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Sex-specific consequences of early life seizures

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Abstract

Seizures are very common in the early periods of life and are often associated with poor neurologic outcome in humans. Animal studies have provided evidence that early life seizures may disrupt neuronal differentiation and connectivity, signaling pathways, and the function of various neuronal networks. There is growing experimental evidence that many signaling pathways, like GABA_A receptor signaling, the cellular physiology and differentiation, or the functional maturation of certain brain regions, including those involved in seizure control, mature differently in males and females. However, most experimental studies of early life seizures have not directly investigated the importance of sex on the consequences of early life seizures. The sexual dimorphism of the developing brain raises the question that early seizures could have distinct effects in immature females and males that are subjected to seizures. We will first discuss the evidence for sex-specific features of the developing brain that could be involved in modifying the susceptibility and consequences of early life seizures. We will then review how sex-related biological factors could modify the age-specific consequences of induced seizures in the immature animals. These include signaling pathways (e.g., GABA_A receptors), steroid hormones, growth factors. Overall, there are very few studies that have specifically addressed seizure outcomes in developing animals as a function of sex. The available literature indicates that a variety of outcomes (histopathological, behavioral, molecular, epileptogenesis) may be affected in a sex-, age-, region- specific manner after seizures during development. Obtaining a better understanding for the gender-related mechanisms underlying epileptogenesis and seizure comorbidities will be necessary to develop better gender and age appropriate therapies.

Keywords

animal models; epilepsy; early life seizures; GABA; sex differences; status epilepticus; development; substantia nigra; hippocampus

Introduction

Early life seizures are among the most common clinical signs of brain dysfunction. They can be caused by genetic abnormalities or acquired insults such as perinatal asphyxia, traumatic brain injury, intracranial hemorrhage, central nervous system infection, malformations of cortical development, metabolic disturbances or just fever (Annegers et al., 1995; Chapman et al., 2012; Huang et al., 1998; Jensen, 2009; Sillanpaa et al., 2008; Tekgul et al., 2006; Verity et al., 1985). In humans, early onset seizures including neonatal seizures not associated with acute metabolic derangements, and particularly status epilepticus (SE), have been linked to poor neurologic outcome and increased risk of subsequent neurodevelopmental dysfunction and epilepsy (Brunquell et al., 2002; Holmes, 2009; Ronen et al., 2007; Sheppard and Lippe, 2012). Animal studies have corroborated these observations showing that the immature brain is more prone to seizures but more resilient to seizure-induced histopathological injury to the hippocampus or epileptogenesis compared with the adult brain (Albala et al., 1984; Friedman and Hu, 2014; Galanopoulou, 2008a; Galanopoulou and Moshe, 2009; Galanopoulou et al., 2002; Holmes, 2005; Nardou et al., 2013; Sperber et al., 1991). Early life seizures may, under certain circumstances, adversely alter the developing brain and disrupt neuronal differentiation, signaling and connectivity, and ultimately the function of specific neuronal networks (Auvin et al., 2012; Ben-Ari and Holmes, 2006; Sankar and Rho, 2007; Scantlebury et al., 2007). Among the factors contributing to the different effects of early life seizures are the incomplete maturation of the operant neurotransmitter systems and networks, metabolic factors, or interactions with environmental or systemic factors. In experimental studies, the consequences of early life seizures are strongly age- and model- dependent.

Incidence studies have indicated a higher incidence of acute symptomatic seizures (excluding febrile seizures) in males than in females, including in pediatric populations (Annegers et al., 1995). The age-specific incidence of acquired (nongenetic) epilepsy, in general, is only mildly higher in males than in females (Hauser et al., 1993; Kotsopoulos et al., 2002) without statistically significant sex differences (Perucca et al., 2014). However interesting sex-specific differences emerge when specific seizure syndromes or epilepsies are considered. Females appear to have greater risk for generalized-onset epilepsy in general (Hauser et al., 1993), childhood absence, or photosensitive seizures of genetic or unknown etiology (Asadi-Pooya et al., 2012; Nicolson et al., 2004; Taylor et al., 2013; Taylor et al., 2004), or febrile status epilepticus (Hesdorffer et al., 2013). In contrast, mild male predominance has been shown in Landau-Kleffner syndrome, West and Lennox Gastaut syndromes, and severe myoclonic epilepsy of infancy (Aicardi and Chevrie, 1970; Galanopoulou et al., 2000; Tsai et al., 2013; Widdess-Walsh et al., 2013). Regarding the consequences of seizures, clinical and epidemiological studies indicate sex-differences in the natural course of epilepsy in adults. However, very few conclusive clinical studies exist on sex-specific outcomes in very young individuals with seizures or epilepsies, due to experimental limitations.

Despite the obvious phenotypic differences between males and females, most of the preclinical studies on the consequences of early life seizures have been performed either only in male animals or the sex distribution of the subjects has not been reported. Increasing

evidence supports that the sexual dimorphism of the brain starts very early in life, differentially affecting molecular signaling pathways, physiologic functions or morphologic attributes, including of brain regions classically involved in seizure expression and control. If the male and female brains operate differently, it would be expected that early seizures should have sex-specific effects. Indeed, few studies have started evaluating the sex-specific effects of early life seizures on neurogenesis, GABAergic or other signaling pathways, behavioral tests, or subsequent injury (Castelhano et al., 2010; Desgent et al., 2012; Galanopoulou, 2008a; Lemmens et al., 2005). It is less clear whether these changes lead to distinct long-term epilepsy or functional outcomes. In this review, we will discuss the animal studies on the sex-specific acute and long-term consequences of early life seizures and will discuss the underlying pathophysiologic mechanisms.

Why look for sex-specific effects of seizures?

Brain maturation is a long process of evolving changes in neurogenesis and migration, gliogenesis, cellular differentiation, synaptogenesis, myelination and synaptic pruning, all of which can be affected by the ongoing developmental processes that occur in systems outside the CNS. It is controlled by species-specific, genetic or non-genetic biological, environmental or epigenetic factors, many of which influence differently males and females. In humans, brain maturation begins about 3 weeks after conception and continues until about 30 years of life (Kolb et al., 2013). To facilitate the comparison between humans and rodents, Table 1 outlines the chronology of developmental stages in male and female rats. Postnatal (PN) 8–10 rodents have been suggested to be developmentally equivalent to human newborn babies, because the rate of brain growth, and its DNA, cholesterol and water contents resemble those of a human full-term neonate (Dobbing and Sands, 1979; Gottlieb et al., 1977). The infantile stage extends until PN21 with peaks of the myelination rate and synaptic density at PN20–21 (Semple et al., 2013). The onset of puberty (early pubertal stage in Table 1) is between PN32–36 (females) and PN35–45 (males), while rodents are considered young adults on PN60 (Ojeda et al., 1980). This is however only a simplified schema of the equivalency of developmental processes between rodents and humans as several developmental processes proceed at different tempos across species (Avishai-Eliner et al., 2002).

Similar to the earlier sexual maturation of females (see Table 1), brain maturation also follows different paths in males and females, which affect protein expression and function, neurogenesis and migration, cellular physiology and differentiation (Table 2) and function (Giorgi et al., 2014), or the interactions of the CNS with other biological systems or environmental factors. Here we will highlight some of the sex-specific features of factors that may influence the sex-specific outcomes of early life seizures.

