane potential and potentially generating an action potential. In mature neurons, intracellular chloride content is low and the driving force for chloride is into the cell. Activation of $GABA_A$ receptors shifts the membrane potential in a negative direction, hyperpolarizes the cell, and GABA is inhibitory. The driving force for chloride is determined by the relative expression of the chloride transporters NKCC1 (blue) and KCC2 (yellow).