Sexual Dimorphism in Health and Disease

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From the Lab Bench to Clinical Investigations

Edited by

Jorge Morales Montor

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I dedicate this book to my lovely wife Karen, my two daughters Casandra and Alexa, and my two boys Iker and Axel. To all my family, the symbol of love and giving, and my friends who encourage and support me. This book is also dedicated to my dear father, who has been nicely my supporter during my whole life, and my beloved mother who, for years past, has encouraged me attentively with her fullest and truest attention to keep up with my career. Also, I am dedicating this book to two beloved people who have meant and continue to mean so much to me: my dear aunt Luz and my beloved maternal grandmother Graciela. Although they are no longer on this world, their memories continue to regulate my life.

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PREFACE

Sexual dimorphism (SD) is the difference in morphology and physiology between sexes of the same species and it has been widely recognized as a very important risk factor in all diseases. Furthermore, SD can reflect the susceptibility associated with gender in human disease. The significance of the present book is that it is of high priority to people interested in an update on sex differences in health and disease, and biological factors of risk, and explains the latest sex-associated factors for different types of diseases. It also delves into the advantage of modifying metabolism in order to obtain potential strategies for restoring the function of effector immune cells. Likewise, the book examines topics such as sex and cardiovascular disease, sex bias in immunity and immunity to infection and sex hormones cancer and autoimmunity. Additionally, it presents basic and clinical data of different diseases.

The scope of the Book is to provide updated information to scientists and clinicians that is valuable in their quest to gather information, carry out new investigations, and develop novel research about sexual dimorphism.

This book is a valuable source for clinical researchers, medical Doctors, Clinicians, and several members of biomedical field who need to understand the mechanisms to understand a hot topic and a very important biological factor in disease: sexual dimorphism. This book addresses the function of different signaling pathways, the modification of the metabolism of the cells as well as chemical compounds with estrogenic activity that are involved in the generation of gender differences in disease. The foregoing is intended to provide understanding to broaden the landscape to comprehend sexual dimorphism and its molecular mechanisms mainly during human pathologies. Thus, the book is an actualized, up to date product that intends to be of use to all people involved in the world of human disease and wishes to know why there is always a sex-associated susceptibility to disease.

SECTION 1.

SEX AND CEREBRAL DISEASE

CHAPTER 1

SEXUAL DIMORPHISM IN EPILEPSY

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Abstract

Epilepsy is the most common central nervous system disease with an incidence of sixty-five million patients and with a 2.4 million of new cases every year around the world. The International League Against Epilepsy (ILAE) classifies epilepsies as focal, generalized or combined that are due to different causes such as genetic, structural, infectious, metabolic, immune, traumatic, or due to unknow origin. A relevant aspect in epilepsy is that are few studies about the different incidence on male and female patients with distinct types of epilepsies. However, it is a very well characterized epilepsy that dependent of the estrogen plasma levels in the catamenial epilepsy a multifaceted neuroendocrine condition in which seizures are clustered around specific points in the menstrual cycle, most often around perimenstrual or periovulatory periods.

In the case of absence seizures, a generalized type of epilepsy that are characterized in the electroencephalogram (EEG) with a spike-wave discharges (SWDs) associated with an unconsciousness with a mean duration of 25 seconds, with a spontaneous recovery. There are two well-characterized rat models of this type of epilepsy the Wistar Albino Glaxo of Rijswijk (WAG/Rij), and the Genetic Absence Epilepsy from Strasbourg (GAERS) in which it has been demonstrated an increased in the incidence of SWDs in the proestrus and estrus phases and with a significant reduction during diestrus and metestrus phases of the estrus cycle. However, less is known about the role of androgens in this type of generalized epilepsy, there is a report in male WAG/Rij rats in which the discharges increase in when they are orchiectomized in adulthood.