GABA signaling

GABA_A receptor (GABA_AR), and to a lesser extent GABA_BR signaling pathway, has been extensively studied as to its sex-specific features. These features include sex-specific differences in (a) the developmental expression patterns of various GABA_AR subunits, (b) kinetics, amplitude, and agonist sensitivity of GABA_AR inhibitory postsynaptic currents

(IPSCs), (c) GABA content in certain brain regions, (d) the timing of the developmental shift from depolarizing to hyperpolarizing GABA_AR signaling, as well as (e) the responsiveness of seizure control networks to GABA_ARs that may modify the strength of the pro- or anticonvulsant effects (Table 2) (Giorgi et al., 2014).

In general, the trend of changes observed in the GABAergic system favor an earlier maturation of certain aspects of the GABA-mediated inhibition in certain sexually dimorphic brain regions of the immature females. For instance, the GABAergic neurons of the substantia nigra pars reticulata (SNR) appear to have an earlier peak of neurogenesis prenatally (Galanopoulou et al., 2001), are more abundant in the developing SNR (Galanopoulou et al., 2001; Ravizza et al., 2003), demonstrate earlier developmental rise in the expression of the $\alpha 1$ GABA_AR subunit (Chudomel et al., 2009; Ravizza et al., 2003), which then renders them more sensitive to $\alpha 1$ -selective GABA_AR agonists in developing females compared with males (Chudomel et al., 2009). As detailed also in the review by Giorgi et al (2014) the earlier maturation of the female SNR extends to the earlier appearance of its muscimol-sensitive anticonvulsant function in the anterior SNR (Veliskova and Moshe, 2001). However, exceptions can be seen in certain regions, where the expression of glutamic acid decarboxylase (GAD; GABA synthesizing enzyme) mRNA in the CA1 region of hippocampus and dorsomedial hypothalamic nucleus is higher in males than in females (Davis et al., 1996).

Of particular interest is the earlier appearance of hyperpolarizing GABA_AR signaling in certain sexually dimorphic brain structures, like the rat substantia nigra, CA1 pyramidal hippocampal neurons, or hypothalamus which results from the higher expression of Cl[−] exporters, like **potassium-chloride cotransporter-2 (KCC2)**, and lower levels of Cl[−] importers, like **sodium-potassium-chloride cotransporter-1 (NKCC1)**, in developing females (Galanopoulou, 2003; Galanopoulou, 2006, 2008a; Kyrozis et al., 2006; Murguia-Castillo et al., 2013; Nunez and McCarthy, 2007). The role of cation Cl[−] cotransporters (CCCs) in the regulation developmental changes in GABA_AR signaling and their impact on neuronal activity and age and sex-specific transcriptional control are depicted in Figure 1. The timing of the shift from depolarizing to hyperpolarizing GABA_AR signaling is different for different cell types and brain regions and appears to be important for normal brain development. Failure to develop hyperpolarizing GABA_AR signaling by KCC2 knockout can be lethal (Hubner et al., 2001). In contrast, early termination of depolarizing GABA_AR signaling in developing cortical neurons may disrupt dendritic arborization and formation of dendritic spines, having severe consequences on functions that depend on sensorimotor gating (Cancedda et al., 2007; Wang and Kriegstein, 2009, 2011).

The prolonged developmental windows when GABA_AR signaling is depolarizing in males compared with females in these structures are likely to directly influence the patterns of responses to afferent GABAergic projections and consequently the neuronal activity and intracellular signaling responses of these sexually dimorphic structures. This has been well illustrated *in vitro* with gramicidin perforated patch clamp studies, where electrical stimulation of GABAergic afferents or bath application of GABA_AR agonists evoked depolarizing responses, intracellular calcium rises, only in males but elicited isoelectric or inhibitory responses in same age immature female GABAergic SNR or dopaminergic

substantia nigra pars compacta (SNC) or CA1 pyramidal neurons (Galanopoulou, 2003; Galanopoulou, 2006, 2008a). As a result, *in vivo* systemic administration of GABA_AR agonists, like muscimol, enhances the activity of calcium-sensitive signaling targets in these sexually dimorphic nuclei of male rats but not in same age females. Thus, muscimol can increase the phosphorylation of *cAMP responsive element binding protein* (pCREB) in the PN15 male SNR and SNC neurons but has no effect in same age females (Galanopoulou, 2003; Galanopoulou, 2006) and may also modify, in a sex-specific pattern, the downstream effects of other signaling that are critical for normal development, as has been described for estradiol (Galanopoulou, 2006; Galanopoulou and Moshe, 2003). Similar observations were also reported in the neonatal rat hypothalamus and hippocampus, where *in vivo* systemic muscimol administration increased pCREB immunoreactivity (-ir) in males but reduced it in females (Auger et al., 2001). Depolarizing GABA_AR can also permit the transcriptional regulation of certain genes (e.g., KCC2) by estradiol, during the perinatal period, facilitating possibly the masculinization of certain brain regions that are exposed to higher local estradiol levels in males derived either through aromatization by the perinatal testosterone surge or from local steroidogenesis. These sex-specific transcriptional effects are therefore likely to produce more persistent effects promoting the sexual dimorphism of the targeted neurons (Galanopoulou, 2008c). GABA_AR signaling also plays a major role in the control of seizures and their consequences, raising the question of whether such sex differences could also set the ground for sex-specific consequences of early life seizures.

Fewer studies have looked into the sex-specific features of GABA_BR signaling and these have focused on the expression of GABA_BR subunits in the hypothalamus and anterior pituitary, where GABRB1a was expressed at higher levels in neonatal females than in males. In the hypothalamus, GABRB1a declined more in peripubertal females than in males, an event that was thought to be linked to the onset of puberty (Table 1) (Bianchi et al., 2001; Bianchi et al., 2005).

Glutamatergic receptors

NMDA receptors (NR) are ligand gated cation channels that are tetramers comprised usually of two NR1 subunits, that bind glycine, and two NR2 subunits which bind L-glutamate (Benarroch, 2011). NR1/NR2A receptors are mostly postsynaptic and their intracellular effects may promote neuroprotection and synaptic plasticity whereas NR1/NR2B receptors are usually extrasynaptic and mediate the pro-apoptotic effects in the setting of excessive NR activation. NR binding in the hippocampus increases with age in a region-specific manner (Insel et al., 1990). Region and subunit specific changes have been reported, with different patterns in mRNA and protein studies (reviewed in (Szczurowska and Mares, 2013)). In many regions including the hippocampus and cerebral cortex, there seems to be predominantly NR1/NR2B expression perinatally followed by a dramatic increase in NR2A during the first three first postnatal weeks (Chang et al., 2009; Ling et al., 2012; Sans et al., 2000; Schurowska and Mares, 2013; Zhong et al., 1995). Interestingly, newborn male rats have higher expression of NR2A and lower expression of NR2B in their hippocampus on PN5 compared to females, whereas such differences are not present at older ages (Damborsky and Winzer-Serhan, 2012). NR1 protein expression in the preoptic nucleus is also higher in males than in females during the embryonic and early postnatal periods, which

has been proposed to be important for the presence of perinatal testosterone surge in males and for reducing apoptosis (Hsu et al., 2000). It is therefore possible that in females, during the early postnatal period, there may be fewer synaptic NRs – and more silent synapses – in these brain regions.

The α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-R) are heteromeric complexes comprised of combinations of GluR1-GluR4 and elicit the fast excitatory postsynaptic currents (EPSCs). The presence of GluR2 renders AMPA-R calcium-impermeable; consequently, the low levels of GluR2 early in life allows for more calcium-permeable AMPA-Rs (Pellegrini-Giampietro et al., 1992; Pellegrini-Giampietro et al., 1997). There is no report of sex-specific expression of GluR2 in the developing brain, although sex-specific regulation by estradiol has been reported in adult rat hypothalamus (Diano et al., 1997). In contrast, GluR1 expression is higher in the male PN0 hippocampus, whereas it becomes predominant in PN7–60 females (Bian et al., 2012).

