Sex Differences in the Epilepsies and Associated Comorbidities: Implications for Use and Development of Pharmacotherapies

Catherine A. Christian, Doodipala Samba Reddy, Jamie Maguire, and Patrick A. Forcelli

Department of Molecular and Integrative Physiology, Neuroscience Program, and Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, Illinois (C.A.C.); Department of Neuroscience and Experimental Therapeutics, College of Medicine, Texas A&M University Health Science Center, Bryan, Texas (D.S.R.); Neuroscience Department, Tufts University School of Medicine, Boston, Massachusetts (J.M.); and Departments of Pharmacology and Physiology and Neuroscience, Georgetown University, Washington, D.C. (P.A.F.)

Abstract——The epilepsies are common neurologic disorders characterized by spontaneous recurrent seizures. Boys, girls, men, and women of all ages are affected by epilepsy and, in many cases, by associated comorbidities as well. The primary courses of treatment are pharmacological, dietary, and/or surgical, depending on several factors, including the areas of the brain affected and the severity of the epilepsy. There is a growing appreciation that sex differences in underlying brain function and in the neurobiology of epilepsy are important factors that should be accounted for in the design and development of new therapies. In this review, we discuss the current knowledge on sex differences in epilepsy and associated comorbidities, with emphasis on those aspects most informative for the development of new pharmacotherapies. Particular focus is placed on sex differences in the prevalence and presentation of various focal and generalized epilepsies; psychiatric, cognitive, and physiologic comorbidities; catamenial epilepsy in women; sex differences in brain development; the neural actions of sex and stress hormones and their metabolites; and cellular mechanisms, including brain-derived neurotrophic factor signaling and neuronal-glial interactions. Further attention placed on potential sex differences in epilepsies, comorbidities, and drug effects will enhance therapeutic options and efficacy for all patients with epilepsy.

Significance Statement——Epilepsy is a common neurological disorder that often presents together with various comorbidities. The features of epilepsy and seizure activity as well as comorbid afflictions can vary between men and women. In this review, we discuss sex differences in types of epilepsies, associated comorbidities, pathophysiological mechanisms, and antiepileptic drug efficacy in both clinical patient populations and preclinical animal models.

I. Introduction to Epilepsy, Seizures, and the Importance of Investigating Sex Differences

Epilepsy is the fourth most common neurologic disorder, and approximately 1 in 26 people will develop epilepsy in their lives (England et al., 2012). "Epilepsy" is an umbrella term referring to a group of disorders characterized by spontaneous recurrent seizures. A seizure is a paroxysmal discharge of hypersynchronous neuronal activity that can either stay confined to one

brain region (focal), start synchronously across many brain regions (generalized), or start as a focal seizure and then generalize (as with focal-to-bilateral tonic-clonic seizures). As described in further detail below, there are many different epilepsy syndromes, classified by the International League Against Epilepsy primarily by whether the seizures are focal, generalized, combined focal-generalized, or unknown, together with behavioral manifestations, electroencephalographic and imaging signatures, and other clinical factors, including age of onset, family history, and probable etiology (Scheffer et al., 2017). Furthermore, seizures and epilepsy can arise from many different causes and etiologies, including genetic mutations, or can be acquired in response to a neural insult such as a fever, infection, stroke, or traumatic brain injury (Lucke-Wold et al., 2015; Pitkanen et al., 2016; Ramantani and Holthausen, 2017). Postinsult epilepsies often arise after a process of epileptogenesis, consisting of myriad neural circuit reorganization and functional changes in which a group of brain cells or a brain region converts from one that will not generate a seizure to one that can spontaneously (that is, without precipitation by an immediate trigger) produce a seizure, the electrical signal of which is referred to as ictal activity.

Boys, girls, men, and women of all ages across the human lifespan are affected by seizures and epilepsy. Current treatment options focus primarily on antiseizure drugs (ASDs), with the selection, number, and dosage depending on the type of epilepsy and the age of the patient. In some cases, typically in children, dietary-based therapies are used as adjunctive treatments in addition to ASDs (Martin-McGill et al., 2018). Unfortunately, a large proportion of patients (at least one-third) are refractory to current treatments (Kwan and Brodie, 2000; Kobau et al., 2008). Although resection of the tissue that is the seizure focus (the area from which the seizures arise) is an option for some patients, it is not feasible for others and can often produce adverse side effects. In addition, a variety of psychiatric, cognitive, and physiologic comorbidities are present at higher rates in people with epilepsy compared with the general population.

In the ongoing quest to identify cures for epilepsies, there is a growing appreciation that sex differences in underlying brain function and in the neurobiology of epilepsy are important factors that should be accounted for in the design and development of new therapies. This review is aimed at providing an overview of the current knowledge on sex differences in epilepsy and associated comorbidities, with particular emphasis on those aspects that should be informative for and taken into consideration in the development of new pharmacotherapies.

A. Note on Terminology: Sex and Gender

Although "sex" and "gender" are often used interchangeably, there are important distinctions between these two terms. As defined by the World Health Organization, "sex" refers to biologic and physical features, from genetic to physiologic to organismal, that are distinct and usually classified as male or female and largely determined by sex chromosomes. "Gender," however, refers to a psychosocial construct of norms, roles, and interactions typically classified as characteristics of men or women but culminating in an overall identification and self-perception of an individual along a spectrum of gender identities (Manandhar et al., 2018). A person may identify with a gender that is not the same as the biologic sex assigned at birth (transgender); therefore, it is erroneous to equate sex and gender. The study of epilepsy in transgender persons is a nascent area of research, and as such, there is an absence of epidemiologic studies in this population (Johnson and Kaplan, 2017). Therefore, at this point in time for the purposes of literature review, we must rely on the descriptions in clinical studies of the gender of subjects as men or women and operate under the supposition that this also reflects the respective sex. In addition, it is important to note that the term "gender" should not be applied to preclinical animal studies, as the gender identification of a given animal cannot be assessed meaningfully. Therefore, the proper usage of terminology in preclinical studies is to describe the sex of the animal (Torgrimson and Minson, 2005). As our goal in this review is to cohesively discuss both clinical and animal studies of epilepsy, we will use the term "sex" throughout for consistency.

II. Sex Differences in Epilepsies and Comorbidities: Clinical Conditions and Animal Models

The epilepsies, as a whole, are slightly more common in males as compared with females (McHugh and Delanty, 2008). This finding has been replicated in several epidemiologic studies (Hauser et al., 1993; Jallon et al., 1997, 1999, 2001; Dogui et al., 2003; Christensen et al., 2007; Adelow et al., 2009; Hesdorffer et al., 2011a; Fiest et al., 2017), although the difference tends to be small and, in some cases, of borderline or no significance. That said, in a population-based study of the Danish National Hospital Register, arguably the largest sample available, a heightened male prevalence was present in most age groups (Christensen et al., 2007).

A variety of factors have been proposed to account for this difference, including lifestyle and environmental considerations. However, the epilepsies are a diverse group of conditions that differ in symptomology, etiology, and prevalence across sex. In the sections below, we first briefly review the human epidemiologic data and then describe relevant animal models of 1) generalized motor seizures, 2) generalized nonmotor (absence) seizures, and 3) focal seizures. We also refer the reader

to several reviews of sex differences in human epilepsies (McHugh and Delanty, 2008; Savic, 2014).

A. Generalized Motor Seizures

In keeping with the current International League Against Epilepsy operational guidelines for classification of seizures and the epilepsies (Fisher et al., 2017), this category of seizure includes tonic-clonic (formerly known as "grand mal"), tonic, atonic, and myoclonic seizures.

1. Clinical Studies. Some studies have reported a greater rate of generalized tonic-clonic seizures in males (Hauser et al., 1993; Kishk et al., 2019), although other studies have found either no sex difference (Carlson et al., 2014) or increased generalized tonic-clonic seizures in females (Mullins et al., 2007). The variability in these effects across studies may reflect relatively small sample sizes and/or differences in reporting across countries. Similarly, atonic seizures were more common in males in the Epilepsy Phenome/ Genome Project data set (Carlson et al., 2014). This finding differs from those regarding generalized genetic epilepsies, including juvenile myoclonic epilepsy, which has been reported to occur in females at higher rates (Kleveland and Engelsen, 1998; Christensen et al., 2005). Some X-linked syndromes, such as Rett syndrome, are more common in females (Fehr et al., 2011) because of high in utero or early life mortality in males; others, such as seizures in Fragile X syndrome, have been reported to be more common in males (Berry-Kravis et al., 2010). For some other early life epilepsies, such as infantile spasms, there are mixed reports, with some suggesting a higher prevalence in males than in females (Cowan et al., 1989; Luthvigsson et al., 1994; Pellock et al., 2010) and others finding no sex difference (Trevathan et al., 1999; Chen et al., 2004; Jia et al., 2018).

2. Animal Models. The animal models described in this section all display some degree of generalized tonic, clonic, tonic-clonic, or myoclonic seizure activity, although for many of the pharmacological models, the seizure phenotype is highly dose-dependent. We do not include common models of status epilepticus (SE) or other models of what have been referred to as "limbic" seizures in this section but rather discuss them in the context of focal seizures.

a. GABAergic chemoconvulsants. GABA antagonists are one of the most commonly families of chemoconvulsants in epilepsy research. Of this class of drugs, pentylenetetrazole (PTZ) remains a component of the National Institute of Neurological Disorders and Stroke–funded Epilepsy Therapy Screening Program (https://panache.ninds.nih.gov/CurrentModels.aspx). PTZ and the other GABA antagonists, in a dose-dependent manner, evoke absence-like generalized spike-and-wave discharges (SWDs), clonic seizures, and generalized tonic-clonic seizures. Surprisingly, although these drugs (PTZ, picrotoxin, bicuculline, etc.) share a common target, they differ in terms of sex differences (Table 1).

The threshold for picrotoxin-induced generalized tonic-clonic seizures is higher in male than female rats (Pericic et al., 1985; Schwartz-Giblin et al., 1989). Similarly, the latency to picrotoxin-induced akinetic seizures is also longer in males than females (Tan and Tan, 2001). This sex difference is also evident in cats, with female cats displaying a greater frequency of spinal motor neuron discharge than males following picrotoxin (Pericic et al., 1986). In mice, males showed increased sensitivity to picrotoxin compared with females (Pericic et al., 1986). In addition, mortality rates after picrotoxin are greater in male mice than female mice (Pericic et al., 1986) and are elevated in proestrous compared with estrous rats (Tan and Tan, 2001). Sex hormones influence picrotoxin responses, with estradiol treatment increasing sensitivity to picrotoxin in male rats (Pericic et al., 1996). By contrast, testosterone increases susceptibility in females and decreases susceptibility in males (Tan and Tan, 2001). Consistent with the picrotoxin effects, female rats display greater sensitivity to allylglycine-induced convulsive seizures than do male rats (Thomas and Yang, 1991). Thus, in rats, females are more sensitive to picrotoxin than males, a pattern that is reversed in mice.

Interestingly, the threshold for PTZ-evoked clonic seizures is ~10% lower in male rats compared with female rats (Kokka et al., 1992); this finding is somewhat counterintuitive when compared with the data on picrotoxin given that these drugs share a similar mechanism of action (i.e., both are GABA receptor channel blockers). In mice, PTZ responses are greater in females (Medina et al., 2001), and female mice display lower thresholds for response (Min et al., 2013), which is generally consistent with the effects of picrotoxin. Chronic administration of PTZ produces a progressive worsening of seizure severity (i.e., a kindling effect).

TABLE 1
Sex differences in models of epilepsy and seizure induction

Generalized (Motor)	Sex Difference
Picrotoxin	Rats: ♀ > sensitivity
	Mouse: ♀ < sensitivity
Pentlyenetetrazole	Rats: ♀ < sensitivity
	Mouse: ♀ > sensitivity
Bicuculline	Rats: ♀ < sensitivity
Electroshock	Rats, mouse: ♀ > sensitivity
Generalized (Absence)	
WAG/Rij	♀ = ♂
GAERS	♀ = ♂
Focal	
Kainic acid	Rats: ♀ < sensitivity
	Mouse: ♀ > sensitivity
Pilocarpine	Rats: ♀ < sensitivity
	Mice: mixed reports
Kindling	Amygdala kindling: ♀ = ♂
	Hippocampus kindling: ♀ < sensitivity

Male and female rats acquire PTZ kindling at similar rates despite differing sensitivities to acute PTZ (Haeri et al., 2016).

As with PTZ, the threshold for myoclonus, running/bouncing clonus, and tonic hindlimb extension following intravenous bicuculline is lower in male rats compared with females (Finn and Gee, 1994; Pericic and Bujas, 1997). Similarly, males show lower thresholds than females to myoclonus, running/bouncing clonus, and tonic hindlimb extension induced by methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate, a negative allosteric modulator of $GABA_A$ receptors (Finn and Gee, 1994).

b. N-methyl-D-aspartate. Injection of the glutamate receptor agonist N-methyl-D-aspartate (NMDA) in rat pups primed by gestational betamethasone exposure is a common model of infantile spasms, and in this model, sex differences in seizures are not evident (Chachua et al., 2011), a pattern consistent with the clinical literature.

c. Electroshock. As with chemoconvulsant models, maximal electroshock remains a vital part of the screening pipeline for antiseizure medications. The endpoint of this model, tonic hindlimb extension, is particularly relevant for tonic and tonic-clonic seizures.

Female rats display lower maximal electroshock thresholds than do male rats (Woolley et al., 1961; Kokka et al., 1992). Consistent with this finding, a comprehensive analysis by sex in 10 strains found that females displayed, on average, a 10%–15% lower threshold for electroshock-evoked minimal, maximal, and psychomotor seizures (Frankel et al., 2001).

d. Inbred genetic models. Two of the most well established genetically epilepsy-prone rat (GEPR) models of epilepsy are the GEPR-3 and GEPR-9 strains. In response to acoustic stimulation, GEPRs display wild running, bouncing clonus, and, depending on the substrain, tonic extension. Neither the GEPR-3 nor the GEPR-9 substrains display sex differences in the ontogeny of audiogenic seizures (Reigel et al., 1989). However, in both the -3 and -9 strains, the penetrance of the audiogenic phenotype is greater in females than males (Kurtz et al., 2001). Moreover, female GEPR-9s display shorter latencies to seizures and more severe seizure scores than do age-matched GEPR-9 males (Mishra et al., 1988a,b). Inhibitors of adenosine metabolism cause heightened mortality in females compared with male GEPR-9s (Kommajosyula et al., 2016). Similarly, in the GEPR-3 strain, females display shorter latencies to wild running than do matched males (Mishra et al., 1989) and greater anticonvulsant responses to transient receptor potential cation channel subfamily V receptor 1 antagonists than males (Cho et al., 2018).

The Ihara's Epilepsy Rat model displays a striking sex difference in penetrance; though essentially all male rats display tonic-clonic seizures, only ~20% of females show similar responses (Amano et al., 1996). The Noda Epileptic Rat model also displays generalized tonic-clonic seizures with a high penetrance (>95%) in both sexes (Noda et al., 1998).

The El/Suz mouse (Suzuki and Nakamoto, 1982) is a selectively bred polygenic model of generalized epilepsy. These mice display handling-induced seizures and behavioral comorbidities (Bond et al., 2003; McFadyen-Leussis and Heinrichs, 2005) and are hyper-responsive to stress (Forcelli et al., 2007). Though the penetrance of the seizure phenotype does not differ as a function of stress in this strain (Leussis and Heinrichs, 2006), female El mice but not male El mice display increased cell density in the amygdala (Forcelli et al., 2007).