Metabotropic glutamate receptors (mGluR) control neuronal activities and differentiation by influencing the activity of other ion channels (e.g., NRs, L-VSCCs) or intracellular signaling pathways. There is currently no report of sex specific expression in the brain for any of the eight known mGluRs.

Catecholaminergic system

The sexual dimorphism of the dopaminergic system perinatally also defines social behavior later in life (Auger and Olesen, 2009). During the neonatal period, males have higher dopamine levels in the cerebral cortex and hypothalamus than female mice (Connell et al., 2004), although such differences appear to be region-specific (Restani et al., 1990). Females have higher densities of dopaminergic axons in the striatum prenatally compared with males (Ovtscharoff et al., 1992) as well as higher dopamine β -hydroxylase plasma levels (Koudelova and Mourek, 1990).

Cholinergic system

The cholinergic system in developing rats also exhibits sexually dimorphic features (lateralization of high affinity choline uptake, nicotine effects on KCC2 and **brain derived neurotrophic factor (BDNF)**) in the rat hippocampus (Damborsky and Winzer-Serhan, 2012; Kristofikova et al., 2004). The participation and role of these factors in the pathogenesis of seizures and epilepsy is known. The role of the sex-specific features of excitatory systems on seizure outcomes needs further investigation.

Signaling pathways involved in epileptogenesis

Many pathways involved in the generation of seizures and epileptogenesis have been studied as to their sex specific influence. **BDNF** modulates synaptic transmission, neurogenesis, and promotes survival, neuronal growth and differentiation and has been proposed as an anti-epileptogenic treatment (Bovolenta et al., 2010). Interestingly, in the hippocampus, BDNF mRNA expression is higher in males than in females on PN5 but not on PN8 (Damborsky and Winzer-Serhan, 2012). BDNF may have age-specific effects on KCC2 expression and GABA_AR inhibition. It decreases KCC2 in mature neurons, potentially switching GABA_AR

signaling to depolarizing, an effect that has been proposed to underlie the neuroprotective effects of BDNF, although it reduces the strength of GABA_AR inhibition (Rivera et al., 2004; Shulga et al., 2008). Immature neurons however respond differently, since BDNF increases KCC2 expression and synaptogenesis (Aguado et al., 2003). The factors that control the way BDNF regulates KCC2 expression and GABA_AR inhibition include the intracellular Cl⁻ concentration and TrkB-activation of calcium-sensitive or insensitive pathways (Huang et al., 2012; Rivera et al., 2004). Given the interplay of BDNF with these intracellular pathways that do show sex-specific patterns of activity, it would be interesting to explore whether BDNF may mediate sex-specific effects of seizures.

Higher expression of Bcl-2 protein, an important factor in the regulation of neuronal cell death and survival of neurons, is reported in preoptic area of male rats (Hsu et al., 2000). Insulin-like growth factor-I (IGF-I) receptor shows sex and laterality differences in an age-dependent manner in hippocampal CA1, CA3 and DG areas (Hami et al., 2013). There is no study known to us that demonstrates sex-specific effects of mTOR activation in the brain of developing animals, although sex-specific mTOR signaling has been reported in the cardiomyocytes of mice (Gurgen et al., 2013).

Morphological differences

Various morphological sex differences in brain attributes have been reported including the number of dendritic segments, the number of cells in certain brain regions, connectivity or volumetric differences. For example, dendritic branching is higher in the ventromedial nucleus of hypothalamus of the male rats during neonatal period (PN2). These differences are hormonally regulated and were abolished by treating female neonates with exogenous testosterone (Todd et al., 2007). In contrast, at PN25 the number of dendritic segments is greater in female than in male rats (Juraska, 1990). MRI studies in humans indicate that the volumes of amygdala and hippocampi exhibit significant age-related changes from infancy to early adulthood, and amygdala reach their peak volume earlier in females than in males (Uematsu et al., 2012).

Age-specific effects of early life seizures

Early life seizures can produce cell injury, metabolic disturbances, affect the developing and plastic neuronal circuitry or neurogenesis, change gene and protein expression and/or function, or lead to epileptogenesis but their effects are age dependent, and often their effects are distinct from seizures occurring in adulthood (Auvin et al., 2012; Ben-Ari and Holmes, 2006; Galanopoulou and Moshe, 2011; Kubova and Mares, 2013; Rakhade and Jensen, 2009; Sankar and Rho, 2007; Wasterlain et al., 2013). Multiple factors affect the outcomes, including the age at seizure induction, timepoint of outcome observation, cell type and brain region studied, model, duration or recurrence of seizures, the species or strain studied, or the environmental conditions. A major limitation however of these developmental studies is the difficulty in extrapolating the bioequivalency of the administered chemoconvulsant doses across ages, given the need to use different doses at age groups that differ substantially as to the type and degree of engagement of their downstream signaling targets.

SE in immature rodents, during the first two weeks of life, does not always induce cell loss or synaptic reorganization but the degree and pattern of injury and histopathological findings increase and change if SE is induced in older animals, in a model-specific manner (Albala et al., 1984; Galanopoulou, 2008a; Kubova et al., 2001; Raol et al., 2003; Sankar et al., 1998; Scholl et al., 2013; Sperber et al., 1991). Unlike adults, in two week old rats, kainic acid SE (KA-SE) or kindling seizures produce less KA-SE induced cell loss, no kindling cell loss, no synaptic reorganization or change in dentate gyrus (DG) inhibition (Haas et al., 2001; Sperber et al., 1991). Lithium–pilocarpine SE induced cell loss appears mostly at the CA1 region if SE is induced in 2 week old rats, but mostly in CA3, hilus and dentate granule cells in 3–4 week old rats (Sankar et al., 1998). The development of mossy fiber sprouting and spontaneous seizures was also more likely to occur when SE was induced at older ages (Haas et al., 2001; Roch et al., 2002; Sankar et al., 2000; Sperber et al., 1991). Nevertheless, histopathological changes may not be limited to the hippocampal region as extra-hippocampal areas appear to be injured and may contribute to both epileptogenesis and associated comorbidities (Galanopoulou and Moshe, 2009; Scholl et al., 2013).

Early life recurrent or prolonged seizures have been reported to either reduce or increase neurogenesis (McCabe et al., 2001; Nunes et al., 2000), whereas in adults seizures usually increase it (Parent et al., 1997; Scharfman et al., 2000). They may alter neurotransmitter signaling pathways in complex manners that may vary according to the downstream target, the cell type or brain region studied, the age at seizure induction or study of each endpoint, or the seizure model. Exposure to early life seizures changes the expression or the distribution of GABA_ARs (Briggs and Galanopoulou, 2011; Goodkin et al., 2008; Ni et al., 2005; Raol et al., 2006; Zhang et al., 2004). For certain subunits, like the $\alpha 1$, SE can have opposite effects on their expression if induced in very young (PN10: increase in $\alpha 1$) or adult rats (decrease in $\alpha 1$) exposed to lithium-pilocarpine SE (Brooks-Kayal et al., 1998; Raol et al., 2006). Also, within the same animals subjected to lithium-pilocarpine SE on PN20, mature and immature DG neurons respond with different changes in the expression of glutamatergic receptors, suggesting that not only the chronological age but also the maturational state of the cell is important in defining outcomes (Porter et al., 2006). Single or repetitive flurothyl seizures may influence in the same way certain outcomes [e.g., number of GABA_AR $\alpha 1$ -ir or NMDA receptor (NR) NR2C-ir neurons] but not others (e.g., cell loss, glutamatergic or GABAergic responses) (Isaeva et al., 2006; Isaeva et al., 2010; Ni et al., 2005; Sogawa et al., 2001). KA-SE in immature rats has age-specific effects on glutamatergic receptors and GABA_ARs that are often opposite to those observed in adult rats (Friedman et al., 1994; Friedman et al., 1997). The effects of seizures on certain signaling pathways are also age-specific. Seizures can increase BDNF or FGF-2 in young animals, but this effect is more pronounced in older age groups (Kim et al., 2010; Kornblum et al., 1997; Talos et al., 2012b). It is therefore important to replicate specific findings to other seizure models as well as evaluate the overall functional impact of these changes to deduce whether these are impactful or compensated.

In contrast to adult rats, most of which develop epilepsy after SE, early life SE does not necessarily result in epilepsy (Galanopoulou and Moshe, 2011; Haas et al., 2001; Holmes et al., 2002; Moshe, 1993; Roch et al., 2002; Stafstrom et al., 1992). There is no report of adult

epilepsy after SE induced in normal rats during the first week of life. Lithium-pilocarpine induced SE on PN12 or PN14 results in epilepsy in approximately 25% of rats, whereas 50–82% of rats develop spontaneous recurrent seizures in adulthood if SE is induced at PN21–25 (Kubova et al., 2004; Roch et al., 2002). A similar age-specific trend is observed when seizure susceptibility to induced seizures is being studied: the older the age at seizure induction is, the greater is its impact on lowering seizure threshold later in life. KA-SE in 2–3 week old rats does not change kindling susceptibility in adulthood (Okada et al., 1984) whereas PN27 rats exposed to KA-SE kindle faster than controls, 3 days later (Holmes and Thompson, 1988). **Early exposure of PN10 male rats to lithium-pilocarpine induced SE does not alter the long-term susceptibility to seizures induced by GABA_AR antagonists or kainic acid during adulthood** (Nehlig et al., 2002). In contrast, adult rats exposed to lithium-pilocarpine SE had higher susceptibility to pentylenetetrazol during the latent phase (Rattka et al., 2011). However, again, model specific differences are observed.

Despite the milder pro-epileptogenic consequences of seizures in very young rats, compared to older age groups, normal rats subjected to single or repeated episodes of neonatal seizures may develop significant cognitive impairments during adolescence or adulthood (Cornejo et al., 2008; de Oliveira et al., 2008; Dube et al., 2009; Holmes et al., 1998; Sayin et al., 2004; Stafstrom, 2002). These include changes in learning and memory, behavioral abnormalities and emotional behavior, may occur both with repetitive brief seizures of SE and are reported to be more severe if SE is induced at older age groups (Huang et al., 1999; Karnam et al., 2009; Kubova et al., 2004; Sogawa et al., 2001). Interestingly, even a single episode of kainic acid induced seizures on PN7 impairs hippocampal-dependent working memory and this have been correlated with changes at the synaptic level such as reduction of GluR1 subunits and NR2A, impairments in long term potentiation (LTP) and enhancement of long-term depression (LTD) in the CA1 region of male rats (Cornejo et al., 2008; Cornejo et al., 2007).

Sex-specific consequences of early life seizures

Most of the above studies have included either males or animals of unspecified sex. Despite the different trajectories of brain maturation in males and females, to date, only few experimental studies have been designed to compare sex specific molecular or functional consequences of early life seizures, as will be discussed below (Table 3). In this review we will refer to induced models of seizures in immature rodents, as genetic models will be addressed elsewhere in this issue.

GABA signaling

Many reports indicate that seizures may alter GABA_AR signaling in immature and mature periods. Following the report of depolarizing GABA_AR responses in human epileptic subiculum (Cohen et al., 2002), multiple studies confirmed or extended these findings that suggest abnormal CCC expression and depolarizing GABA_AR in human epileptic tissues (Aronica et al., 2007; Cepeda et al., 2007; Conti et al., 2011; Huberfeld et al., 2007; Jansen et al., 2010; Munakata et al., 2007; Munoz et al., 2007; Palma et al., 2006; Talos et al., 2012a). Several animal studies also indicated that seizures or the epileptic state in adult

rodents may lead to re-appearance of depolarizing GABA_AR due to either increase in NKCC1 or decrease in KCC2 activity (Benini and Avoli, 2006; Okabe et al., 2002; Okabe et al., 2003; Pathak et al., 2007; Rivera et al., 2002). **Until** recently, it had been unclear how GABA_AR signaling is affected in immature animals exposed to seizures when these occur during the period when GABA_ARs are normally depolarizing. Of equal importance, do male and female neonatal pups respond in the same manner, given the known sexual dimorphism of GABA_AR signaling? The only report demonstrating abnormal switch to depolarizing GABA_AR as a result of seizures in immature neurons was in PN6–7 male Wistar rat hippocampi induced to generate spontaneous seizures *in vitro*, using the mirror focus formation method (Khalilov et al., 2003). However, in this study, PN6–7 seizure-naïve hippocampal CA3 pyramidal neurons had isoelectric or mildly hyperpolarizing reversal potentials, rather than depolarizing, as seen in neonatal neurons.

In our lab, we evaluated the effects of early life kainic acid induced SE on GABA_AR signaling of CA1 rat pyramidal neurons during the first week of life, when GABA_ARs elicit depolarizing responses in males and isoelectric or hyperpolarizing responses in females (Galanopoulou, 2008a). This allowed us to study the effects of seizures on neonatal male and female rats of the same chronological age (PN4–6) but with distinct modes of GABA_AR responses, given their known sex differences. After inducing 3 episodes of kainic acid status epilepticus (3KA-SE) at PN4–6, we determined the reversal potential of GABA_AR responses (E_{GABA}) in CA1 pyramidal neurons during the subsequent 2 weeks, using gramicidin-perforated patch clamp. In males that had depolarizing E_{GABA} at the time of 3KA-SE(PN4–6) induction, seizures caused a precocious appearance of hyperpolarizing E_{GABA} , 4 days earlier than expected. In contrast, in females that had isoelectric or hyperpolarizing E_{GABA} at the time of 3KA-SE(PN4–6), a transient re-emergence of depolarizing E_{GABA} was observed between PN8–13, but this was not persistent. Interestingly, GABA_AR blockade with bicuculline during 3KA-SE reversed the effects of 3KA-SE on E_{GABA} , suggesting that the effects of seizures on E_{GABA} are GABA_AR mediated. The 3KA-SE induced changes in E_{GABA} also correlated with altered activity of KCC2 and NKCC1.

We have also extended these findings to the SNR. Both male and female SNR GABAergic neurons have depolarizing E_{GABA} at the time of 3KA-SE(PN4–6) induction (Kyrozis et al., 2006). Similar to our observations in the male hippocampus, seizures also accelerated the appearance of hyperpolarizing GABA_AR responses in GABAergic SNR neurons and abolished the muscimol-induced intracellular calcium rises that are normally observed at that age (Kyrozis et al., 2003). Both our hippocampal and SNR studies support that neonatal seizures tend to reverse the polarity of GABA_AR responses in developing neurons, during the days following seizure exposure, in a manner that depends upon their E_{GABA} at the time of seizure induction.