The DBA/1 inbred strain of mice has been used as a model of sudden unexpected death in epilepsy (SUDEP) (Faingold et al., 2010). In response to acoustic stimulation, these animals display tonic seizures, which end with hindlimb extension and respiratory arrest. Although the rate of SUDEP is higher in males than females in clinical populations (Hesdorffer et al., 2011b), the rate of SUDEP in DBA/1 mice is equivalent across sexes (Faingold and Randall, 2013).

e. Other genetic models. The explosion of genetic models of epilepsy over the last three decades makes a thorough characterization of effects difficult for several reasons. First, many models for the same "syndrome" exist, and in many cases, these models differ from one another. Second, differing genetic backgrounds of mice exert strong modulatory effects on seizure phenotype (Hawkins et al., 2016; Kang et al., 2018). Thus, only a few examples are provided below.

In the $Brd2^{+/-}$ model of juvenile myoclonic epilepsy, males show reduced clonic seizure threshold, whereas females have reduced tonic-clonic threshold (Velíšek et al., 2011). Males and females also differ in open-field performance, with males displaying decreased anxiety-like behavior compared with wild-type littermates and females displaying increased anxiety-like behavior (Chachua et al., 2014). In models of Dravet syndrome (e.g., $Scn1a^{+/-}$ mice), there have been reports of females showing greater mortality than males (Niibori et al., 2020) as well as reports of no sex differences (Kang et al., 2018). Therefore, more comprehensive screening for sex differences across genetic models is clearly needed.

B. Generalized Nonmotor Seizures (Absence Seizures)

1. Clinical Studies. Absence seizures are particularly well studied in the context of childhood and juvenile absence epilepsy syndromes. These seizures are characterized by disrupted consciousness and generalized SWDs at a characteristic 3-Hz frequency (Panayiotopoulos, 2008). Absence epilepsies have been reviewed elsewhere (Tenney and Glauser, 2013). Among the available clinical epidemiologic data for

sex differences in epilepsy, those for absence epilepsy are stronger and generally more consistent than other epilepsy types. Specifically, multiple studies have reported childhood absence epilepsy and juvenile absence epilepsy both occur at higher rates in females compared with males (Hauser et al., 1993; Camfield et al., 1996; Waaler et al., 2000; Larsson and Eeg-Olofsson, 2006; Mullins et al., 2007).

2. Preclinical Genetic Models. Sex differences in preclinical absence epilepsy models have been reviewed in detail previously (van Luijtelaar et al., 2014) and are summarized in brief here. Two of the most common models for absence epilepsy are the Wistar Albino Glaxo/Rijswijk (WAG/Rij) strain of inbred rats (van Luijtelaar and Coenen, 1986) and the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) (Vergnes et al., 1982). Both of these strains display a high (near 100%) penetrance of SWDs that have been proposed to model generalized nonconvulsive seizures in humans. In both these strains, unlike typical absence epilepsy, seizures increase in frequency with age. Although human absence epilepsy is more common in females than males, in the WAG/Rij rats, the SWD burden is the same across sexes (Coenen and Van Luijtelaar, 1987).

As in the WAG/Rij, in the GAERS, the penetrance and frequency of SWDs does not differ as a function of sex (van Luijtelaar et al., 2014). Though seizures do not differ, however, comorbid social behavior impairments do; female, but not male, GAERS display deficits in sociability (Henbid et al., 2017). By contrast, the Brown Norway rat strain also displays SWDs, albeit with a different penetrance than the other models. In these rats, the phenotype is more common in males than females (Jando et al., 1995). SWDs discharges have also been reported in wild-type Sprague-Dawley rats, with discharges emerging earlier in females than in males (Pearce et al., 2014).

In addition to the above rat strains, several mouse strains display absence-like seizures, including mice with deletion of the $\alpha 1$ subunit of the $GABA_A$ receptor (Arain et al., 2012), the stargazer mouse (Noebels et al., 1990), and mice lacking *gria4* (Beyer et al., 2008). Though for these latter strains, to the best of our knowledge, sex differences have not been carefully examined, both the stargazer and *gria4* models show absence seizures in both sexes. In $\alpha 1$ subunit of the $GABA_A$ receptor–knockout mice, sex differences depend on genetic background of the strain; in animals on a C57 background, females display a greater incidence of absence seizures than males but the incidence does not differ as a function of sex when the animals are on a DBA/2J background (Arain et al., 2012).

3. Pharmacological Models. Inhibition of cholesterol synthesis in the first month of life produces a permanent alteration in brain development that leads to the emergence of atypical SWDs in rats. Sex differences in this model emerge before puberty, with females

showing two- to threefold increases in SWD duration compared with males (Persad et al., 2002). Consistent with the enhanced sensitivity of females in this model, females show profound downregulation of $\alpha 1$ and $\gamma 2$ subunits of the $GABA_A$ receptor in the somatosensory thalamus, whereas males show no significant change (Li et al., 2007).

The gamma hydroxybutyrate and gammabutyrolactone (GBL) models of absence epilepsy are acute, drug-induced models with cross-species validity (Venzi et al., 2015). In the GBL model, males display a greater seizure burden than females. The increased sensitivity in males is primarily due to longer discharge duration as compared with an increase in the frequency of discharges (Santos et al., 2018).

C. Focal Seizures and Temporal Lobe Epilepsy

1. Clinical Studies. Though some studies have reported greater incidence of focal seizures in males (Hauser et al., 1993), others have reported similar occurrence of focal seizures in both sexes (Manford et al., 1992). This pattern also holds true for temporal lobe epilepsy (TLE), with reports of increased frequency in males (Hauser and Kurland, 1975), no differences as a function of sex in symptomatic TLE (Christensen et al., 2005), and increased cryptogenic cases of TLE in females (Christensen et al., 2005). Focal seizure semiology has also been reported to differ by sex, with one report suggesting that autonomic, visceral, epigastric, and psychic symptoms, as well as déjà vu, are more common in females. Many other symptoms, however, displayed no difference between sexes in this study (Carlson et al., 2014). This profile differed from that observed in a smaller study, which found increased occurrence of isolated auras in females but no other sex differences (Janszky et al., 2004). Beyond semiology, it has also been suggested that the pattern of ictal spread can differ as a function of sex. For example, in a small study incorporating both clinical electroencephalographic and postictal positron emission tomography imaging, both hypometabolism and ictal spread to the ipsilateral frontal cortex were reported to be more common in males, whereas hypometabolism and ictal spread in contralateral temporal cortex was more common in females (Savic and Engel, 1998). Consistent with this observation, a more recent voxel-based morphometry study of 120 persons with TLE reported greater predominance of temporal lobe changes in females and higher rates of frontal lobe changes in males (Santana et al., 2014). Although these findings rely on smaller samples than large epidemiologic studies, they are intriguing and merit follow-up efforts.

Although status epilepticus (SE) is not confined to TLE, we include it in this section because it is one of the most common models used to induce TLE in rodents (see below). Clinical data regarding sex differences in SE are varied, with some studies reporting higher rates of SE

in males compared with females (DeLorenzo et al., 1996; Hesdorffer et al., 1998; Coeytaux et al., 2000; Knake et al., 2001) and others reporting a slight female preponderance (Vignatelli et al., 2003, 2005; Leitinger et al., 2019).

2. Kainic Acid. Kainic acid (KA) is a glutamate receptor agonist and has, for the past three decades, been commonly used in modeling TLE (Ben-Ari et al., 1979). KA, when administered either intracerebrally or systemically, produces repeated "limbic" seizures characterized by clonus of the face and forelimbs as well as loss of postural control. Prolonged seizure activity (i.e., SE) triggered by KA results in the emergence of spontaneous recurrent seizures in the days to weeks following the initial insult.

Female Wistar rats display shorter latencies but fewer limbic seizures after KA administration (Mejias-Aponte et al., 2002). Unlike rats, female C57BL/6 mice display greater sensitivity to KA than males. This is marked by greater mortality, more severe behavioral seizures, and more neurodegeneration (Zhang et al., 2008).

In the intrahippocampal KA model of TLE in mice, males show greater seizure severity, greater hippocampal cell loss, increased hippocampal gliosis, poorer survival, and more severe long-term memory deficits than females (Li and Liu, 2019). Another report shows that across three strains of mice, intrahippocampal KA results in faster onset of spontaneous seizures in females than in males; indeed, in females, there is no obvious latent period (Twele et al., 2016). Electrographic seizure patterns in the model also differ as a function of sex. Males display frequent focal hippocampal discharges in addition to convulsive seizures and nonconvulsive spike-and-wave discharges. By contrast, focal hippocampal discharges are absent in females despite a similar rate of convulsive seizures and spike-and-wave discharges (Twele et al., 2016).

3. Pilocarpine. Like KA, treatment with the muscarinic agonist, pilocarpine (or alternatively lithium and pilocarpine), produces repetitive seizures and SE (Honchar et al., 1983; Turski et al., 1983). As with KA, this model also produces spontaneous recurrent seizures after a period of epileptogenesis (Leite et al., 1990).

In the lithium-pilocarpine model, female rats display longer latencies to seizure onset compared with male littermates (Persinger et al., 1988; Peternel et al., 2009). Similar results have been reported after pilocarpine alone (Mejias-Aponte et al., 2002), with females displaying longer latency to seizures, a lower rate of SE, and a lower rate of mortality. In addition to lower susceptibility to pilocarpine-evoked status, females, as compared with males, display reduced lipid peroxidation and reduced glutathione peroxidase activity after pilocarpine (Peternel et al., 2009).

In mice, there have been mixed reports, with some showing equivalent susceptibility to pilocarpine-induced SE between sexes (Muller et al., 2009; Oliveira et al., 2015) and another showing a greater rate of status in males than in females across the lifespan (Buckmaster and Haney, 2012). Both of these studies reported equivalent survival between sexes. However, it is clear that strain, and even substrain, differences can influence seizure susceptibility, which may account for these disparate findings (Muller et al., 2009).

4. Kindling. Originally described in the late 1960s, kindling is a process by which repeated stimulation of the brain produces permanent reductions in seizure threshold (Goddard et al., 1969). Kindling can be accomplished through electrical stimulation of a variety of brain regions (most commonly amygdala or hippocampus), through repeated chemoconvulsant treatment, and even through repeated electroshock seizures. In electrical kindling models, the threshold current needed to produce an epileptiform pattern of activity that outlasts the stimulation (i.e., an afterdischarge), is a common dependent measure. Across models, behavioral seizure severity is also a common measure (Racine, 1972).

In the amygdala kindling model, female rats display a greater reduction in afterdischarge threshold over the course of kindling than males and display a greater response rate to phenytoin treatment (Ebert et al., 1994; Borowicz et al., 2003). In response to callosal stimulation, female rats kindle at a similar rate to male rats but display a more rapid dendritic reorganization and simplification in the neocortex than males (Teskey et al., 1999). Pharmacological kindling through repeated administration of subconvulsant doses of nicotine (Bastlund et al., 2005) is faster in female rats than male rats, and females show greater kindling-associated oxidative stress responses throughout hippocampus, cortex, and striatum (Gomes et al., 2013). Unlike nicotine kindling, repeated ethanol withdrawal, which also produces a kindling-like phenomenon, is only evident in males. Interestingly, the protective effect in females in the repeated ethanol withdrawal model is independent of ovarian hormones (Veatch et al., 2007). Sex differences are also apparent in epileptogenic seizures produced from hippocampal kindling, with males exhibiting a significantly faster progression to a fully kindled state as well as greater behavioral and electrographic seizure activity (Reddy et al., 2019).

Corneal (electroshock) kindling in mice is an alternative to traditional kindling methods (e.g., amygdala or hippocampal kindling). In this model, kindling is induced by repeated transcorneal minimal electroshock seizures (Matagne and Klitgaard, 1998). Although the ultimate rate of kindling in this model does not differ as a function of sex, female mice are more sensitive to the initial stimuli and show an early emergence

of behavioral seizures than do males (Potschka and Loscher, 1999).

D. Comorbidities

1. Psychiatric and Affective Disorders.

a. Clinical studies. Psychiatric comorbidities are common in epilepsy (Josephson and Jetté, 2017) and estimated to occur in 25%–50% of patients (LaFrance et al., 2008), although higher incidence has been reported, with the highest prevalence observed in association with TLE (Bragatti et al., 2010) and drug-resistant epilepsy (depression = 55%; anxiety = 28.7%) (Kwon and Park, 2014; Jansen et al., 2019). The incidence of depression in epilepsy has been reported in 11%–80% of patients compared with 4.9%–17% in the general population (LaFrance et al., 2008). Similarly, the incidence of anxiety is higher in patients with epilepsy (15%–20%) (Brandt et al., 2010) compared with 5%–7% in the general population (LaFrance et al., 2008; Kanner, 2011; Kwon and Park, 2014; Brandt and Mula, 2016; Salpekar and Mula, 2018). It is important to note that the incidence of psychiatric comorbidities is even more prevalent in epilepsy as compared with other chronic illnesses such as cancer, diabetes, or asthma (Hermann et al., 1996). Furthermore, patients with psychiatric disorders, such as depression, are at a higher risk for developing epilepsy (Josephson and Jetté, 2017). Therefore, it is thought that psychiatric disorders and epilepsy may share a common underlying pathophysiological mechanism (Kanner, 2003).

Despite the high incidence of psychiatric comorbidities in epilepsy and the well established sex differences in anxiety and depression in the general population (Altemus et al., 2014), few studies have investigated sex differences in psychiatric comorbidities in epilepsy. The incidence of anxiety and depression is twice as common in females than in males in the general population (Altemus et al., 2014), and this is also true for patients with epilepsy. A retrospective study found that the incidence of depression was 15.5% in males and 26.8% in females with epilepsy (Chan et al., 2015). Similarly, anxiety disorders were found in 9.65% of males and 17.4% in females with epilepsy (Chan et al., 2015). These few studies suggest that there may be sex differences in psychiatric comorbidities in epilepsy. This represents a significant concern for epilepsy treatment given that psychiatric comorbidities are associated with worse epilepsy outcomes (Josephson and Jetté, 2017). Further studies are required to understand the extent of sex differences in psychiatric comorbidities in epilepsy and the impact on epilepsy outcomes.

b. Animal models. Similar to clinical studies, the majority of studies investigating sex differences in psychiatric disorders in experimental epilepsy models have focused solely on males, leaving us with limited information regarding sex differences in psychiatric comorbidities in preclinical epilepsy models. Existing studies suggest that there are sex differences in seizures in experimental animals as highlighted above [for review, see Scharfman and MacLusky (2006)]. In the few studies that have examined associated psychiatric comorbidities, increased anxiety- and depression-like behaviors have been observed in numerous experimental epilepsy models, including both genetic (Aguilar et al., 2018) and acquired epilepsy models (Mazarati et al., 2008; Becker et al., 2015; Hooper et al., 2018; Zeidler et al., 2018). However, none of these studies to date have demonstrated sex differences in anxiety- or depression-like behaviors in epilepsy models, although only a few have looked. In fact, Zeidler et al. (2018) state that no significant sex differences in depression- or anxiety-like behaviors were observed in chronically epileptic mice in the intrahippocampal KA model of epilepsy but provide the disclaimer that their experiments were not designed to test for sex differences. One study demonstrated subtle sex differences in behavior in a genetic epilepsy model, including differences in head dips off the open arms, an effect primarily driven by females (Aguilar et al., 2018). Some outcome measures also showed a main effect of sex but not a strain-by-sex interaction (Aguilar et al., 2018). Although overall these findings do not support sex differences in psychiatric comorbidities in epilepsy by using traditional behavioral tests and assessments of standardized outcome measures, there is an increased appreciation that males and females may exhibit different behaviors in these tests, and care should be taken in how to properly interpret these data (Shansky, 2018). Furthermore, the limited information on this subject is evident from this review, and additional studies are required for a better understanding of the mechanisms contributing to sex differences in comorbid psychiatric disorders in epilepsy.