These 3KA-SE induced changes in E_{GABA} and CCCs are distinct from those induced simply by the separation stress of the animals, which tends to cause hyperpolarizing shifts in both males and females (Galanopoulou, 2008a). If indeed depolarizing GABA_AR signaling is associated with the epileptic state, it would be tempting to hypothesize that the inability of

neonatal 3KA-SE to cause persistent appearance of depolarizing GABA_AR could potentially contribute to the low pro-epileptogenic diathesis of newborn rats.

To address whether the disruption of E_{GABA} might also affect the related developmental processes that control seizure susceptibility, we looked at the effects of early life 3KA-SE upon the muscimol-sensitive anticonvulsant function of the anterior SNR and on the susceptibility to flurothyl seizures. For more details on the seizure control function of the SNR please see (Giorgi et al., 2014). Interestingly, 3KA-SE also disrupted the muscimol-sensitive anticonvulsant function of the anterior SNR in males, when it was induced during the developmental period of depolarizing GABA_AR signaling in GABAergic SNR neurons (3KA-SE(PN4–6)) but not after the time of E_{GABA} shift (3KA-SE(PN14–16)) (unpublished data). However, neither 3KA-SE(PN4–6) nor 3KA-SE(PN14–16) affected flurothyl seizure thresholds in PN32 male rats, in the absence of intranigral muscimol infusions, suggesting that other compensatory factors overcame the impaired seizure-controlling function of the SNR (Akman et al., 2014). In line with the hypothesis that the early termination of depolarizing GABA_AR signaling may produce developmental abnormalities, we recently found learning delay in male rats exposed to 3KA-SE at PN 4–6 but not in females (Akman et al., 2014).

Overall, these observations suggest that neonatal seizures may impact on the GABA_AR-sensitive brain development, including its sexually dimorphic features, and that their effects appear to be region, network, and sex-specific. However, complex age-, sex-, and network-specific homeostatic mechanisms may compensate for or aggravate some of the anticipated deficits.

Neurogenesis

Neurogenesis in the dentate gyrus of young rats may be influenced by sex hormones (Ormerod et al., 2003), hence shows differences between males and females. PN17 or 4 week old male rats exhibit more newborn neurons in the dentate than same age females, yet their survival is better in females (Lemmens et al., 2005; Perfilieva et al., 2001). Exposure to 30-min hyperthermic seizures on PN10 improves survival of newborn DG neurons in males till PN66, but had no effect in females (Lemmens et al., 2005). It would also be important to investigate how neonatal exposure to seizures may differentially affect neurogenesis in the DG, given the prior studies that indicate reduction of newborn cells after neonatal flurothyl seizures (McCabe et al., 2001). In addition, investigation as to how environmental or nutritional factors, or life experiences may alter the effects of early life seizures on DG neurogenesis, as a function of age and sex, is also warranted, given the evidence that malnutrition increases seizure-induced neurogenesis after PN15 flurothyl seizures (Nunes et al., 2000). Although seizure-induced neurogenesis has been associated with either epileptogenesis or cognitive dysfunction, a clear causative link with the ensuing epileptogenesis or associated comorbidities has not been established. Future research into the mechanisms involved in seizure-induced neurogenesis and how age and sex-specific factors control this process will be needed.

Histological changes

Sex-specific differences in the pattern of seizure-induced injury in adult rats have been described and could potentially reflect differences in hormonal milieu (Galanopoulou et al., 2003a). There are no studies, known to us, documenting sex-specific seizure-induced injury shortly after early life induced seizures, although this has not been systematically pursued. Intrahippocampal injection of kainic acid to induce seizures in PN10 rats caused hippocampal sclerosis in both sexes by PN55, without any discernible sex differences (Dunleavy et al., 2010). In contrast, daily administration of low doses of domoic acid in PN8–14 rats, reduced parvalbumin (PRV) and GAD positive cells in a sex and region specific manner in the adult hippocampus (Gill et al., 2010). Although domoic acid is a structural analog of kainic acid, it is unclear if, in this study, seizures had occurred and might have contributed to the observed findings. However, it still warns that potential chemoconvulsants may have sex-specific effects on the observed pathology.

Sex-specific factors could potentially interfere with the neuroprotective effects of tested drugs. For example, gabapentin reduced injury and seizures in PN12 CD1 mice subjected to acute ischemic seizures (Traa et al., 2008). A study on the apoptosis induced by anticonvulsant drugs (lamotrigine alone or in combination with phenobarbital or phenytoin) in seizure-naïve neonatal male and female PN7 rats did not report any sex differences in apoptosis (Katz et al., 2007). Administration of estradiol in PN0–1 rats that had been given kainic acid, prevented hippocampal neuronal loss by PN7 in females but not in males (Hilton et al., 2003). Neonatal Wistar female rats subjected to cortical freezing to induce microgyria had less pronounced neuropathology in the medial geniculate nucleus compared to males or androgenized females (testosterone-treated), suggesting again the gonadal hormones may modify the expected histopathological outcomes in certain procedures utilized in epilepsy research (Rosen et al., 1999). The interplay of gonadal hormones with many neurotransmitter systems and the fact that during development, and particular the neonatal period, steroid hormones may play critical role in the neuronal survival and differentiation (see (Giorgi et al., 2014)) emphasize the importance of accounting for sex-related factors when looking at seizure outcomes.

Behavioral tests

Overall, males appear to be more vulnerable to subsequent behavioral comorbidities than females when exposed to early life seizures. Castelhana et al (2010) reported that a single episode of pilocarpine-induced SE at PN9 produces social impairment in males but not in females after PN30 (Castelhana et al., 2010). In contrast, early life SE enhanced self-grooming only in females. Using the 3KA-SE model in PN4–6 rats, we observed selective learning delay in males but not in females at the Barnes maze test performed between PN16–19 (Akman et al., 2014). Reduced susceptibility to short-term social-memory deficits induced by neonatal freezing model of microgyria was observed in female mice in adulthood, compared to males (Rial et al., 2009). Daily administration of domoic acid in PN8–14 rats (uncertain if seizures were induced) impaired Morris water maze and water H-maze performance in males but not in females during adulthood (Gill et al., 2012).

Seizure susceptibility, mortality, epileptogenesis in induced models of seizures

Review of the literature did not reveal any sex-specific differences in seizure susceptibility or resultant mortality in immature rats acutely induced to develop seizures (see Table 2), although such reports are present in adult animals (Galanopoulou, 2008a; Manev et al., 1987; Young et al., 2006). In rats with prior experience of 3KA-SE (PN4–6) no change in flurothyl seizure threshold on PN32 was observed, even if prior alterations in GABAergic system and GABA-sensitive seizure controlling networks had been documented (Akman et al., 2014). These may indicate that in spite of known sex differences in the expression or function of specific neurotransmitter systems, these may be (a) either compensated by other homeostatic mechanisms or (b) overridden by the strong proconvulsant effects of exogenous seizure-inducing methods.

In contrast, a recent study by Desgent and coworkers (2012) reported sexual dimorphism in the long-term vulnerability to develop spontaneous recurrent seizures (epileptogenesis) in the two-hit model of mesial temporal lobe epilepsy (MTLE). In this model, a cortical malformation induced at PN1 with freeze-lesioning of the cortex leads to enhanced susceptibility to a second insult, the hyperthermic seizure (HS), at PN10. Male or androgenized (with prenatal testosterone) female but not female rats with initial cortical malformation displayed spontaneous recurrent seizures after experiencing hyperthermic seizures at PN10. This correlated with a rise in corticosterone levels following the lesion at PN1 in male or androgenized female rats only. Cortical lesion volumes in adulthood were similar between male and female adult rats (Desgent et al., 2012).

Conclusions

There is solid evidence in support of the sexual dimorphism of the brain, including regions and networks that control seizures. The current evidence strongly suggests potent and age-dependent effects of early life seizures on the developing brain at cellular and network levels, including neurotransmitter signaling pathways, neuronal proliferation, differentiation and connectivity, long-term behavioral or epilepsy outcomes. Despite the differences of brain maturation in males and females, to date, the sex specific molecular or functional consequences of early life seizures have not been fully elucidated, largely because most of the available studies have been done in males or rodents of unclear sex specification or because the low sample sizes and variability in outcomes in animal studies may not allow the detection of certain sex differences. Among the studies that have addressed sex differences in the consequences of early life seizures, a number of outcomes have shown statistical significance, including: molecular or functional alterations in certain neurotransmitter systems (e.g., GABA_AR, cholinergic), survival of newborn DG cells, injury induced by seizures or their induction methods, neuroprotective effects of selected drugs (e.g., gabapentin) or hormones (e.g., gonadal hormones), long-term behavioral or epilepsy outcomes. A variety of factors have been implicated in the expression of sex-specific outcomes, including but not limited to GABA_AR signaling, growth factor signaling, gonadal hormones, network differences, although more needs to be known. In contrast, there is also sufficient evidence that in many cases, and despite documented sex-specific abnormalities, subsequent pathology or seizure susceptibility may be unaltered, emphasizing the

importance and power of homeostatic mechanisms that may overcome these deficits. However, even if this is the case, gender-focused research into the mechanisms of epileptogenesis will be necessary in the identification of gender-appropriate therapies that will minimize side effects and improve patient care and quality of life.

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Highlights

- The sexual dimorphism of the brain is present very early in life
- The substantia nigra and hippocampus are sexually dimorphic
- Early life seizures may have age- and sex-specific consequences.
- GABA_A receptors mediate sex-specific effects of neonatal seizures
- More studies are needed to elucidate the sex-specific effects of seizures

Fig 1:

Sex-specific developmental changes in the direction of GABA_AR signaling are determined by cation chloride cotransporters.

Panel A: Early in life, in most studied neurons, the intracellular Cl^- concentration is higher than in adults, due to the functional dominance of cation chloride cotransporters (CCCs) that import Cl^- , like the $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter NKCC1, over cotransporters that export Cl^- , like the K^+/Cl^- cotransporter KCC2 (Plotkin et al., 1997; Rivera et al., 1999). As a result of this intracellular accumulation of Cl^- , activation of GABA_AR triggers Cl^- efflux, neuronal depolarization that activates L-type voltage-sensitive calcium channels (L-VSCCs) or NMDA receptors (NRs) triggering intracellular calcium increases that eventually activate calcium-sensitive processes that are important in neuronal migration, proliferation, differentiation and synaptogenesis (Ben-Ari, 2002; Farrant and Kaila, 2007; Galanopoulou, 2008b). For example, GABA_AR-induced calcium rises activate the phosphorylation of certain transcriptional factors, like *cAMP responsive element binding protein* (CREB), and genes (e.g. KCC2). Furthermore, 17β -estradiol can downregulate KCC2 mRNA expression only in neurons that demonstrate depolarizing GABA_AR signaling. In certain sexually dimorphic brain regions, like the hypothalamus, CA1 pyramidal hippocampal neurons and

substantia nigra, the developmental period when depolarizing GABA_AR signaling is observed is protracted, compared to same age females and this possibly contributes to the sex-specific differentiation of these regions.

Panel B: During development, a gradual shift in the balance of these transporters from NKCC1 dominant to KCC2 dominant state is responsible for the switch of GABA_AR signaling from depolarizing to hyperpolarizing (Galanopoulou, 2008c; Plotkin et al., 1997; Rivera et al., 1999). The increased KCC2 and decline in NKCC1 activity reduce the intracellular Cl⁻ levels. Therefore, GABA_AR activation results in hyperpolarization. This strengthens the GABA_AR inhibition but also abolishes the transcriptional effects observed by depolarizing GABA_AR signaling.

This Figure has been modified with permission from (Galanopoulou, 2008c).

Table 1

Developmental stages in rats, based on hypothalamus-pituitary-gonadal maturation

Stage	Females		Males	
	<i>PN</i>	<i>Features</i>	<i>PN</i>	<i>Features</i>
Neonatal	0–6		0–6	
Infantile	7–21		7–21	
Juvenile	21–32		21–35	
Early pubertal	32–36	Anestrus to early proestrus; pulsatile gonadotropin release in sleep only	35–45	Pulsatile gonadotropin release in sleep only
Puberty	34–38	1 st proestrus, first estrus; 1 st surge of gonadotropins and vaginal opening	45–60	Gonadotropin release also in wakefulness; Maximal testicular response to gonadotropins; Increase in Leydig cells and steroidogenesis
Adult	>60		>60	

The table is based on reference (Ojeda et al., 1980). Please note that these age groups have been defined based on the endocrine changes occurring during maturation and not brain maturation. In general, PN8–10 rats are considered equivalent to full-term newborn humans, based on gross brain growth and its DNA, cholesterol and water contents that resemble those of a human full-term neonate (Dobbing and Sands, 1979; Gottlieb et al., 1977).

Table 2

Sex-specific features in the brain of immature rodents.

Feature	Species, brain region	Age	Sex-specific findings	Reference
GABA signaling				
GABRA1 mRNA	Sprague-Dawley Rat SNR	PN15, PN30	F>M	(Ravizza et al., 2003)
GABRA1 mRNA	Long Evans Rat SS cortex and SS thalamus	PN7, PN14, PN35, PN60	F=M (PN7, PN14) F<M (PN35, PN60)	(Li et al., 2007)
GABRA1 protein and zolpidem sensitivity of IPSCs	Sprague-Dawley Rat SNR	PN5, PN15, PN30	F>M F=M	(Chudomel et al., 2009)
GABRA3 protein	Sprague-Dawley Rat SNR	PN5, PN15, PN30	F>M F=M	(Chudomel et al., 2009)
GABA _A R IPSCs - baseline IPSCs	Sprague-Dawley Rat SNR	PN5–9 PN28–32	F<M: frequency, amplitude, charge/ event F<M: amplitude F>M: decay and rise times	(Chudomel et al., 2009)
GABRG2 mRNA	Long Evans Rat SS cortex	PN7, PN14, PN35, PN60	<u>Somatosensory Cortex:</u> F=M (PN7, PN14) F<M (PN35, PN60) <u>Somatosensory Thalamus:</u> F=M (PN7, PN14, PN60) F<M (PN35)	(Li et al., 2007)
GAD mRNA	Sprague-Dawley Rat, dorsomedial nucleus, arcuate nucleus, CA1	PN1 PN15	F<M F=M	(Davis et al., 1996)
GABA content	Sprague-Dawley Rat SNR	PN15, PN30	F>M	(Ravizza et al., 2003)
GABA-ir cells	Sprague-Dawley Rat striatum	E16–22	F>M	(Ovtscharoff et al., 1992)
GABA-ir cells or parvalbumin GABAergic neurons	Sprague-Dawley Rat SNR	PN15, PN30	F>M	(Galanopoulou et al., 2001; Ravizza et al., 2003)
GABA _A R switch to hyperpolarizing	Sprague-Dawley Rat SNR	PN4–26	F: PN10 M: PN17	(Kyrozos et al., 2006)
GABA _A R switch to hyperpolarizing	Sprague-Dawley Rat CA1 pyramidal neurons	PN4–18	F: isoelectric or hyperpolarizing responses from PN4 M: PN14	(Galanopoulou, 2008a)
Muscimol effects on V _m , <i>in vitro</i>	Sprague-Dawley Rat SNC	PN15	F: no effect M: depolarization	(Galanopoulou, 2006)
Muscimol effects on pCREB, <i>in vivo</i>	Rat hypothalamus, CA1 pyramidal neurons	Neonatal	F: decrease in pCREB-ir M: increase in pCREB-ir	(Auger et al., 2001)
Muscimol effects on pCREB, <i>in vivo</i>	Rat SN	PN15	F: no effect M: increase in pCREB-ir and KCC2 mRNA	(Galanopoulou, 2006; Galanopoulou et al., 2003b)
GABRB1	Sprague-Dawley Rat hypothalamus	PN1–38, adult	<u>GABRB1a</u> F>M (PN1) F<M (PN38) <u>GABRB1b</u> F=M <u>GABRB2</u> F=M	(Bianchi et al., 2005)