2. Reproductive Endocrine Disorders and Sexual Dysfunction.

a. Clinical studies. Both men and women with epilepsy are at increased risk for reproductive endocrine disorders compared with the general population (Herzog, 2008; Koppel and Harden, 2014). Up to 90% of men with epilepsy may present semen abnormalities, including low sperm count or abnormal sperm morphology or motility, and a population-based study quantified a 40% lower birth rate in men with epilepsy (Taneja et al., 1994; Artama et al., 2004; Herzog, 2008). It should be noted, however, that lower birth rates can be affected by many social factors (Mameniškienė et al., 2017) and are not necessarily a direct representation of fertility. Furthermore, changes in semen quality and sperm count are highly associated with the use of certain enzyme-inducing ASDs, such as valproic acid, carbamazepine, and oxcarbazepine (Roste et al., 2003; Isojarvi et al., 2004; Hamed et al., 2015; Ocek et al., 2018) and may thus be independent of epilepsy-induced

changes. In women, common ailments include anovulatory menstrual cycles, oligomenorrhea (irregular cycle periodicity and infrequent menstruation), polycystic ovaries, and polycystic ovarian syndrome (PCOS) (Cummings et al., 1995; Bauer et al., 1998, 2000a,b; Morrell et al., 2002; Herzog et al., 2003a; Lofgren et al., 2007). Specifically, an estimated 10%–25% of women with TLE are diagnosed with PCOS compared with 4%–6% of the general population (Webber et al., 1986; Herzog and Schachter, 2001), and another 12% of women with TLE develop hypothalamic amenorrhea compared with 1.5% of the general population (Herzog et al., 1986b). Although much focus has been placed on these comorbidities in the context of TLE and other focal epilepsies, several studies have also documented these comorbidities in women with generalized epilepsy disorders (Morrell et al., 2002; Lofgren et al., 2007). In addition, although ASD treatment is highly associated with development of reproductive endocrine problems in both men and women (for examples, see Isojarvi et al., 1993, 2004; Murialdo et al., 1997; Harden, 2005a; Ocek et al., 2018), several lines of evidence indicate that seizure activity itself is a primary driver of these comorbid issues. In particular, multiple groups have documented a pattern in which women with TLE and seizures originating on the left side of the brain (i.e., left-sided TLE) show increased rates of PCOS, whereas women with TLE seizures originating in the right hemisphere (right-sided TLE) exhibit higher rates of hypothalamic amenorrhea (Herzog, 1993; Drislane et al., 1994; Herzog et al., 2003a,b; Kalinin and Zheleznova, 2007; Quigg et al., 2009). The findings indicate that distinct reproductive endocrine disorders may be related to the area of the brain primarily affected by seizure activity and suggest that specific neural pathways act as substrates to drive altered endocrine functions.

Investigations of the pathophysiology of reproductive endocrine comorbidities of epilepsy have naturally focused on the hypothalamic-pituitary-gonadal (HPG) axis, which links the brain to the control of fertility and reproduction in all mammalian species. Gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus release the GnRH peptide to stimulate gonadotrope cells of the anterior pituitary to produce the gonadotropin hormones, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). In both sexes, LH and FSH act on the gonads to induce gametogenesis and production of sex steroid hormones. LH and FSH can be readily detected in the peripheral bloodstream. Therefore, measurements of these hormones are commonly used as surrogate readouts of activity at the hypothalamic level of the HPG axis in humans, including in patients with epilepsy. An important feature of both LH and FSH is that these hormones are released in intermittent boluses, or pulses. Therefore, the pattern of pulsatility of these hormones as well as changes in mean basal levels are important indicators of disruptions to the hypothalamic-pituitary axis in people with epilepsy. In brief, several studies of both women and men with epilepsy have documented changes in the frequency of LH pulses (Meo et al., 1993; Drislane et al., 1994; Quigg et al., 2002; Herzog et al., 2003a), although others did not find a difference in LH pulsatility (Murialdo et al., 1995). Furthermore, impaired LH response to exogenous GnRH treatment is also commonly observed in both women and men with epilepsy (Herzog et al., 1982; Bilo et al., 1988; Murialdo et al., 1995), along with changes in mean LH or FSH levels (Bilo et al., 1988; Meo et al., 1993; Morrell et al., 2002; Herzog et al., 2003a). These changes in pituitary gonadotropin levels suggest that activity at the hypothalamic level, as well as further upstream in the brain, is altered such that pituitary response to GnRH and/or gonadotropin synthesis and release are commonly affected in both women and men with epilepsy, particularly TLE. These pathophysiological changes are challenging to assess in human patients but are beginning to be addressed in animal models.

As described in further detail below, the sex steroid hormones estradiol, progesterone, and testosterone exert strong effects on neural function with major implications for seizure activity. Therefore, disruptions to normal patterns of sex steroid synthesis and release can have profound impacts on seizure control. In this regard, it is notable that both women and men with epilepsy commonly show changes in circulating levels of these hormones. Although these changes often arise as side effects of treatment with ASDs, particularly valproic acid (Isojarvi et al., 1993, 2004; Harden, 2005a; Roste et al., 2005; Isojarvi, 2008; Ocek et al., 2018), epilepsy and seizure activity have also been independently associated with changes in hormone levels, particularly regarding testosterone. In men, for example, decreased testosterone has been documented in men with TLE, with recovery to normal levels often seen after temporal lobe resection and improved seizure control (Bauer et al., 2000c, 2004). It should be noted, however, that not all studies have found changes in testosterone in male patients (Mikkonen et al., 2004). Furthermore, testosterone may be lower in men with TLE compared with men with seizure foci outside of the temporal lobe (Bauer et al., 2004), suggesting specific etiologies related to downstream hormone changes dependent on the neural circuits primarily affected by the seizure activity. Conversely, elevated testosterone is often observed in women with TLE (Herzog et al., 2003a) or idiopathic generalized epilepsy (Lofgren et al., 2007). Particularly interesting findings were that women with left-sided TLE showed higher testosterone compared with women with right-sided TLE (Herzog et al., 2003a) and that women with right-sided TLE showed significantly decreased estradiol levels compared with women with left-sided TLE (Herzog et al., 2003b).

These results further suggest that downstream hormone changes can reflect impairments and disruption to specific neural circuits.

Another pituitary hormone critical to proper regulation of HPG axis function is prolactin (PRL), which is produced under the control of dopaminergic synaptic inputs projecting from the hypothalamic arcuate nucleus into the posterior pituitary. This direct synaptic connection is thus anatomically poised to provide an uninterrupted link between seizure activity in the brain and altered pituitary PRL release. Because elevated PRL levels suppress the GnRH-LH axis, hyperprolactinemia can be another mechanism of impaired reproductive endocrine function in patients with epilepsy. With respect to changes in PRL in women with epilepsy, some studies have documented increased levels (Bilo et al., 1988), whereas others have not found baseline differences (Dana-Haeri et al., 1984; Herzog et al., 2003a). Acute postictal increases in prolactin have also been reported in women with epilepsy (Collins et al., 1983; Dana-Haeri et al., 1983; Pritchard et al., 1985). Approximately 10% of men with TLE may show signs of hyperprolactinemia (Herzog et al., 1986a), although other studies have documented acute increases in PRL immediately after seizure activity without a change in mean levels (Abbott et al., 1980; Collins et al., 1983; Dana-Haeri et al., 1983; Pritchard et al., 1985; Quigg et al., 2002). Still other studies documented similar postictal increases in PRL, but the sex of the subjects was not specified (Trimble, 1978; Sperling et al., 1986). Therefore, seizure-associated changes in PRL levels may be seen in both men and women with epilepsy, particularly TLE. Indeed, acute measurement of serum PRL postictally has been suggested as a possible diagnostic tool to distinguish epileptic seizures from psychogenic nonepileptic seizures (Chen et al., 2005a).

In addition to impairments of reproductive endocrine systems, many men and women with epilepsy also complain of sexual dysfunction. Although some studies have failed to find higher rates of such complaints in people with epilepsy (Jensen et al., 1990), others have documented a variety of symptoms [for comprehensive reviews, see Harden (2005b), Luef and Madersbacher (2015)]. For example, several studies have documented hyposexuality, decreased sexual drive, and lower arousability in substantial proportions of men and women with epilepsy (Demerdash et al., 1991; Christianson et al., 1995; Murialdo et al., 1995; Morrell and Guldner, 1996; Daniele et al., 1997; Duncan et al., 1997; Silveira et al., 2001; Baird et al., 2003; Kuba et al., 2006; Henning et al., 2019). Furthermore, deficits in arousability, genital blood flow, and erectile function appear to reflect physiologic impairments (Morrell et al., 1994; Guldner and Morrell, 1996; Morrell and Guldner, 1996). Disproportionate rates of sexual dysfunction observed in patients with right-sided TLE compared with left-sided TLE also suggest primary neural components

drive these comorbidities (Daniele et al., 1997; Herzog et al., 2003b) in addition to more frequent observations of temporal lobe seizure foci in women with epilepsy that exhibit sexual dysfunction compared with patients that do not exhibit the comorbidity (Demerdash et al., 1991). It should be noted, however, that sexual dysfunction can also be associated with ASD treatment (Herzog et al., 2005; Luef and Madersbacher, 2015), although some studies have not observed such an effect (Henning et al., 2019). In summary, various presentations of sexual dysfunction are commonly observed in both men and women with epilepsy.

b. Animal models. The investigation of reproductive endocrine comorbidities of epilepsy is one of the few areas of preclinical research in which the studies investigating female animals far outnumber those examining males. This is primarily due to the commonalities between the estrous cycle of female rodents and menstrual cycle in humans [Fig. 1; see also Fig. 2 in Scharfman and MacLusky (2014b)] such that disruptions to the estrous cycle are useful experimental models with which to investigate the pathophysiological mechanisms of reproductive endocrine disorders in epilepsy. In this regard, female rodents tested in various models of epilepsy have also shown high propensity for developing disrupted estrous cycles and other indicators of reproductive endocrine dysfunction. For example, female Wistar rats tested in the intrahippocampal KA model of TLE were found to spend decreased time in the stages of proestrus and estrus and increased time in metestrus (Amado et al., 1987). In recent studies examining female mice in a similar intrahippocampal KA model, prolongation of estrous cycle period, typically characterized by more time spent in diestrus, was observed in the majority of mice (Li et al., 2016, 2018). Furthermore, these differences did not appear for at least 6 weeks after the KA injection, indicating that it was the chronic epileptic condition, and not the acute effects of KA excitotoxicity, that drove the cycle disruption (Li et al., 2016). In studies employing the pilocarpine post-SE model of TLE, Wistar rats showed more time spent in diestrus from 2 to 6 weeks after injection (Amado and Cavalheiro, 1998), and Sprague-Dawley rats showed a variable response, with one-third showing immediate cessation of cycling after pilocarpine-induced SE, one-third developing irregular cycles over time, and the remaining animals maintaining regular cyclicity (Scharfman et al., 2008). Disrupted estrous cycles have also been described in pilocarpine-treated mice (Fawley et al., 2012). Lastly, amygdala kindling models have also been linked with estrous cycle disruption, producing increased time in estrus and/or decreased time in diestrus in Sprague-Dawley and Wistar rats in some studies (Edwards et al., 1999d; Pan et al., 2013) and cycle period lengthening without induction of persistent estrus in others (Hum et al., 2009). In summary, these studies indicate that disruptions to the
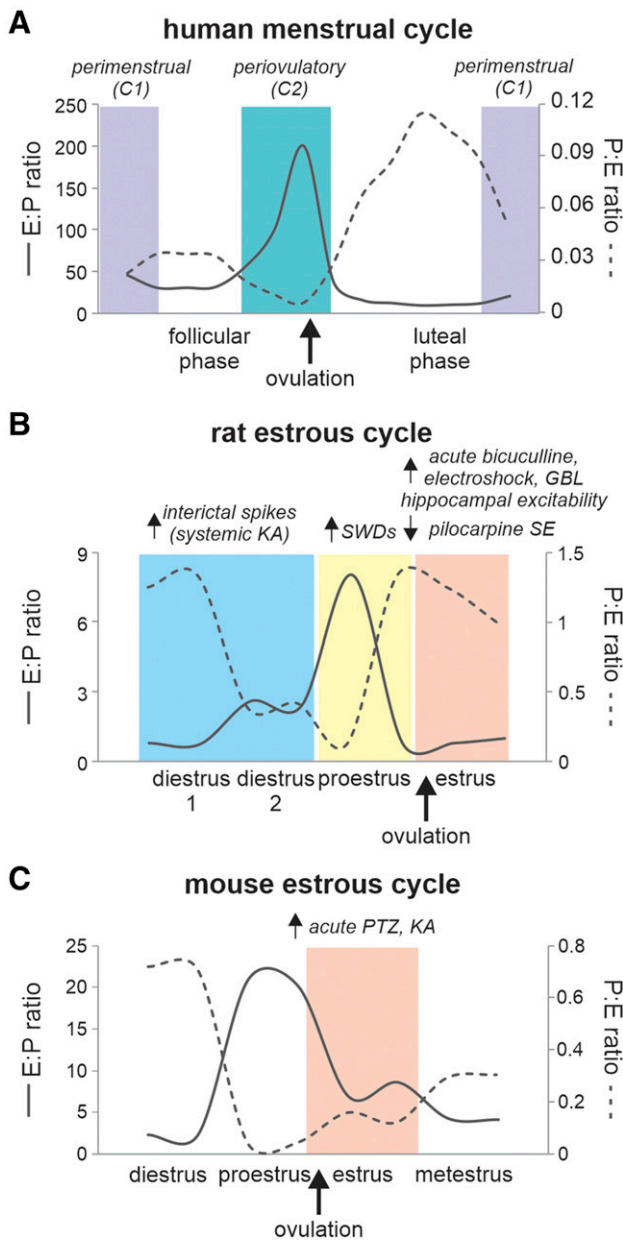
Fig. 1. Changes in seizure susceptibility associated with fluctuations in ratios of estradiol (E, pg/ml) and progesterone (P, ng/ml) levels in the human menstrual and rodent estrous cycles. (A) E:P (solid line) and P:E (dashed line) ratios in the human menstrual cycle and associated catamenial seizure clustering. Hormone data are adapted from Thorneycroft et al. (1971). See section *III. Catamenial Epilepsy: Fluctuations in Seizure Occurrence across the Menstrual Cycle* for discussion of catamenial seizure clustering. (B) E:P and P:E ratios in the rat estrous cycle. Hormone data are adapted from Smith et al. (1975). (C) E:P and P:E ratios in the mouse estrous cycle. Hormone data are adapted from Walmer et al. (1992). See section *IV.B.1. Estrous Cycle–Associated Changes in Seizure Susceptibility* for discussion of changes in seizure susceptibility associated with the estrous cycle in rats and mice.

estrous cycle are commonly observed in rodent models of epilepsy.

Changes in pituitary gonadotropin (LH and FSH) release or content, as observed in human patients, have also been documented in several rodent models of chronic epilepsy and acute seizure induction in both males and females (Bhanot and Wilkinson, 1982; Fujii

et al., 1984; Amado and Cavalheiro, 1998), although changes in LH pulsatility patterns have not yet been tested in either sex. These changes appear to be at least partially due to paroxysmal activity in limbic structures, as several studies have shown changes in LH and/or FSH release following electrical stimulation of hippocampus and/or amygdala in both male and female animals (Velasco and Taleisnik, 1969; Gallo et al., 1971; Kawakami et al., 1973a,b).