Feature	Species, brain region	Age	Sex-specific findings	Reference
GABRB1	Sprague-Dawley Rat anterior pituitary	PN4-PN38, adults	<u>GABRB1a</u> F>M (PN4, 12) <u>GABRB1b</u> F: very low M: barely detectable <u>GABRB2</u> F, M: barely detectable	(Bianchi et al., 2001)
Effects of intranigral muscimol infusions, <i>in vivo</i>	Sprague-Dawley Rat SNR	PN15-PN60	Sex specific effects on flurothyl seizure threshold	(Veliskova and Moshe, 2001)
GABAergic neurons	Sprague-Dawley Rat striatum	E26, E18, E20, E21	F>M	(Ovtscharoff et al., 1992)
Cation Cl⁻ Cotransporters				
KCC2 protein	Sprague-Dawley Rat CA1 pyramidal hippocampal neurons	PN10	F>M	(Galanopoulou, 2008a)
KCC2 protein	Wistar Rat hippocampus	PN1–15	F>M (PN5–15)	(Murguia-Castillo et al., 2013)
KCC2 protein	Wistar Rat entorhinal cortex	PN1–15	F>M (PN9–15)	(Murguia-Castillo et al., 2013)
KCC2 mRNA	Sprague-Dawley Rat hippocampus (CA1, CA3, DG)	PN5, PN8	F=M	(Damborsky and Winzer-Serhan, 2012)
KCC2 mRNA	Sprague-Dawley Rat SNR	PN15, PN30	F>M	(Galanopoulou et al., 2003b)
KCC2 protein	Sprague-Dawley Rat hypothalamus	E20, PN0, PN5	F>M (PN5)	(Perrot-Sinal et al., 2007)
KCC2 mRNA	Sprague-Dawley Rat hypothalamus	E20, PN0, PN5	F>M (PN0)	(Perrot-Sinal et al., 2007)
NKCC1 mRNA	Sprague-Dawley Rat hippocampus (CA1, CA3, DG)	PN5 PN8	F<M F=M	(Damborsky and Winzer-Serhan, 2012)
NKCC1 protein	Wistar Rat hippocampus	PN1–15	F<M (PN9) F>M (PN7, 13)	(Murguia-Castillo et al., 2013)
NKCC1 protein	Wistar Rat entorhinal cortex	PN1–15	F<M (PN5, PN9, PN11)	(Murguia-Castillo et al., 2013)
NKCC1 protein	Sprague-Dawley Rat hypothalamus	E20, PN0	F<M	(Perrot-Sinal et al., 2007)
NKCC1 mRNA	Sprague-Dawley Rat hypothalamus	E20, PN0, PN5	F<M (PN0)	(Perrot-Sinal et al., 2007)
Glutamatergic signaling				
NR1 protein	Long Evans Rat preoptic area	E16, PN1, PN3	F<M	(Hsu et al., 2000)
NR2A mRNA	Sprague-Dawley Rat hippocampus (CA1, CA3, DG)	PN5 PN8	F<M F=M	(Damborsky and Winzer-Serhan, 2012)
NR2B mRNA	Sprague-Dawley Rat hippocampus (CA1, CA3, DG)	PN5 PN8	F>M F=M	(Damborsky and Winzer-Serhan, 2012)
GluR1	C57BL/6 mice Hippocampus	PN0, PN7, PN14, PN30, PN60	F>M (PN0) F<M (PN7, PN14, PN30, PN60)	(Bian et al., 2012)
Cholinergic system				
High affinity choline uptake	Wistar Rat hippocampus	PN7, PN14, 3 months	F: no lateralization M: left lateralization of Vmax [left > right] (PN14, 3 months)]	(Kristofikova et al., 2004)

Feature	Species, brain region	Age	Sex-specific findings	Reference
Nicotine effects on KCC2 and BDNF	Sprague-Dawley Rat hippocampus (CA1, CA3, DG)	PN5, PN8	<i>Daily nicotine po</i> F: no effect M: increase in KCC2, BDNF mRNA	(Damborsky and Winzer-Serhan, 2012)
Muscarinic receptors	Sprague-Dawley Rat cortex (cingulate, parietal), hippocampus	Adult	M1 receptor binding: M>F (CA1) M3 mRNA: M>F	(Potier et al. 2005)
Catecholaminergic system				
Tyrosine hydroxylase positive axons	Sprague-Dawley Rat striatum	E26, E18, E20, E21	F>M	(Ovtscharoff et al., 1992)
Dopamine levels	BALB/cByJ mice, cerebral cortex, hypothalamus	PN3	F<M	(Connell et al., 2004)
Dopamine, Serotonin (5-HT), Dihydroxyphenylacetic acid, Homovanillic acid, 5-hydroxyindoloacetic acid (5-HIAA)	Rat Caudate	PN0–30	F=M	(Restani et al., 1990)
Dopamine	Rat	PN35	F=M (midbrain, prefrontal cortex, striatum)	(Llorente et al., 2010)
5HT	Rat	PN35	F>M (striatum) F=M (midbrain, prefrontal cortex, hippocampus, cerebellum)	(Llorente et al., 2010)
Dopaminergic neurons	CBA/J mice Substantia nigra Ventral tegmental area	E21 – PN90	F=M	(Lieb et al., 1996)
Dopamine beta-hydroxylase	Rat Plasma	PN5-adulthood	F>M	(Koudelova and Mourek, 1990)
Other				
BDNF mRNA	Sprague-Dawley Rat hippocampus (CA1, CA3, DG)	PN5, PN8	F<M (PN5)	(Damborsky and Winzer-Serhan, 2012)
BDNF, NGF, NT-3, trkB mRNA	Rat Ventromedian nucleus of hypothalamus)	E20, PN4	F=M	(Sugiyama et al., 2003)
Anti-NGF treatment	Wistar Rat	PN0–12 qod anti-NGF treatment	<i>PN16 choline acetyltransferase in cortex</i> F: no effect M: reduced	(Ricceri et al., 1997)
Bcl-2 protein	Long Evans Rat preoptic area	PN8	F<M	(Hsu et al., 2000)
IGF-1R positive cells	Wistar Rat hippocampus	PN0 PN7 PN14	F>M (CA1, CA3, DG) Right: F<M (DG) Left: F>M (CA1, CA3, DG) Right: F<M (CA1, DG) Left: F<M (CA1, CA3, DG)	(Hami et al., 2013)
Morphology, neurogenesis				
Dendritic spine number	Rat ventromedian nucleus of hypothalamus	PN2	F<M	(Todd et al., 2007)
NeuN positive cells	Hippocampus	PN7	F<M (CA1, CA2/3, DG)	(Hilton et al., 2003)