The major advantage of animal models is increased direct access to the hypothalamic components of the HPG axis, particularly the GnRH neurons. In this regard, earlier studies limited to immunocytochemical staining for the GnRH peptide yielded conflicting results, with decreased GnRH immunoreactivity found in female rats after intra-amygdalar injection of KA or systemic pilocarpine induction of SE (Amado et al., 1993; Friedman et al., 2002) but no difference in GnRH staining in pilocarpine-injected female mice (Fawley et al., 2012). A recent study, however, provided the first direct documentation of functional changes in GnRH neuron activity and excitability, showing that in female mice, the rate and pattern of GnRH neuron firing was altered in the intrahippocampal KA model of TLE and that the direction of change in KA-injected mice compared with controls shifted from diestrus to estrus. Specifically, GnRH neurons from KA-injected mice showed higher firing on diestrus and suppressed firing on estrus, and these differences were most profound in the mice that concomitantly showed prolonged cycle length. Moreover, male mice tested in the same model showed a more modest effect, with only a subset of GnRH neurons (those with cell bodies in the medial septum) displaying elevated firing activity (Li et al., 2018) (Fig. 2).

As in human patients with epilepsy, several animal studies have documented changes in circulating levels of the sex steroids in preclinical models of TLE, with variations dependent on the species and specific epilepsy induction model (Table 2). For example, amygdala-kindled female Wistar rats that became acyclic developed higher baseline estradiol levels along with higher estradiol and testosterone levels following progesterone treatment (Edwards et al., 1999d). Decreased progesterone levels were also documented in female rats tested in the intrahippocampal KA model of TLE (Amado et al., 1987). Sprague-Dawley rats treated in the pilocarpine post-SE model of TLE, however, did not show differences in estradiol or progesterone when measured on diestrus but did show elevated testosterone (Scharfman et al., 2008), whereas Wistar rats tested in the pilocarpine model displayed increased estradiol and decreased progesterone (Amado and Cavalheiro, 1998). By contrast, in a recent study using the intrahippocampal KA model of TLE in female mice, the pattern of change in estradiol or progesterone levels as measured 2 months after KA injection changed with
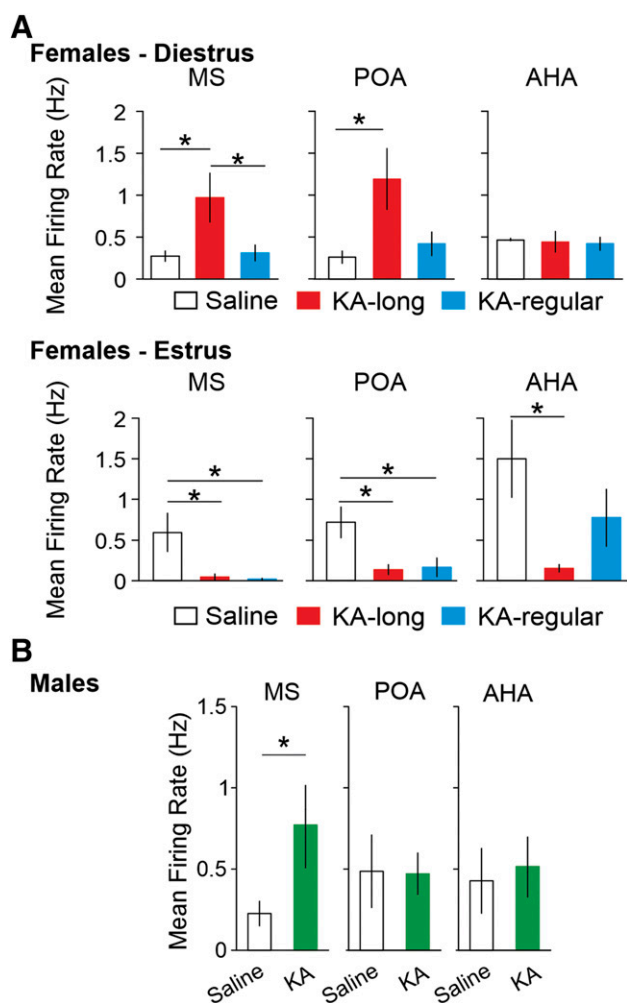
Fig. 2. Sex differences in impacts of epilepsy on GnRH neuron firing activity. Mean ± S.E.M. for mean firing rate of GnRH neurons from saline-injected controls (white bars) and KA-injected female mice recorded on diestrus and on estrus (A) and male mice (B). KA-injected females are divided into KA-long (red bars) and KA-regular (blue bars) groups based on estrous cycle length (long = ≥7 days, regular = 4–6 days). Cells are classified based on anatomic location of somata in medial septum (MS), preoptic area (POA), and anterior hypothalamic area (AHA). Adapted from Li et al. (2018).

estrous cycle stage and depended on whether the mice developed prolonged, disrupted estrous cycles. Specifically, progesterone levels were suppressed in KA-injected mice that developed long cycles but not in

KA-injected mice that retained normal cycle periodicity, and this pattern was observed on both diestrus and estrus. Regarding estradiol, however, no differences were observed between groups in levels of this hormone on diestrus, but KA-injected mice, independent of estrous cycle period, displayed elevated levels compared with controls (Li et al., 2018). With respect to male animals, in one study, amygdala-kindled rats appeared to show increased testosterone, but acute maximal electroshock-induced seizures transiently decreased testosterone levels (Edwards et al., 1999a). These findings suggested that focal and generalized seizures may have different downstream impacts on testosterone production and/or metabolism. Male mice treated in the intrahippocampal KA model of TLE, however, did not show a change in testosterone levels when measured 2 months after KA injection (Li et al., 2018). So far, PRL has received less attention in studies of animal models of epilepsy, with conflicting reports of higher PRL levels described in amygdala-kindled female rats (Edwards et al., 1999d), decreased levels observed in male rats following acute electrical stimulation in the amygdala (Kawakami et al., 1973a), and no change observed in pilocarpine-treated female rats (Scharfman et al., 2008). In summary, although the specific directions and degrees of change in sex steroid levels may display species differences and vary across epilepsy models, these effects may have important implications for understanding and interpreting features of epileptiform activity that are both distinct between and common to male and female animals.

To date, sexual dysfunction in animal models of epilepsy has received less investigatory emphasis than reproductive endocrine disorders. In one study, male cats displayed reduced sexual behavior for at least 6 months following intra-amygdalar injection of aluminum hydroxide to induce focal epilepsy (Feeney et al., 1998). In studies using the pilocarpine post-SE model of TLE in Wistar rats, males showed longer latency to initiate sexual activity and reduced numbers of mounts and intromissions, and females displayed reduced sexual interest in a male rat (proceptivity) and suppressed receptivity (readiness to allow copulation as exhibited by the lordosis response) following estradiol

TABLE 2
Summary of observed changes in circulating sex steroid levels in various rodent models of TLE

TLE model	Species	Estradiol	Progesterone	Testosterone
Amygdala kindling				
Male	Rats	–	–	↑ Edwards et al., 1999a
Female	Rats	↑ Edwards et al., 1999c	–	–
Systemic pilocarpine				
Female	Rats	↑ Amado and Cavalheiro, 1998 ↔ Scharfman et al., 2008	↓ Amado and Cavalheiro, 1998 ↔ Scharfman et al., 2008	↑ Scharfman et al., 2008
Intrahippocampal KA				
Male	Mice	–	–	↔ Li et al., 2018
Female	Rats	–	↓ Amado et al., 1987	–
	Mice	↑ (Estrus) Li et al., 2018	↓ (Diestrus and estrus) Li et al., 2018	–

and progesterone priming treatment sufficient to induce estrus in control rats (Andersen et al., 2012; Alvarenga et al., 2013). Therefore, in line with the common observation of hyposexuality in people with epilepsy, animal models also appear to display at least some degree of sexual dysfunction in both sexes. Given the increased feasibility of measuring sex behavior in rodent models of epilepsy in the absence of confounding antiepileptic drug treatment, further investigation in this area is likely to yield insights into physiologic links between epileptiform and seizure activity and altered sexual function.

3. *Cognitive and Learning Deficits.*

a. Clinical studies. A large body of work has accumulated evidence for functional sex differences in human cognition and various forms of learning and memory, at least when assessed on average, although the specific features and underlying mechanisms are a matter of ongoing debate (Andreano and Cahill, 2009; Hamson et al., 2016; Asperholm et al., 2019). As many forms of epilepsy reflect seizure activity and, in some cases, pathologic damage to brain regions that participate in these cognitive functions, it is not surprising that cognitive and learning deficits are major comorbidities of epilepsy. Comprehensive discussion of the links between epilepsy and cognition across the lifespan, and the various forms of memory and neurocognitive function that can be affected, are beyond the scope here and are available in many recent reviews (Bell et al., 2011; Lin et al., 2012; Helmstaedter and Witt, 2017; Semple et al., 2019). It should be noted as well that there is growing appreciation for a comorbid relationship between epilepsy and Alzheimer disease, as discussed in other recent reviews (Chin and Scharfman, 2013; Sen et al., 2018). Assessing sex differences in the relationship between epilepsy and Alzheimer disease, however, is somewhat complicated by differential incidence and progression of Alzheimer disease and other forms of aging-related dementia in women and men (Hebert et al., 2013; Pike, 2017; Ferretti et al., 2018). The documented increase in risk for Alzheimer disease in postmenopausal women compared with age-matched men (Ferretti et al., 2018), however, underscores the need to identify causal mechanisms that produce sex-specific outcomes as well as features common to both sexes. Such insights will require studies aimed at understanding the mechanisms of sex differences in epilepsy and Alzheimer disease separately as well as the comorbid interactions between the two neurologic disorders in the context of both aging-dependent and -independent changes. In this section, we will focus on those studies that suggest sex differences in the presentation of cognitive comorbidities in children and adults, without the confounds of dementia-related disorders that could lead to cognitive impairment independently of the effects of epilepsy and seizures. Furthermore, we will focus particularly on those studies

that specifically assessed and described whether a sex difference was observed.

Children with epilepsy are at particular risk for cognitive impairments and learning deficits, and sex can play a role in the presentation and features of these comorbidities. For example, in a study of children and adolescents with intractable epilepsy examining impairments in episodic verbal and visual memory, girls exhibited better delayed recall of stories and learning of a word list, but no sex difference was observed in performance on delayed recall of words or in visual tasks (Smith et al., 2009). Both boys and girls with epilepsy exhibit higher rates of autism, intellectual disability, and attention-deficit/hyperactivity disorder compared with children in the general population (Socanski et al., 2013; Aaberg et al., 2016; Williams et al., 2016). Although some studies document higher numbers of boys with epilepsy affected than girls (Aaberg et al., 2016), these findings may reflect an underlying male bias of these neurodevelopmental disorders in the general population, independent of specific effects of epilepsy and seizure activity (Rucklidge, 2010; Baron-Cohen et al., 2011; May et al., 2019). Other studies, however, have found similar rates of attention-deficit/hyperactivity disorder in both boys and girls with epilepsy [for review, see Williams et al. (2016)]. In addition, an analysis of data on children with epilepsy from the National Longitudinal Survey of Children and Youth in Canada found a slight preponderance of learning disabilities in girls (Prasad et al., 2014). The outcomes of epilepsy in children are also of interest given the increased potential for plasticity and functional compensation early in development. In this regard, it is interesting that patients who exhibit left-sided temporal lobe seizures by 1 year of age, when examined in early adulthood, can display sex-specific outcomes in cognitive impairment, and this effect is influenced by the lateralization of language function. Specifically, males were found to show generalized cognitive impairment of both verbal and nonverbal functions, independent of whether the left hemisphere remained dominant for speech. This effect contrasted with the outcomes in female patients examined, in which only some verbal functions were impaired, but nonverbal functions were largely unaffected if the left hemisphere remained dominant for speech (Strauss et al., 1992). Altogether, these and other studies suggest that neurobehavioral outcomes are prominent comorbidities in children with epilepsy, that these comorbidities can exhibit sex-specific features or prevalence, and that the long-lasting outcomes later in life can continue to exhibit sex differences in presentation and/or underlying mechanism.

Neuropsychological testing of adults with epilepsy has yielded interesting insights into sex differences in various forms of learning and memory, both in relation to epilepsy and to human cognition in general. To a large

extent, and perhaps as to be expected, most of the focus in this domain has been in studying patients with TLE. In general, most studies indicate that women with epilepsy typically display better verbal memory than men with epilepsy (Berenbaum et al., 1997; Helmstaedter et al., 1999, 2004; Berger et al., 2017, 2018), although it should be noted that not all studies show a sex difference (Davies et al., 1998). After surgical resection of epileptogenic tissue of the anterior temporal lobe, women often continue to display superior performance (Trenerry et al., 1995; Berenbaum et al., 1997; Davies et al., 1998; Bengtson et al., 2000; Bjornaes et al., 2005; Berger et al., 2017). Overall, therefore, it appears that women in general display better performance on verbal learning and memory tests, and this sex difference confers a degree of resilience to decline in these measures in the face of epilepsy. Similarly, women without epilepsy, women with TLE, and women with generalized epilepsy all show better delayed face recognition memory when compared with men in these respective groups (Bengner et al., 2006). With regard to spatial memory as measured by hippocampal activation during an object location memory task in a virtual environment, it appears that in healthy controls, women display a left-lateralized activation pattern, whereas men display a right-lateralized pattern (Frings et al., 2006). In patients with TLE, by contrast, the lateralization pattern in this same task appears determined by the side of the seizure focus, not sex (Frings et al., 2008). Taken together, these studies suggest that various forms of memory and/or the organization of underlying neural substrates for these functions are changed in epilepsy, with impacts and features reflecting the sex of the patient.

b. Animal models. As would be expected from the observations of prominent cognitive comorbidities in patients with epilepsy, similar changes have also been well documented in various animal models of epilepsy. For a recent comprehensive review of behavioral comorbidities in preclinical models of epilepsy, see Holmes (2015). As is the case regarding other aspects of preclinical studies of epilepsy and seizure activity, however, few studies have specifically investigated the potential impact of sex as a biologic variable on these measures. Two recent studies of different genetic mouse models, however, have yielded some interesting sex-specific differences. First, the EL mouse, which displays autism-like behavioral features and comorbid epilepsy, appears to respond to ketogenic diet treatment in a sex-specific manner, with females showing greater improvement in sociability and stronger reduction in stereotyped repetitive self-grooming behavior (Ruskin et al., 2017). In addition, a recent study investigating heterozygous potassium voltage-gated channel subfamily Q member 2 knockout mice, which do not show spontaneous seizures but do show a reduced seizure threshold, observed male-specific increases in compulsive marble-burying and social dominance (Kim et al., 2020). By contrast, a recent study investigating both males and females in the GEPR-3 rat model documented that these rats display impaired novel object recognition, and a greater proportion of animals failed to explore the objects compared with Sprague-Dawley control counterparts, but it did not observe a sex difference in this effect (Aguilar et al., 2018).

With respect to pharmacological models of seizure induction and epilepsy, one study examining the behavioral consequences of KA-induced SE in newborn (4–6 days of age) rat pups documented that males showed stronger deficits in spatial learning as tested in a Barnes maze at 16–19 days of age than did the corresponding female pups (Akman et al., 2015). Conversely, however, in another study in which newborn rat pups were exposed to subconvulsive doses of domoic acid [which can lead to behavioral abnormalities suggestive of focal seizures as well as a lowering of seizure threshold (Gill et al., 2010)], the female, but not male, rats showed impaired spatial learning in a Morris water maze (Doucette et al., 2007). A series of studies all testing C57BL/6J mice have also documented varying effects. For example, one study of adult mice tested 2 months after pilocarpine-induced SE showed male-specific impairments in object exploration and reduced immobility in a forced swim test but no sex differences on other parameters, including open-field exploration and object recognition (Oliveira et al., 2015). Another recent study examining the short-term effects (within 5 days) of systemic KA induction of SE documented that male mice showed worse performance than females on novel object recognition, but it should be noted that the males also showed increased seizure susceptibility in response to the KA injection (Li and Liu, 2019). Therefore, the sex differences in behavioral consequences may simply reflect sex differences in the initial seizure severity. Conversely, direct unilateral injection of KA into the hippocampus can produce deficits in spatial memory in mice in a manner that does not appear to differ between males and females (Zeidler et al., 2018).

Although most investigations have focused on the neurobehavioral consequences of epilepsy and seizure activity, one recent study provides an intriguing potential sex-specific link between underlying performance on the Morris water maze in control conditions and subsequent seizure susceptibility. When Wistar rats were tested first in the Morris water maze and then challenged with PTZ seizure induction, the female rats displayed a correlation between maze performance and later seizure susceptibility that was absent in the males, with better maze performance correlating with reduced subsequent seizure susceptibility (Haeri et al., 2016). Altogether, only a few studies of animal models of epilepsy document sex differences in cognitive comorbidities, and others document the lack of a sex difference in a given behavioral parameter tested. However, there

have not been sufficient studies performed to suggest that sex differences in cognitive comorbidities in animal models are largely absent. Systematic inclusion of both male and female animals, and formal statistical testing for the presence of any sex differences, would greatly help to improve our understanding of which cognitive comorbidities, if any, show a sex-specific predominance.

4. Migraine and Others.

a. Clinical studies. In addition to the prominent psychiatric, reproductive, and cognitive comorbidities described in the previous sections, people with epilepsy are also at increased risk for a variety of other somatic conditions. Among these, urinary tract infection, hypothyroidism, and migraine are in the top 10 conditions for women, whereas cancer, coronary artery disease, and gastroesophageal reflux disease are in the top 10 for men (Wilner et al., 2014). Of these, the best studied is migraine, which also exhibits a higher prevalence in women in the general population worldwide (Stewart et al., 1992, 2008; Sakai and Igarashi, 1997; Vetvik and MacGregor, 2017).

The relationship between migraine and epilepsy is of particular interest for several reasons. First, both are episodic disorders (Haut et al., 2006; Rogawski, 2012); second, migraine is the most common type of headache observed in patients with epilepsy (Mainieri et al., 2015); third, the presence of comorbid migraines can negatively impact the prognosis of becoming seizure-free (Velioğlu et al., 2005); and fourth, many ASDs are effective as prophylactic treatments for migraines, producing a reduction in the number of episodes (Sprenger et al., 2018). Of note, migraine is far more commonly observed in patients with epilepsy than epilepsy is observed in migraine sufferers, simply because migraine on its own is far more prevalent (Bigal et al., 2003). Several studies have documented higher rates of migraine in people with epilepsy (for review, see Bigal et al., 2003; Haut et al., 2006; Rogawski, 2012), although there are exceptions (Brodtkorb et al., 2008; Tonini et al., 2012). Intriguingly, however, the sex difference in migraine prevalence observed in the general population appears to be largely equalized in the face of epilepsy. Whether focusing on acute postictal headache or peri-ictal headache, or migraine in general, relatively similar rates have been observed in both men and women with epilepsy (Forderreuther et al., 2002; Karaali-Savrun et al., 2002; Ito et al., 2004; Syvertsen et al., 2007; Mameniskiene et al., 2016), and the sex of the patient does not appear to influence the type of headache (e.g., migraine, tension, or unclassified) associated with seizure activity (Leniger et al., 2001). This does not exclude the possibility, however, that the underlying mechanisms and triggers of migraine and other headaches in epilepsy may be different between men and women.

b. Animal models. There is growing investigation of the underlying neurobiological mechanisms of migraine in animal models (for review, see Bolay et al., 2011; Ferrari et al., 2015; Pavlovic et al., 2017). Most of the focus in animal models has been on measures of cortical spreading depression (CSD), which is postulated to underlie the migraine aura (Charles and Baca, 2013). In this regard, it is intriguing that CSD susceptibility in rodent models also exhibits certain sex differences. For example, the threshold for triggering CSD in wild-type C57BL/6 mice appears to be lower in females, in both the potassium chloride and tetanic stimulation models of CSD induction (Brennan et al., 2007). Furthermore, mouse models of familial hemiplegic migraine type 1, which harbor mutations in the *Cacna1a* gene encoding the α1A subunit of Cav2.1 channels, exhibit sex differences, with females exhibiting increased susceptibility to CSD and neurologic motor deficits than males and increased propagation of CSD into subcortical structures (Eikermann-Haerter et al., 2009, 2011).

In terms of potential links between migraine and epilepsy, only a handful of studies have addressed this issue in animal models. Nevertheless, a study of Wistar audiogenic rats indicated intriguing sex differences that reversed with exposure of the rats to audiogenic kindling stimulation. Specifically, when tested prior to audiogenic kindling, the female and male rats showed higher and lower CSD conduction velocity, respectively, compared with controls of the same sex. After audiogenic kindling, however, the female rats showed lower CSD propagation than controls, whereas males showed higher CSD propagation (Guedes et al., 2009). Conversely, mice that harbor heterozygous or homozygous knockout mutations of proline-rich transmembrane protein 2, which underlie a group of disorders including epilepsy and migraine, do not appear to exhibit sex differences in PTZ or audiogenic seizure induction or in different motor or cognitive behaviors (Michetti et al., 2017). Lastly, a pair of recent studies examining a mouse model of familial hemiplegic migraine type 2, a subtype of severe migraine with aura and comorbid epilepsy, have documented sex differences in some parameters but not others. These mice, which carry a mutation in the astrocyte-specific α2-isoform of the Na+/K+ ATPase, may exhibit sex differences in certain behavioral comorbidities, including open-field exploration, and elevated cortical and hippocampal glutamate levels in adult females (Bottger et al., 2016). In tests of CSD susceptibility, a sex difference was not observed in young mice, but susceptibility was lowered specifically in aged, reproductively senescent females; however, these mice do not appear to show a sex difference in susceptibility to epileptiform activity (Kros et al., 2018). Altogether, the few studies that have accounted for seizures or epilepsy have primarily focused on models of rare, more severe subtypes of migraine. Therefore, it is unclear how the findings may relate more broadly to heterogeneous epilepsies and to the milder forms

of migraine observed more generally in people with epilepsy.

III. Catamenial Epilepsy: Fluctuations in Seizure Occurrence across the Menstrual Cycle

Perhaps the most prominent sex difference in epilepsy is that women with epilepsy commonly show a pattern of cyclical occurrence of seizure exacerbations during particular phases of the menstrual cycle (Newmark and Penry, 1980; Reddy, 2004b; Herzog and Fowler, 2008). In this section, we will review this phenomenon, "catamenial epilepsy," with particular focus on the clinical presentations and ongoing development of preclinical models to study this sex-specific aspect of epilepsy etiology and treatment.

A. Types and Prevalence

The types of epilepsies and seizures that are susceptible to catamenial fluctuations are not yet thoroughly defined. However, it appears that seizures in both focal epilepsies (such as TLE) and certain primary generalized epilepsies (such as juvenile myoclonic epilepsy) can exhibit catamenial exacerbations. Catamenial epilepsy is a widespread condition that affects between 25% and 70% of women with epilepsy who are of reproductive age (Reddy, 2009). The reason for the large range is due to differences in definition or diagnostic criteria. As a result of cyclic fluctuations in hormones and subsequent changes in the levels of neurosteroids, women suffering from catamenial epilepsy experience exacerbations of epileptic seizures associated with particular phases in the menstrual cycle (Herzog, 1999; Reddy, 2004b). Presently, there is no specific Food and Drug Administration–approved drug therapy for the treatment of catamenial epilepsy. In many cases, women diagnosed with epilepsy who are experiencing increased cyclic-related seizures are prescribed conventional ASDs. Unfortunately, many patients still experience menstrual cycle–related seizures despite drug treatment, indicating their condition is not responding to conventional ASDs; these seizures can thus be classified as pharmacoresistant.

Catamenial epilepsy is observed in women with both ovulatory and anovulatory cycles. In one study (Herzog et al., 2004), about 16.5% of subjects were found to have anovulatory cycles and an inadequate luteal phase. Altogether, three types of catamenial seizures have been identified, perimenstrual, periovulatory, and inadequate luteal phase, based on seizure exacerbation in relation to the menstrual cycle (Herzog et al., 1997; Reddy, 2009) [see Fig. 2 in Harden and Pennell (2013)]. The specific pattern of incidence can be identified simply by charting menses and seizures, along with measuring mid-luteal–phase serum progesterone levels to distinguish between normal and inadequate luteal phase cycles (Herzog and Fowler, 2008; Quigg et al., 2009). The diagnosis of catamenial epilepsy is mainly based on the assessment of menstruation and seizure records. Using the first day of menstrual bleeding as the first day of a regular 28-day cycle, the menstrual cycle is divided into four phases: 1) menstrual phase, days −3 to +3; 2) follicular phase, days +4 to +9; 3) ovulatory phase, days +10 to +16; and 4) luteal phase, days +17 to −4. The number of seizures in each phase is checked for at least two cycles, and a twofold or greater increase in frequency during a particular phase of the menstrual cycle can be used as diagnostic criteria of catamenial epilepsy. In perimenstrual catamenial epilepsy, the most common clinical type, women with epilepsy experience an increase in seizure activity on days −3 to 3 of the cycle (Reddy, 2009).

B. Preclinical Models

Preclinical models have been developed that mimic the perimenstrual seizures of catamenial epilepsy. This seizure condition can be induced with pharmacologic agents or by electrical stimulation in rodents with suitable manipulation of neuroendocrine milieu (Reddy, 2009; Scharfman et al., 2009; Reddy et al., 2012). Neurosteroid levels fluctuate during the ovarian cycle phases. They are generally found in high concentrations during the luteal phase and lower concentrations during the perimenstrual phase. Furthermore, perimenstrual catamenial epilepsy could be triggered by physiologic reductions of neurosteroids. There is some clinical evidence stating that some neurosteroids have been found to be deficient in blood plasma of patients with perimenstrual catamenial seizures (El-Khayat et al., 2008; Tuveri et al., 2008). Such premises have been used to model neurosteroid withdrawal in rodents to obtain a better understanding of the underlying mechanism.

There are several features for an ideal catamenial epilepsy model (Scharfman et al., 2005, 2008; Reddy, 2016). It should reflect pathophysiology similar to those of catamenial seizures in women with epilepsy, exhibit appropriate menstrual seizure phenotype consistent with the neuroendocrine fluctuations of women with epilepsy, exhibit appropriate latency following steroid hormone fluctuations or withdrawal period, and respond to drug therapy with resistance to certain anticonvulsants. Because catamenial epilepsy is a complex neurologic disorder that encompasses many causes and seizure phenotypes, it is highly unlikely that any single animal model will truly recapitulate the full spectrum of clinical catamenial seizure features. Therefore, it is necessary to screen potential therapeutic products and investigate pathologic mechanisms in a battery of animal models prior to clinical trials. In this regard, both rat and mouse models were recently developed with the basic premise of creating a hormonal milieu of the perimenstrual period (Reddy, 2016), using both healthy rats and epileptic rats as well as healthy mice and kindled (epileptic) mice. This was done by creating

a variety of manipulations through the use of pseudo-pregnancies, the exogenous administration of progesterone, and the utilization of a spontaneous seizure model. For example, gonadotropin is used to increase endogenous neurosteroid levels (Reddy et al., 2001). When gonadotropin is administered, it induces superovulation and the release of progesterone, followed by its conversion to allopregnanolone (Fig. 3). Concurrently, when allopregnanolone levels peak, they can be blocked by administration of the neurosteroid synthesis inhibitor, finasteride, to create a state of neurosteroid withdrawal. During this neurosteroid withdrawal phase, the seizure threshold in these animals drops significantly lower before returning to normal levels within 72 hours.

The above paradigms were recently replicated in mouse models, which allow for more mechanistic studies, and two distinct mouse models of perimenstrual catamenial epilepsy were developed (Gangisetty and Reddy, 2010; Reddy et al., 2012). These models are based on the premise that seizure susceptibility decreases when neurosteroid levels are high (luteal phase) and increases during their withdrawal (perimenstrual periods) in females in association with specific changes in the GABA$_A$ receptor subunit plasticity. First, a chronic seizure condition was created by using the hippocampus kindling model in female mice. Second, the fully kindled mice were subjected to fluctuating levels of neurosteroids, mimicking the ovarian cycle. Then, two distinct pharmacological approaches were used to induce elevated neurosteroid levels: 1) chronic exogenous progesterone treatment protocol and 2) gonadotropin regimen for induction of endogenous synthesis. The gonadotropin-induced neurosteroid synthesis and withdrawal paradigm appears more physiologically relevant than the exogenous progesterone treatment. In this model, elevated neurosteroid levels are induced by sequential gonadotropin treatment and withdrawal induced by finasteride (Reddy et al., 2012). This mouse model of perimenstrual catamenial epilepsy is useful for the investigation of disease mechanisms and exploring the efficacy of new therapeutic approaches. However, there are some limitations with these preclinical models because the actual endocrine conditions that exist in the menstrual cycle are different from those observed in rodent models. As discussed above, in rodents, the estrous cycle duration is 4–7 days, and the menstrual cycle in women lasts about 28 days. In addition, rodents do not exhibit a true luteal phase as observed in the menstrual cycle. This is a major concern with most animal models developed in rodents.

IV. Potential Neurobiological Bases for Sex Differences in Seizure Susceptibility and Epilepsy

The underlying mechanisms that give rise to the sex differences in various types of epilepsy and associated comorbidities are ongoing areas of investigation. In this section, while not exhaustive, we will discuss some of the developmental, neurobiological, endocrinological, and metabolic effects that are likely to be involved in driving sex-specific outcomes in the context of epilepsy and/or comorbidities.

A. Sex Differences in Brain Development

Sex differences are evident throughout the full trajectory of brain development. In humans, brain volume differences between males and females are evident from birth and are present throughout the lifespan [see review in Paus (2010)], including highly ictogenic regions such as hippocampus and amygdala.

Early in development, gonadal hormone–mediated organizational effects lead to terminal differentiation of neurons and circuits to adopt sex-specific patterns [for review, see McCarthy and Arnold (2011)]. This was first described in the landmark paper of Phoenix et al. (1959), in which guinea pigs were exposed to testosterone in utero, a treatment that resulted in masculinization of females as measured by adult copulatory behavior. The window for these organizational effects is developmentally restricted but permanent [for review, see Arnold (2009)]. These organizational effects are complemented by activational effects, i.e., hormone-mediated changes in function that are reversible with removal of the hormonal influence. These effects set up dimorphic patterns that may influence both the expression and treatment of the epilepsies.

Given that infancy is one of the peak periods of new onset seizure occurrence, sex differences in brain development may have stark impacts on responses to treatment. In the sections below, we describe sex differences in the context of early-life epilepsies, with a focus on preclinical models to understand mechanisms.

1. Neurogenesis. One of several ways sex differences manifest is through divergent rates of neurogenesis. This topic has been previously reviewed extensively (Porter, 2008) and is presented in brief below. First, basal rates of neurogenesis differ during development as a function of sex; newborn male rats display a higher rate of neurogenesis in the hippocampus than females (Bowers et al., 2010). In contrast to the hippocampus, newborn female rats display higher rates of neurogenesis in the amygdala than male rats (Krebs-Kraft et al., 2010). This baseline difference in neurogenesis may, in turn, set up divergent responses to epileptogenic insults.