Feature	Species, brain region	Age	Sex-specific findings	Reference
Amygdalar, hippocampal growth	Humans	0–25 years	<u>Age of amygdala peak volume:</u> F: L/R = 9.6/11.4 years; earlier subsequent growth deceleration in F M: L/R = 11.1/12.6 years <u>Rightward laterality:</u> F: L<R hippocampus M: L<R hippocampus and amygdala <u>Hippocampal volume:</u> F<M	(Uematsu et al., 2012)
Dendritic tree	Long Evans hooded Rat dentate granule cells of hippocampus	PN25, adults	<u>Number of dendritic segments:</u> F>M (PN25 only) <u>Length of individual dendritic segments:</u> F=M (PN2, adults)	(Juraska, 1990)
Neurogenesis in dentate gyrus	Sprague-Dawley Rat	4 week old	F<M	(Perfilieva et al., 2001)

Abbreviations: BDNF: Brain-derived neurotrophic factor; DG: dentate gyrus; F: females; GABA: gamma-aminobutyric acid; GABRA1: GABA_A receptor α 1 subunit; GABRA3: GABA_A receptor α 3 subunit; GABRB1: GABAB receptor 1 subunit; GABRG2: GABA_A receptor γ 2 subunit; GAD: glutamic acid decarboxylase; GluR1: AMPA-type glutamate receptor 1; IGF-1R: insulin-like growth factor 1 receptor; IPSCs: inhibitory postsynaptic currents; -ir: -immunoreactivity; KCC2: potassium/chloride co-transporter 2; L: left; M: males; NGF: Nerve growth factor; NKCC1: sodium/potassium/chloride co-transporter 1; NR1: NMDA receptor subunit 1; NR2A: NMDA receptor subunit 2A; NR2B: NMDA receptor subunit 2B; pCREB: phosphorylation of cAMP responsive element binding protein at Ser133; R: right; SNC: substantia nigra pars compacta; SNR: substantia nigra pars reticulata; SS: somatosensory.

Table 3

Consequences of early life seizures as a function of sex in induced rodent models of seizures.

Endpoint	Model	Species, Region	Age at seizures	Observation	Reference
Seizure susceptibility and induced mortality	Picrotoxin seizures	CBA/HZgr mice	PN20, 2.5–3 mo, 2 yrs	<u>Seizure occurrence, mortality</u> F=M: PN20, 2 yrs F<M: 2.5–3 mo	(Manev et al., 1987)
	Bicuculline seizures	CBA/HZgr mice	PN20, 2.5–3 mo, 2 yrs	<u>Seizure occurrence, mortality</u> F=M: PN20, 2 yrs F<M: 2.5–3 mo: (mortality)	(Manev et al., 1987)
	3KA-SE	Sprague-Dawley Rat, CA1	PN4–6	F=M: Latency to seizure onset	(Galanopoulou, 2008a)
DG Neurogenesis	Kindling (left CA1)	Sprague-Dawley Rat	PN14–15	F=M: Kindling parameters	(Young et al., 2006)
	Hyperthermic seizure	Sprague-Dawley Rat, DG	PN10	F: no effect M: Increased survival of BrdU-positive DG cells by PN66;	(Lemmens et al., 2005)
	3KA-SE	Sprague-Dawley Rat, CA1	PN4–6	F: no change in KCC2; increased NKCC1 activity; EGABA : depolarizing shift 4 days post-SE (transient) M: increased KCC2; decreased NKCC1 activity; EGABA : hyperpolarizing shift 4 days post-SE	(Galanopoulou, 2008a)
GABAergic system	3KA-SE	Sprague-Dawley Rat, SNR	PN4–6	M, F: increased KCC2; EGABA : hyperpolarizing shift	(Kyrozis et al., 2003)
	Domoic acid (unknown if seizures occurred)	Sprague-Dawley Rat, hippocampus	PN8–14 (daily inj)	M: reduced PRV counts in mid-DG and CA3; reduced GAD counts in CA3 ventral (PN90) F: no change in PRV; reduced GAD in DG ventral (PN90)	(Gill et al., 2010)
	Penyltensetr azole seizure	Sprague-Dawley rat, cortex (cingulate, parietal) and hippocampus	PN20	F<M: MI receptor binding: (CA1) F<M: M3 mRNA F>M: [³⁵ S]GTPγS incorporation <u>Early PTZ seizures (adult)</u> F>M: [³⁵ S]GTPγS incorporation: (cingulate, parietal cortex)	(Potter et al., 2005)
Cholinergic system (Muscarinic receptors)					
Other neurotransmitter systems	Domoic acid (unknown if seizures occurred)	Sprague-Dawley Rat, hippocampus	PN8–14 (daily inj)	<u>Adrenergic receptors (adult)</u> F: no change M: elevated α2A and α2C <u>Mineralocorticoid receptors (MR, adult)</u> F: no change M: elevated	(Gill et al., 2012)
	Kainic acid (unknown if seizures occurred)	Sprague-Dawley Rat, hippocampus	PN0–1	F: estradiol protected from KA-induced neuronal loss (PN7) M: no effect of estradiol	(Hilton et al., 2003)
	Acute ischemic seizures	CD1 mice	PN12	F<M: Seizure and injury reduction	(Traa et al., 2008)
Learning	3KA-SE	Sprague-Dawley Rat	PN4–6	<u>Barnes maze (PN16–19)</u> F: no effect M: learning delay (PN16–19)	(Akman et al., 2014)
	Domoic acid (unknown if seizures occurred)	Sprague-Dawley Rat, hippocampus	PN8–14 (daily inj)	<u>Morris water maze or Water Hole maze (adult)</u> M: delayed latencies F: no effect	(Gill et al., 2012)
Social behavior, anxiety	Pilocarpine	Wistar Rat	PN9	M: less attack and defensive play behaviors than F	(Castelhano et al., 2010)
Locomotor activity	Pilocarpine	Wistar Rat	PN9	F=M: reduced after PN30	(Castelhano et al., 2010)
Hippocampal sclerosis	Intrahippocampal KA-SE	Sprague-Dawley Rat, hippocampus	PN10	F=M: Hippocampal sclerosis (PN55)	(Dunleavy et al., 2010)
Subsequent seizure susceptibility	3KA-SE	Sprague-Dawley Rat	PN4–6	M, F: no change in flurothyl seizure thresholds (PN32)	(Akman et al., 2014)
Adult epilepsy	Two-hit model of MTL	Sprague-Dawley Rat,	PN1: freeze-lesion; PN10: hyperthermic seizures	M: Epilepsy after PN90	(Desgent et al., 2012)

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Endpoint	Model	Species, Region		Age at seizures	Observation	Reference
					F: No epilepsy	

Abbreviations: DG: dentate gyrus; GAD: glutamic acid decarboxylase; F: females; PN: post-natal; GABA: gamma-aminobutyric acid; KA-SE: kainic acid induced SE; KCC2: potassium/chloride co-transporter 2; M: males; M1 and M3: muscarinic receptors; MR: mineralocorticoid receptor; MTL: mesial temporal lobe epilepsy (MTLE); NKCC1: sodium/potassium/chloride co-transporter 1; PRV: parvalbumin; SE: status epilepticus; TLE: temporal lobe epilepsy.