Aberrant neurogenesis is well characterized as a pathologic feature of TLE in adult animal models (Parent et al., 1997; Gray and Sundstrom, 1998; Scharfman et al., 2000). Though similar findings have been reported in younger animals (Porter et al., 2004), this differs substantially across models and age, with some studies reporting decreased cell birth (McCabe et al., 2001; Liu et al., 2003), some reporting increased
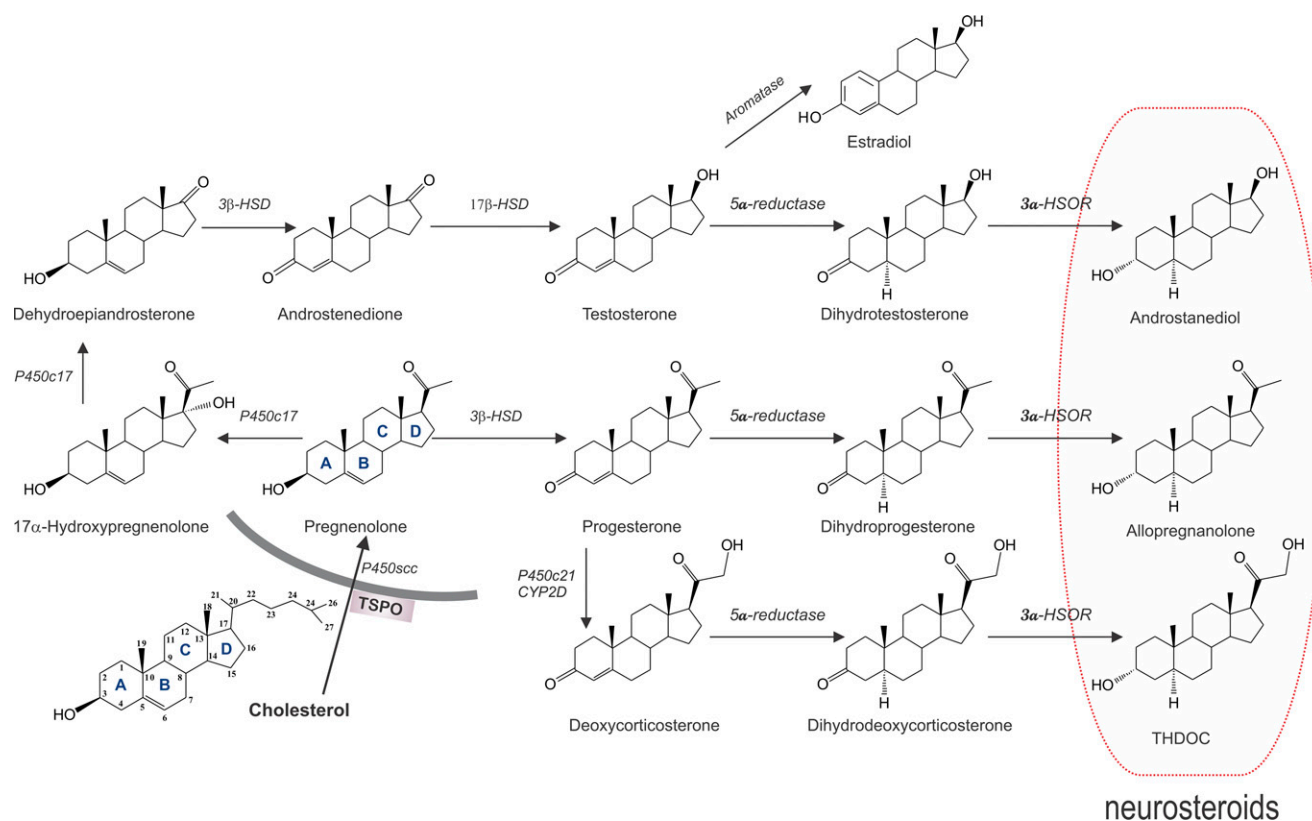
Fig. 3. Biosynthetic pathways of neurosteroid synthesis in the brain and nervous system. Enzymatic pathways for the production of three prototype neurosteroids allopregnanolone (now called brexanolone), allotetrahydrodeoxycorticosterone (THDOC), and androstanediol are illustrated from cholesterol and intermediate steroid precursors. Cholesterol is converted to pregnenolone by P450scc in the inner mitochondrial membrane. Pregnenolone is the precursor for progesterone and other neurosteroids. Progesterone, deoxycorticosterone, and testosterone undergo two sequential A-ring reduction steps catalyzed by 5α-reductase and 3α-HSOR to form the 5α, 3α-reduced neurosteroids. The conversion of progesterone, deoxycorticosterone, or testosterone into neurosteroids occurs in several regions within the brain. The 5α-reductase, 3α-HSOR, and other enzymes are present in the brain. *3β-HSD*, 3β-hydroxysteroid dehydrogenase; *3α-HSOR*, 3α-hydroxysteroid oxidoreductase; *17β-HSD*, 17β-hydroxysteroid dehydrogenase; *P450c21*, cytochrome P450 21-hydroxylase; *P450scc*, cholesterol side-chain cleavage enzyme. Adapted from Reddy (2013).

(Sankar et al., 2000), and some reporting no change (Bender et al., 2003). There are only sparse reports examining sex differences in survival of neurons after early-life seizures. For example, female rats that received febrile seizures at postnatal day 17 displayed greater survival of newborn cells in the hippocampus than males (Lemmens et al., 2005), but the rate of neurogenesis did not differ by sex. In a different model (KA-induced SE), female newborn rats showed less survival of cells in the dentate gyrus than males (Hilton et al., 2003). Ultimately, the number of neurons present after injury or stimulation is determined by the balance of proliferation and survival. The role of neurogenesis in epilepsy is complex, with recent studies in adult animals showing that basal neurogenesis is protective against seizures (Iyengar et al., 2015; Jain et al., 2019b), whereas aberrant neurogenesis following SE contributes both to adverse cognitive outcomes and seizure burden (Cho et al., 2015). How these findings will translate to the developing brain, and how these findings are modulated by sex, remains to be determined.

2. Chloride Homeostasis and GABAergic Neuro transmission. Sex differences in the development of GABAergic neurotransmission have been extensively reviewed by others (Akman et al., 2014) and are summarized below, with a focus on features of particular relevance to epilepsy and pharmacotherapy.

First, several GABA$_A$ receptor subunits (α1, α3, and γ2) display differing expression profiles as a function of sex and brain region (Ravizza et al., 2003; Li et al., 2007; Chudomel et al., 2009). Consistent with this observation, a study of children undergoing positron emission tomography imaging prior to epilepsy surgery found greater flumazenil binding in females compared with males (Chugani et al., 2001). Moreover, in postmortem tissue from adults, α1, α2, α5, and β3 subunit expression is greater in males than females (Pandya et al., 2019). As subunit composition impacts both the kinetics of GABAergic neurotransmission and the pharmacology of GABA$_A$ receptors (Olsen and Sieghart, 2009), a deeper understanding of sex differences in receptor expression and function may enable more appropriate targeting of pharmacotherapy.

Perhaps one of the best-explored sex differences during development in the context of epilepsy is the shift from depolarizing to hyperpolarizing GABA, which is regulated by the expression of solute transporters that set the chloride gradient. Of particular interest

are the sodium-potassium-chloride cotransporter 1 (NKCC1) and the potassium-chloride cotransporter 2 (KCC2). NKCC1 transports chloride into the cell, whereas KCC2 transports chloride out of the cell. Early in development, NKCC1 is highly expressed relative to KCC2, which results in a net chloride loading of neurons. Thus, in immature neurons, $GABA_A$ receptor activation results in chloride efflux and depolarization (Ben-Ari et al., 2007). This depolarizing GABA has been suggested to contribute to the relatively poor efficacy of first-line antiseizure medications (e.g., phenobarbital) for the treatment of neonatal seizures (Dzhala et al., 2005). Over the course of early postnatal development, expression of NKCC1 decreases, expression of KCC2 increases, and an adult-like chloride equilibrium potential is reached. However, the relative timing of this "switch" from depolarizing to hyperpolarizing GABA differs as a function of both brain region (Glykys et al., 2009) and sex (outlined below).

Although the developmental time course of KCC2 and NKCC1 expression has been characterized across brain regions in rats (Wang et al., 2002), it has only been done so in a thorough manner for males. Similarly, though spatio-temporal trajectories for both transcripts have been reported for the human brain, and samples for both males and females were included, the data were not disaggregated by sex (Kang et al., 2011). Fortunately, a handful of studies have directly compared expression in at least a subset of brain regions across sexes in animal models. In the hypothalamus of newborn rats, NKCC1 expression is significantly greater in males compared with females, and KCC2 expression is higher in female rats than males at postnatal day 5 (Perrot-Sinal et al., 2007). Similarly, KCC2 expression in cortex increases earlier in females than males (Kang et al., 2015). In the *substantia nigra pars reticulata*, KCC2 mRNA levels are regulated in a hormone-dependent manner, with females displaying higher KCC2 levels than males at postnatal day 15 (Galanopoulou and Moshe, 2003). Consistent with this pattern of expression, gramicidin-perforated patch recordings demonstrated hyperpolarizing GABA in female rats and depolarizing GABA at the same developmental stage (Galanopoulou et al., 2003). In adult animals, however, infusion of GABA agonists into the substantia nigra potently suppresses seizures, and during development, a sex difference appears; in males, a proconvulsant effect is observed, whereas in females, either no effect or an anticonvulsant effect is observed (Veliskova and Moshe, 2001). This effect may be explained by the earlier emergence of hyperpolarizing GABA in female rats.

Likewise, in the entorhinal cortex and hippocampus, KCC2 levels are higher in females than in males (Murguia-Castillo et al., 2013). Functionally, this expression results in hyperpolarizing GABA in females at ages as young as postnatal day 4, whereas in males,

the majority of neurons display depolarizing GABA responses until postnatal days 14–18 (Galanopoulou, 2008). In sum, females display a more "adult-like" expression pattern of NKCC1/KCC2 at earlier developmental ages, which results in a mature chloride gradient and hyperpolarizing GABA signaling. In the hippocampus, these divergent patterns of maturation of GABAergic transmission interact with seizure history in a complex manner. KA-induced seizures on postnatal days 4–6 induce an early shift toward hyperpolarizing GABA in males and cause a regression toward depolarizing GABA in females (Galanopoulou, 2008). In humans, NKCC1 expression in cortex peaks around birth and then falls to adult levels in the months after birth. By contrast, KCC2 levels rise slowly and consistently over the first year of life (Dzhala et al., 2005). Though expression during development has not been stratified by sex in human tissue, this overall expression is consistent with rodent studies, with a heightened ratio of NKCC1 to KCC2 expression early in development.

As several common classes of antiseizure medications used in neonates (e.g., barbiturates, benzodiazepines) exert seizure-suppressive effects through GABA-mediated inhibition, sex differences may be of particular importance in understanding effects (or lack thereof) as randomized controlled trials in these populations become more common. At the very least, subgroup analyses based on sex, which have not been reported in the few recent trials comparing phenobarbital to other therapies in neonates, are warranted.

3. Hypoxia and Hypoxia-Ischemia. In classic pharmacological models used in preclinical epilepsy research, little to no evidence exists for sex differences in immature animals with respect to induction, seizure threshold, or mortality [see Table 3 in Akman et al. (2014)]. This effect differs from models of hypoxia or hypoxia/ischemia (HI). HI encephalopathy is one of the most common causes of neonatal seizures. Growing clinical evidence suggests that females display more favorable outcomes after HI (Smith et al., 2014). Similarly, survival and long-term outcomes are enhanced in female, as compared with male, low-birth-weight infants (Ito et al., 2017). For a thorough review of this topic, see Hill and Fitch (2012).

What mechanisms may underlie these differences in outcome? In mice, 1) females display smaller infarct volume and fewer seizures, 2) males display increased microglial activation and inflammatory cytokine production, and 3) females display less severe behavioral impairment (Mirza et al., 2015; Al Mamun et al., 2018). Moreover, males show a transient but significantly heightened seizure burden following HI at postnatal day 7, an effect that is not observed in females (Kang et al., 2015). Similar findings after neonatal HI have been reported in rats, with males displaying more severe brain damage and neurologic deficits than

females (Hill et al., 2011). This enhanced sensitivity in males appears to be at least partly mediated by testosterone, as treatment with testosterone worsens outcomes in females (Hill et al., 2011). Moreover, it has been suggested that the X-linked inhibitor of apoptosis may confer a protective advantage to females (Hill and Fitch, 2012).

Clinically, phenobarbital remains the first-line therapy for neonatal seizures following hypoxia or hypoxia-ischemia (Rennie and Boylan, 2007). However, as described above, early in postnatal development, GABA action can result in excitation of neurons, and drugs such as phenobarbital can thus result in a paradoxical increase in activity. In rodent models, phenobarbital displays a range of efficacy; after graded global hypoxia in rats (Cleary et al., 2013), it displays modest efficacy at suppressing seizures, but this action is significantly potentiated by the NKCC1 inhibitor bumetanide. By contrast, in a carotid ligation model in mice, phenobarbital suppressed seizures, and the addition of bumetanide was either without effect or worsened seizure activity (Kang et al., 2015). Interestingly, the exacerbation of seizure activity by bumetanide was preferential to females (Kipnis et al., 2019). In other models, sex has not been explicitly addressed; however, phenobarbital alone failed to suppress seizure activity but abolished seizures in combination with bumetanide in in vitro hippocampal seizures evoked by either low magnesium (Dzhala et al., 2008) or high potassium (Dzhala et al., 2005). Similarly, in vivo seizures triggered by KA are poorly controlled by phenobarbital in postnatal day 10 rats but are significantly reduced by bumetanide (Cleary et al., 2013). Based largely on the hypothesis that the immature chloride gradient in the developing brain contributes to the high level of refractoriness seen with phenobarbital (Painter et al., 1999), the Neonatal Seizure Treatment with Medication Off-patent consortium trial (Pressler et al., 2015) examined adjunctive treatment with bumetanide for neonatal seizures. The trial was ultimately terminated because of a combination of adverse reactions (ototoxicity) and little evidence for seizure reduction. Given the small sample size (4 female, 10 male), it is difficult to draw any conclusions regarding sex differences, but it is interesting to note that decreases in seizure burden were evident in a subset of subjects of both sexes. Although the trial failed to demonstrate clear efficacy, future studies targeting this mechanism in early-life seizures may still be merited that take into account etiology of seizure given that bumetanide appears to have different responses across animal models based on the etiology of the seizure and the effect of sex.

B. Hormonal and Neurosteroid Mechanisms in Adulthood

1. Estrous Cycle–Associated Changes in Seizure Susceptibility.
Fluctuations in seizure susceptibility across the estrous cycle, akin to catamenial patterns of seizure clustering, have been documented in both acute models of seizure induction and models of chronic epilepsy in female rodents (Fig. 1). With respect to acute induction of seizures, most studies have documented increased susceptibility on proestrus and estrus. For example, PTZ threshold differs across the estrous cycle, with the lowest threshold seen during estrus and the highest seen during diestrus (Riazi et al., 2004). Similarly, thresholds to bicuculline-induced myoclonus are lower during estrus than during diestrus (Finn and Gee, 1994), and susceptibility to seizures induced by systemic KA injection is also higher on estrus compared with diestrus (Maguire et al., 2005). Moreover, sensitivity to GBL peaks during estrus and is lowest during metestrus and diestrus (Santos et al., 2018). After discharge, threshold with kindling stimulation changes over the estrous cycle and is lowest in proestrus (the time of peak circulating estradiol levels) and highest during metestrus (during the peak of progesterone secretion). Hippocampal excitability measured in ex vivo slices from rats is higher on proestrus and estrus relative to metestrus (Scharfman et al., 2003). Similarly, the number of SWDs increases during proestrus in WAG/Rij rats (van Luijtelaar et al., 2001). By contrast, however, a significantly lower percentage of females reach SE as an endpoint after systemic pilocarpine injection during estrus (Scharfman et al., 2005), and the frequency of interictal spikes observed following systemic KA injection in rats is higher on metestrus and diestrus (D'Amour et al., 2015). Furthermore, minimal and maximal electroshock thresholds are also modulated as a function of estrous cycle stage in female rats. Minimal seizure threshold is elevated during diestrus, lower during proestrus, and lowest during estrus. The maximal seizure severity, as measured by the duration of tonic flexion, is greatest during diestrus and shortest during estrus (Woolley and Timiras, 1962a). Together, these studies indicate dynamic fluctuations in seizure susceptibility across estrous cycle stages.

2. Effects of Estradiol, Progesterone, and Testosterone on Seizure Susceptibility.
Steroid hormones, specifically estradiol, progesterone, and testosterone, are intimately involved in sex differences in epilepsy. Estradiol is one of the three molecules that comprise estrogen and has been known to play a role in the exacerbation of seizures in women with epilepsy (Logothetis et al., 1959; Bäckström, 1976; Bäckström et al., 1984; Jacono and Robertson, 1987; Younus and Reddy, 2016). Plasma estradiol levels are found to increase during both the follicular and luteal phase of the normal menstrual cycle, and in the late luteal phase, there is a precipitous decline in progesterone that triggers menstruation. Therefore, an increase in the ratio of estrogen to progesterone during the perimenstrual period may possibly contribute to the development

of perimenstrual seizures (Bonuccelli et al., 1989; Herzog et al., 1997).

a. Estradiol. Proconvulsant effects of estradiol have been observed across diverse animal models of seizure susceptibility (Table 3). Estradiol facilitates chemoconvulsant PTZ kindling in ovariectomized female rats (Hom and Buterbaugh, 1986). Estradiol decreases the latency to KA-evoked status in ovariectomized rats (Woolley, 2000) but interestingly decreases post-status cell loss (Reibel et al., 2000). Estradiol also reduces electroshock seizure threshold (Woolley and Timiras, 1962c) and accelerates both hippocampal and amygdala kindling (Edwards et al., 1999c). Similarly, in ovariectomized females, estradiol facilitates kindling from the anterior neocortex (Buterbaugh, 1989), dorsal hippocampus (Buterbaugh and Hudson, 1991), and amygdala (Hom and Buterbaugh, 1986; Buterbaugh, 1987). Estradiol also facilitates amygdala kindling in male rats (Saberi and Pourgholami, 2003). It should be noted, however, that some anticonvulsant effects of estradiol have also been observed. For example, in ovariectomized rats, the latency to NMDA-evoked seizures can be increased by estradiol (Vathy et al., 1998). In addition, ovariectomy results in an increased sensitivity to NMDA marked by longer total seizure duration and greater numbers of seizures than control animals; this effect is normalized by estradiol (Kalkbrenner and Standley, 2003).

b. Progesterone. Progesterone has primarily anticonvulsant and antiepileptic properties in animals and humans, although in a randomized placebo-controlled trial, progesterone was primarily effective in treating women with a perimenstrual catamenial pattern only (Jacono and Robertson, 1987; Herzog, 1999; Reddy, 2009). Progesterone has long been known to have antiseizure activity in a variety of animal models of epilepsy (Table 3). In recent years, numerous studies have confirmed the powerful anticonvulsant activity of progesterone in diverse animal seizure models (Reddy et al., 2004, 2010). For example, progesterone suppresses PTZ-evoked seizures in female mice (Frye et al., 2002) and protects against NMDA-evoked seizures in male mice (Członkowska et al., 2000). Consequently, seizure susceptibility is typically low during physiologic conditions associated with high progesterone. In women with epilepsy, natural cyclic variations in progesterone during the menstrual cycle could influence catamenial seizure susceptibility, as detailed above. It should be noted, though, that progesterone receptor (PR) agonists may increase the number of spontaneous seizures in female chronically epileptic rats (Shiono et al., 2019).

Progesterone acts on the brain through two different mechanisms. The first pathway involves binding to PRs and exerting effects through both genomic and nongenomic mechanisms. The second pathway involves modulating GABA$_A$ receptors via synthesis of neurosteroids (Fig. 3). Neurosteroids rapidly alter neuronal excitability through direct interaction with GABA$_A$ receptors (Macdonald and Olsen, 1994; Belelli et al., 2002). In the process of neurosteroidogenesis, progesterone is converted to allopregnanolone by sequential A-ring reductions. Another neurosteroid, tetrahydrodeoxycorticosterone (THDOC), is produced by reduction of deoxycorticosterone. It was demonstrated that PRs require longer periods of time to exhibit their effects, whereas neurosteroid synthesis via progesterone conversions occur rapidly, suggesting that these conversions could potentially be more relevant to developing pharmaceutical treatments. This hypothesis was supported by comparing wild-type and PR knockout mice to show that progesterone can still produce anticonvulsant effects in mice that lack PRs. Blockade of progesterone conversion to neurosteroids by finasteride treatment prevented the anticonvulsant effects, indicating that these outcomes were mediated by allopregnanolone (Reddy et al., 2004).

Because endocrine fluctuations in plasma levels of progesterone and other steroids can mediate neurosteroid availability, there are apparent differences between sexes concerning concentrations of neurosteroids in the brain. Though neurosteroids are capable of shaping inhibition and producing behavioral effects in both males and females, the regulation of neurosteroid activity may be sex-specific (Gulinello and Smith, 2003). Differences in maximal GABA$_A$ receptor potentiation are observed between male and female rats for THDOC

TABLE 3
Effects of sex steroid treatment on seizure susceptibility in preclinical rodent models

Generalized (Motor)	Estrogen	Progesterone	Testosterone
Picrotoxin	♀ = Anticonvulsant	♂ = Increased sensitivity	♀ = Proconvulsant ♂ = Anticonvulsant
Pentlyenetetrazole	Rats, ♀ = proepileptogenic	Rat: anticonvulsant	Rat: anticonvulsant
NMDA	Sensitivity: OVX > control ♀ = Anticonvulsant	Mice, ♂ = anticonvulsant	
Electroshock	Proconvulsant	Anticonvulsant	Both pro- and anticonvulsant effects
Generalized (Absence)			
WAG/Rij	More seizures during proestrus		
Focal			
Kainic acid	Rat: proconvulsant		Rat: castration is anticonvulsant
Pilocarpine		Rats, ♀: agonists increase seizure frequency	
Kindling	Rats: proepileptogenic	Rats, ♀ = proepileptogenic	Proepileptogenic

but not for allopregnanolone or androgenic neurosteroids (Wilson and Biscardi, 1997). Sex differences in the expression of 3α-hydroxysteroid dehydrogenase are evident during puberty but subside in the brain as it matures into adulthood; sex-specific gonadal and adrenal endocrine activity have a significant effect on the ability of allopregnanolone to modify anxiolytic (i.e., anxiety-reducing) actions based on variations in biosynthesis of steroid hormones (Mitev et al., 2003). Sex differences are evident in the anticonvulsant activity of neurosteroids; however, the potential mechanisms remain unclear. It is likely that differences in postsynaptic or extrasynaptic GABA$_A$ receptor expression and function may underlie the sex differences in seizure sensitivity and the anticonvulsant activity of neurosteroids (Reddy et al., 2019) (Fig. 4). In this regard, progesterone has also anecdotally been reported to increase typical absence seizures in humans (Grunewald et al., 1992). Moreover, allopregnanolone, which is synthesized from progesterone, significantly increases SWDs in WAG/Rij rats (Budziszewska et al., 1999), consistent with postulated roles for neurosteroid-sensitive extrasynaptic GABA$_A$ receptors in the thalamus in driving absence seizure activity (Banerjee and Snead, 1998; Cope et al., 2009; Errington et al., 2011).

c. Testosterone. Testosterone is synthesized by both the testes and the ovaries and, to a much lesser degree, the adrenal gland, and it can also be synthesized de novo in the brain from cholesterol. Testosterone is converted in the brain to estradiol by aromatase or to the nonaromatizable androgen dihydrotestosterone (DHT) by 5α-reductase (Meinhardt and Mullis, 2002; Swerdloff et al., 2017). The aromatase and 5α-reductase enzymes are expressed by both neurons and glia, although there are regional differences in which cell types are the dominant sources of each enzyme (MacLusky et al., 1987; Martini et al., 1993; Zwain et al., 1997; Melcangi et al., 1998; Zwain and Yen, 1999; Hojo et al., 2004). Castration of male rats as well as testosterone replacement produces both pro- and anticonvulsant effects in the model depending on timing and duration of treatment (Woolley and Timiras, 1962b) (Table 3). On balance, it appears that the actions of estradiol on neuronal excitability and seizure susceptibility are opposite those of DHT, with estradiol and DHT increasing and decreasing these parameters, respectively. Accordingly, aromatase inhibition has demonstrated some efficacy in improving seizure control in men with epilepsy (Harden and MacLusky, 2004, 2005). Several findings support this working model. For example, estradiol treatment of gonadectomized male Wistar rats lowered the threshold to electrical kindling stimulation of the amygdala, an effect that could be mimicked by testosterone treatment but not DHT. Furthermore, in gonad-intact males that produced endogenous testosterone, aromatase inhibition blocked the progressive decrease in threshold typically observed

over the kindling period (Edwards et al., 1999b). Similarly, testosterone treatment of male Sprague-Dawley rats was observed to increase susceptibility to seizures induced by either KA or pilocarpine injection (Mejias-Aponte et al., 2002), and gonadectomy of CF/1 mice increased susceptibility to seizures induced by combined PTZ and strychnine treatment, an effect that could be reversed by testosterone replacement (Pesce et al., 2000). In another study, testosterone treatment also increased seizure susceptibility in male rats and mice, and this seizure exacerbation could be blocked by treatment with the aromatase inhibitor, letrozole (Reddy, 2004c). Conversely, however, testosterone-treated wild-type mice exhibited an increased latency to systemic PTZ-induced seizures, but knockout mice lacking 5α-reductase did not show this effect of testosterone (Frye et al., 2001), indicating that the anticonvulsant effects of testosterone were likely mediated by 5α-reduction to DHT. DHT was also observed to be anticonvulsant against PTZ-induced seizures in male mice (Reddy, 2004c). It should be noted, however, that in studies examining the effects of gonadectomy and hormone replacement on excitatory synaptic spine density in the rat hippocampus, males exhibited a reduction in spine density following gonadectomy that was reversed by testosterone, but intriguingly, the effect of testosterone appeared to be mediated entirely by DHT (Leranth et al., 2003). This effect was in marked contrast to that of females, in which an upregulation of spine density by testosterone was almost completely blocked by letrozole, and DHT produced only a small effect (Leranth et al., 2004). Altogether, it appears that testosterone can exert robust effects on seizure susceptibility, and the end resulting effect depends critically on the balance of conversion to estradiol or DHT.

DHT can be further converted to the neurosteroid androstanediol (5α-androstan-3α,17β-diol, or 3α-Diol), which itself can be converted to androsterone (5α-androstan-3α-ol-17-one) (Kaminski et al., 2005; Reddy and Jian, 2010) (Fig. 3). Similar to progesterone-derived allopregnanolone, androstanediol acts as a positive allosteric modulator of GABA$_A$Rs (Reddy and Jian, 2010) and is thus poised to exert anticonvulsant effects. Indeed, androstanediol treatment has been observed to be protective in various forms of seizure induction models, including hippocampal kindling, PTZ, picrotoxin, and β-carboline ester (Reddy, 2004a,c; Ryan and Frye, 2008; Frye et al., 2009; Reddy and Jian, 2010). There are conflicting reports regarding efficacy against KA-induced seizures (Frye and Reed, 1998; Reddy, 2004a), but these discrepancies may reflect species differences between rats and mice. Intriguingly, a recent study suggests that female mice are more sensitive to the anticonvulsant effects of androstanediol and that this sex effect reflects differences in the expression of δ subunit-containing GABA$_A$Rs in dentate gyrus granule cells (Reddy et al., 2019). Similarly, androsterone
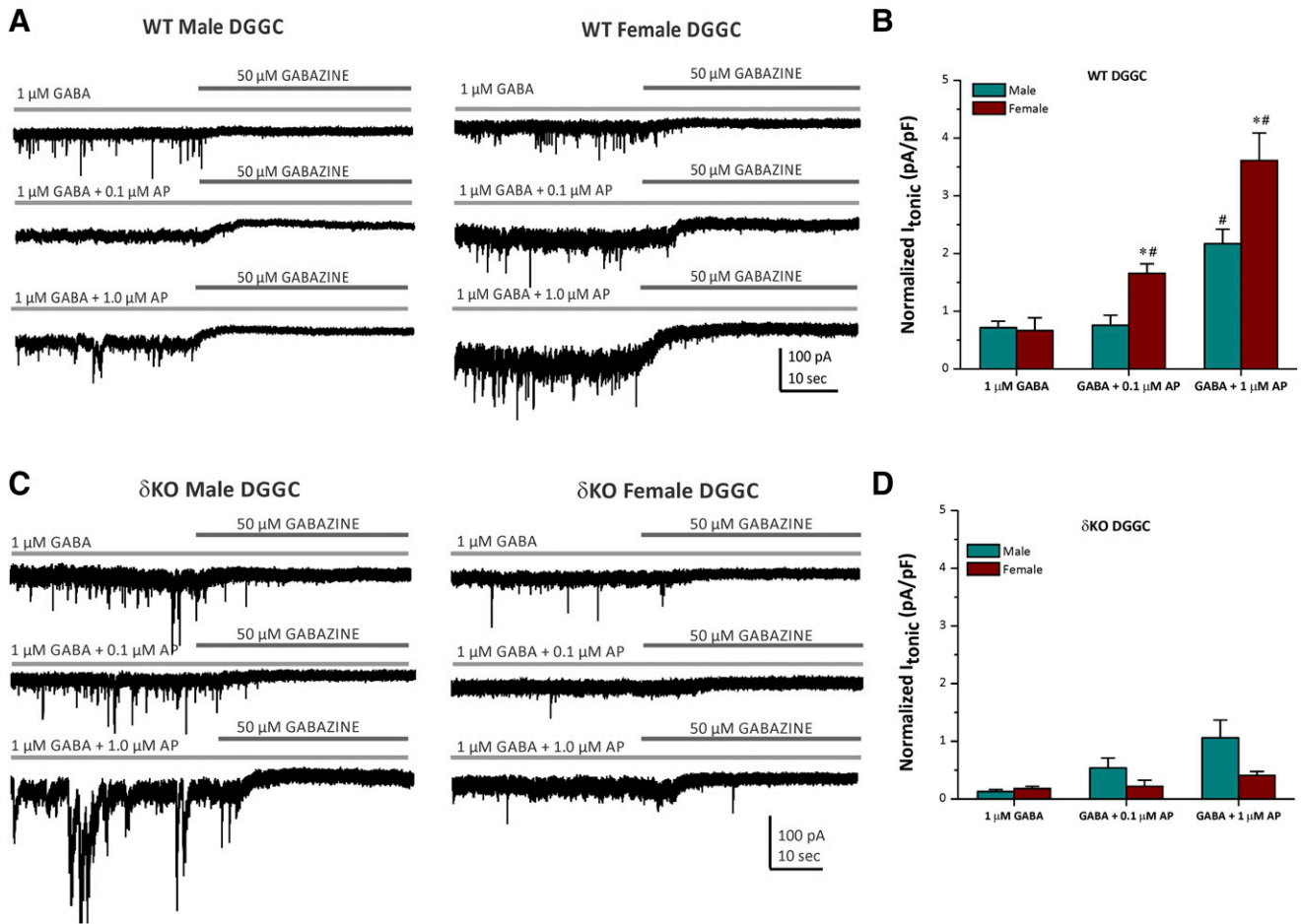
Fig. 4. Sex differences in allopregnanolone potentiation of tonic GABAAR-mediated currents in hippocampal dentate gyrus granule cells (DGGCs) in wild-type (WT) and δ GABAAR subunit knockout mice (δKO). (A) Representative tonic current recordings from DGGCs in slices from WT male and female mice. Allopregnanolone (AP, 0.1–1.0 μM) was applied to the bath in addition to 1 μM GABA to measure allosteric enhancement of tonic current. (B) Summarized data of AP concentration response from male and female DGGCs in WT mice. (C) Representative tonic current recordings in the presence of AP or/and GABA from DGGCs in slices from δKO mice. (D) Summarized data of AP concentration response from male and female DGGCs in δKO mice. Tonic current was normalized to cell capacitance (pA/pF) as a measure of current density. The GABA-A receptor tonic current was expressed as the outward shift in holding current after the application of gabazine (50 mM). $*P < 0.05$ vs. males; $\#P < 0.05$ vs. 1 μM GABA baseline current in the same sex ($n = 6-8$ cells per group). Adapted from Reddy et al. (2019).

treatment appears to produce antiseizure effects across multiple seizure models in male mice and rats, including 6-Hz corneal stimulation, PTZ, 4-aminopyridine, pilocarpine, and maximal electroshock (Kaminski et al., 2005), although actions of androsterone to lower seizure threshold in response to KA have been reported (Mroz et al., 2009). Overall, it appears that both androstanediol and androsterone exert potent anticonvulsant effects; thus, upregulating the downstream multistep conversion of testosterone to either androstanediol or androsterone, or direct treatment with these metabolic products, is a promising avenue of future ASD development. It should be noted, however, that the antiseizure effects of testosterone-derived neurosteroids outlined above may not entirely be the case for absence seizures, reflecting the mechanism of neurosteroid enhancement of inhibition producing stronger postinhibitory rebound burst firing in thalamocortical neurons (van Luijtelaar et al., 2014). In particular, it appears that testosterone itself (and/or DHT) may exert

antiabsence effects, but androstanediol, particularly through enhancement of inhibition mediated by δ subunit-containing GABA_ARs, produces proabsence effects. In this regard, castrated male WAG/Rij rats, a genetic model of absence seizures, display more SWD than intact males, suggesting that on balance, the overall effects of testosterone are seizure-suppressive (van Luijtelaar et al., 1996).

C. Brain-Derived Neurotrophic Factor

Several aspects of brain-derived neurotrophic factor (BDNF) function and regulation suggest roles in epilepsy and neural excitability. For example, BDNF application increases the excitatory:inhibitory ratio of synaptic transmission and elevates neuronal excitability in dentate granule cells resected from patients with TLE (Zhu and Roper, 2001), and levels of BDNF mRNA and protein often appear changed in resected hippocampal tissue from patients with TLE (Murray et al., 2000; Chen et al., 2016; Martinez-Levy et al., 2016). The

evidence for general roles of BDNF in epilepsy has been comprehensively reviewed elsewhere (McNamara and Scharfman, 2012; Harte-Hargrove et al., 2013; Scharfman and MacLusky, 2014a). Therefore, in this section, we will briefly discuss the aspects of BDNF biology that would suggest potential roles in sex differences in epilepsy. Interestingly, the presence and/or pattern of sex differences in BDNF content across brain areas exhibits prominent species differences. Female rats exhibit higher BDNF content in several regions highly relevant to epilepsy and seizure activity, including hippocampus, cortex, and amygdala (Bland et al., 2005; Bakos et al., 2009; Snigdha et al., 2011), and male rats exhibit lower levels of hippocampal BDNF immunoreactivity compared with females, especially in the mossy fiber pathway (Scharfman et al., 2003). This sex difference, however, is reversed in mice, with males showing higher hippocampal BDNF content (Szapacs et al., 2004). Although humans do not appear to show a sex difference in hippocampal BDNF content, women may have higher BDNF in the prefrontal cortex (Hayley et al., 2015). Furthermore, BDNF expression appears to be highly sensitive to steroid hormone signaling, with estradiol, progesterone, and testosterone treatment, as well as removal of such hormones by gonadectomy, all producing changed BDNF levels in the brain (Solum and Handa, 2002; Franklin and Perrot-Sinal, 2006; Li et al., 2012). BDNF exerts its neurotrophic effects through activation of tropomyosin receptor kinase B (TrkB) receptors; heterozygous BDNF knockout mice exhibit a sex difference in the TrkB receptor pathway, with greater TrkB phosphorylation, and thus increased activation of downstream extracellular signal-related kinase signaling, in the frontal cortex and striatum of males compared with females (Hill and van den Buuse, 2011). Of particular relevance to potential sex differences in post-traumatic epileptogenesis, controlled cortical impact injury can produce sex-specific changes in BDNF content. Specifically, male Sprague-Dawley rats, but not females, exhibit increased BDNF in the frontal cortex ipsilateral to the injury, whereas female rats show increased BDNF in the contralateral hippocampus, an effect that was not observed in males (Chen et al., 2005b). In summary, baseline and dynamic differences in BDNF signaling are poised to produce sex differences in various aspects of neural functioning relevant to seizures and epilepsy.

D. Glial Mechanisms: Astrocytes and Microglia

There are nearly as many glial cells as neurons in the human brain (von Bartheld et al., 2016), and neuron-glia interactions are essential to normal brain function [for review, see Khakh and Sofroniew (2015)]. Despite the increased interest in the role of glia, particularly astrocytes and microglia, in the pathophysiology of epilepsy [for recent comprehensive reviews, see Eyo et al. (2017), Patel et al. (2019)], there is a lack of

information regarding the potential role of glia in sex differences in epilepsy. There is emerging evidence, however, for roles of astrocytes and microglia in sex differences in brain function. For example, several studies have documented sex differences in expression of the astrocyte marker glial fibrillary acidic protein (GFAP) in the hippocampus. GFAP expression in various hippocampal areas in Wistar rats appears to show sex-specific differences that shift from the prepubertal period into adulthood (Conejo et al., 2003, 2005; Arias et al., 2009). The number and morphology of astrocytes in the posterodorsal medial amygdala also exhibits sex differences, with higher numbers of astrocytes and greater astrocyte morphologic complexity in tissue from male rats (Johnson et al., 2008). Hippocampal GFAP expression may also shift with the estrous cycle in female rats, with higher numbers of GFAP-immunopositive cells detected on proestrus compared with diestrus in cornu ammonis 1 and cornu ammonis 3 (Arias et al., 2009). Changes in glial phenotype and morphology across the estrous cycle have also been described (Luquin et al., 1993; Klintsova et al., 1995). It should be noted that mice may not show similar sex differences in hippocampal GFAP immunoreactivity, but GFAP expression appears to be sensitive to estradiol and testosterone (via aromatization to estradiol) (McQueen et al., 1992). Cultures of astrocytes and microglia prepared from female and male rat pups also display sex-specific functional differences of high potential relevance to seizure activity, including increased resistance to oxygen-glucose deprivation and increased clearance of glutamate from extracellular space by cells from females (Liu et al., 2007; Morizawa et al., 2012) as well as increased expression of the inflammatory marker interleukin-1β in cells prepared from males (Loram et al., 2012).

Unfortunately, direct examination of sex differences in glial biology and neuronal-glial interactions in animal models of epilepsy is lacking. In one recent study, increased expression of GFAP was detected in the hippocampi of male C57BL/6J mice compared with females 5 days after systemic KA injection, but this effect may have reflected the increased acute seizure severity observed in the same male mice in that cohort (Li and Liu, 2019). With respect to potential roles in post-traumatic epileptogenesis, two studies have documented increased microglia activation in proximity to controlled cortical impact and penetrating cortical injury wounds in male mice, as detected by immunoreactivity for the microglial marker ionized calcium-binding adaptor molecule 1 (Acaz-Fonseca et al., 2015; Villapol et al., 2017). It should also be noted, however, that an increased expression of ionized calcium-binding adaptor molecule 1 in males at early time points after the injury may equalize later, such that no sex difference is seen 30 days later (Villapol et al., 2017). Finally, male mice may show a worsened outcome in experimental autoimmune

encephalitis, a model of multiple sclerosis, and this sex difference is abolished by astrocyte-specific knockout of sodium voltage-gated channel alpha subunit 5 (Pappalardo et al., 2018). With increased development of tools to specifically manipulate the function of glial cells and selectively change the expression of certain genes and proteins in astrocytes and microglia, incorporation of specific assessment of sex differences in the effects of these manipulations, and especially in relation to synaptic function, neuronal excitability, and seizure susceptibility, would likely yield important insights.

E. Stress Response and the Hypothalamic-Pituitary-Adrenal Axis

Stress is a known risk factor for seizures (Neugebauer et al., 1994; Frucht et al., 2000; Haut et al., 2003, 2007; Nakken et al., 2005; Sperling et al., 2008) [for review, see Lai and Trimble (1997)]. The physiologic response to stress is mediated by the HPA axis, which coordinates the neuroendocrine response to stress through release of corticotropin-releasing hormone from the paraventricular nucleus of the hypothalamus, triggering the release of adrenocorticotropic hormone from the pituitary, which then signals the release of cortisol from the adrenal cortex (corticosterone in rodents). Numerous preclinical studies have demonstrated that stress and stress hormones are proconvulsant [for review, see Joëls (2009)]. Given this information, it is concerning that stress hormones have been shown to be elevated in patients with epilepsy and are positively correlated with seizure frequency (Culebras et al., 1987; Galimberti et al., 2005). Both clinical and preclinical studies support a role for stress, HPA axis activation, and elevated stress hormones in epilepsy [for review, see Maguire and Salpekar (2013)].

In addition to the impact of stress on epilepsy outcomes, HPA axis dysfunction has been demonstrated in preclinical epilepsy models. Seizures have been shown to activate the HPA axis (OToole et al., 2014), which can negatively impact epilepsy outcomes independent of stress. For example, HPA axis dysfunction, characterized by increased plasma corticosterone and deficits in the dexamethasone suppression test, is positively correlated with depression-like behaviors in an acquired epilepsy model (Mazarati et al., 2009). Similarly, seizure-induced activation of the HPA axis increased seizure frequency and comorbid depression-like behaviors in a preclinical epilepsy model (Hooper et al., 2018). Based on the fact that hypercortisolism is a hallmark feature of depression (Zobel et al., 2004; Kondziella et al., 2007), HPA dysfunction associated with epilepsy has been suggested to contribute to comorbid depression in epilepsy (Pineda et al., 2010). In fact, the HPA axis and glucocorticoids have been implicated in the bidirectional relationship between epilepsy and depression (Kanner, 2009), and social defeat stress has been shown to predispose chronically epileptic mice to depression-like behaviors (Becker et al., 2015). However, limited studies have directly tested

the mechanistic underpinnings contributing to psychiatric comorbidities in epilepsy.

Clinical and preclinical evidence points to a clear relationship between stress, HPA axis dysfunction, and stress hormones in worsening epilepsy outcomes, including psychiatric comorbidities in epilepsy. There are also well established sex differences in stress reactivity and HPA axis function (Bale and Epperson, 2015; Bangasser et al., 2019) as well as the expression of glucocorticoid receptors (Bourke et al., 2012; Bangasser, 2013), which could impact the relationship between stress, epilepsy, and psychiatric comorbidities. However, to-date, there have not been any studies investigating sex differences in the role of stress, HPA axis, or stress hormones in mediating sex differences in psychiatric comorbidities in epilepsy. Further research is necessary to understand the underlying neurobiology contributing to these comorbidities and potential treatments suitable for both sexes.

V. Considerations for Antiseizure Pharmacotherapies, Drug Screening, and Development

Although the response to antiseizure medications is generally not considered to differ as a function of sex, there are well recognized clinical challenges associated with the use of ASDs that can differ by sex. This issue has been reviewed elsewhere (Perucca et al., 2014) and therefore is described only in brief here. First, though sex per se is not associated with clear pharmacokinetic differences for most ASDs, some small differences have been reported. Therefore, sex differences in drug metabolism, clearance rates, volume of distribution, and protein binding are important variables to consider both for existing therapeutics and those under development. For example, diazepam has been reported to be more highly protein-bound in males (Routledge et al., 1981), with a larger volume of distribution in females (Greenblatt et al., 1980). Levetiracetam clearance has been reported to be greater in females (Alzueta et al., 2018), although this effect was reduced when normalized for weight. Moreover, the half-life and clearance of carbamazepine have also been reported to differ as a function of sex, with males displaying longer half-life and lower clearance (Marino et al., 2012), consistent with the reports of greater hepatic CYP3A4 expression in females (Wolbold et al., 2003). Importantly, even in the absence of baseline differences in pharmacokinetics, these parameters change for many ASDs during pregnancy (Pennell, 2003). These issues underscore the importance of therapeutic drug monitoring and appropriate sex- or state- (e.g., pregnancy) specific dosing. Even in the absence of clear sex differences in large clinical trials, postapproval suggestions to adjust dosing have been issued on occasion, as in the case of zolpidem [for review, please see Farkas et al. (2013)].

Above and beyond pharmacokinetic issues, drug safety concerns may also differ as a function of sex. For example, many ASDs are associated with teratogenic effects (Tomson et al., 2018). Even in the absence of frank teratogenesis, gestational exposure to ASDs may cause long-lasting cognitive changes in offspring of women with epilepsy (Meador et al., 2011; Meador and Loring, 2016). Several ASDs can impact the metabolism of contraceptive agents, and some contraceptive agents, in turn, can impact ASD metabolism (Crawford, 2002; O'Brien and Guillebaud, 2010). There is also a major need for further studies examining the potential for changes in ASD efficacy and pharmacokinetics with age to address the growing need for treatments for patients in middle-aged and elderly populations of both sexes.

It is common for initial screening and subsequent differentiation screening of ASDs to occur in male rodents. However, some of these models, as reviewed above, display sex differences in response to ASDs. Moreover, hormonal influence (sex, estrous cycle stage) impacts responses in many screening models. Given these established differences, screening across sex should be considered, and, likewise, the relative parameters of the model should be adjusted to ensure sensitivity. For example, enhanced sensitivity to a chemoconvulsant in one sex or the other could produce a suprathreshold response and obscure subsequent effects of a putative antiseizure compound. Furthermore, despite ample evidence for sex differences in early development, sex as a biologic variable in the context of preclinical or clinical treatments for early-life epilepsy has been almost completely ignored. Given the robust evidence regarding sex differences in development of inhibitory neurotransmission, neurophysiology, and response to injury, careful assessment of sex in studies in developing animals is a clear priority.

VI. Concluding Remarks and Future Directions

The epilepsies and associated comorbidities prominently affect both sexes, but the specific features and treatment options have some clear distinctions. Overall, our review of the clinical literature in epilepsy thankfully found many studies that included both sexes in patient cohorts, whether by design or by convenience. Unfortunately, the current picture for preclinical animal studies is more skewed. In keeping with much of the preclinical and basic research conducted in neuroscience over the last several decades, most studies have primarily used male animals (Prendergast et al., 2014; Will et al., 2017). Emerging directives from funding agencies, such as the U.S. National Institutes of Health mandate to address sex as a biologic variable (Clayton and Collins, 2014), are aimed at correcting this imbalance. Going forward in both clinical and preclinical studies, we suggest that more emphasis needs to be placed on systematic inclusion of both males and females within the same studies to facilitate direct comparisons and encourage funding and publishing support for studies designed to evaluate the extent to which findings previously obtained in male animals are replicated in females.

Although in our review we were able to find many pieces of literature that included both male and female subjects, there are still gaping holes in our knowledge regarding specific sex differences in the underlying neurobiology of seizures, pharmacokinetics of ASDs, and interactions between the brain and endocrine systems, just to name a few aspects. It should also be noted that emerging evidence suggests that some sex differences are "latent," such that the emergent phenotype does not necessarily display a major sex difference, but the underlying mechanisms that give rise to the phenotype are distinct (Koss et al., 2018; Jain et al., 2019a). This prospect has so far received little attention in the field of epilepsy but is an important possibility to consider, particularly given the implications for efficacy of pharmacotherapies and other forms of treatment. Proper assessment of sex differences in underlying neurobiological mechanisms relevant to epilepsy and drug efficacy will improve epilepsy treatment of as many patients as possible, no matter their sex or gender.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Christian, Reddy, Maguire, Forcelli.

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