In our laboratory at the Institute of Physiology of the Benemérita Universidad Autónoma de Puebla we obtained *taiep* rats, the name is the acronym of the signs that characterized them tremor, ataxia, immobility episodes, epilepsy, and paralysis of the hindlimbs. The disease had a recessive dominant trait and recently we demonstrated using magnetic resonance imaging (MRI) that taiep rats showed a hypomyelination, ventriculomegaly and atrophy of the putamen and the cerebellum. The disease is due to a mutation in the tubulin β 4A (TUBB4a) that are equal to human leukodystrophy named hypomyelination with the atrophy of basal ganglia and cerebellum (H-ABC).

In 24 hours, EEG recordings *taiep* rats showed SWDs similar to that obtained WAG/Rij and GAERS rats that are equal to human patients with absence seizures. In *taiep* rats we demonstrated a sexual dimorphism in the incidence of SWDs being the male more susceptible with respect to female *taiep* rats and male *taiep* rats suffer SWDs at younger ages. Recently, we demonstrated that neonatal orchiectomy significantly decreased the incidence of SWDs when evaluated at 3 months of age. But adult orchiectomy produced the opposite effect, that is a significant increase in the SWDs along the circadian cycle. These results showed that testicular androgens are capable to modify the thalamo-cortical circuit that is responsible to the characteristic discharges in this type of generalized epilepsy in *taiep* rats.

Epilepsy and sex hormones

Epilepsy refers to a broad spectrum of disease states, including both genetic and acquired disorders, which can be associated with varying pathogenic

mechanisms, with different electroencephalographic discharges, frequency of seizure activity and with sex-dependent susceptibility.

The International League Against Epilepsy (ILAE) describe different types of epilepsy as focal, generalized, or combined onset with different levels of awareness or motor or non-motor signs. Epilepsy is a chronic noncommunicable disease of the brain that affects around 65 million people worldwide. It is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve a part of the body that is partial, or the entire body called generalized and are sometimes accompanied by loss of consciousness and loss of control of gut or bladder functions (Berg et al., 2010).

The number of seizure episodes are a result of excessive electrical discharges in group of brain cells. Seizures can vary from the briefest lapses of attention or muscle jerks to severe and prolonged tonic or clonic seizures. The convulsions can also vary in frequency, from less than one per year to several episodes per day. From developmental point of view vary along lifetime from neonatal to adulthood (see Table 1).

Neonatal period:

1. Benign neonatal crises
2. Benign neonatal familial epilepsy
3. Ohtahara syndrome
4. Early myoclonic encephalopathy

Infant

1. Febrile seizures
2. Benign infant epilepsy
3. Benign familial epilepsy of infant
4. West's syndrome
5. Dravet syndrome
6. Myoclonic epilepsy of infancy
7. Myoclonic epilepsy in non-progressive encephalopathy
8. Infant epilepsy with migratory focal seizures

Childhood:

1. Febrile seizures
2. Early-onset childhood occipital epilepsy
3. Epilepsy with atonic myoclonus seizures
4. Childhood absence epilepsy
5. Benign epilepsy with centrotemporal spikes
6. Autosomal dominant nocturnal frontal epilepsy
7. Late-onset childhood occipital epilepsy (Gastaut)
8. Epilepsy with myoclonic absences
9. Lennox-Gastaut syndrome
10. Epileptic encephalopathy with spike-wave pattern during sleep
11. Landau-Kleffner syndrome

Adolescence and adulthood:

1. Juvenile absence
2. Juvenile myoclonic
3. Generalized tonic-clonic seizures only
4. Autosomal dominant with auditory features
5. Other familial temporal lobe epilepsies

Variable starting age:

1. Familial focal epilepsy with variable foci
2. Progressive myoclonic epilepsies
3. Reflex epilepsies

Table 1. Types of epilepsies in the different periods of life.

It is quite important to emphasize that up to 10% of people worldwide have one seizure during their lifetime but this is not imply that he/she had epilepsy because it is necessary the recurrent characteristic of discharges in at least one semester to diagnose this neurological disease. Epilepsy is one of the world's oldest recognized conditions, with written records dating back to Egyptians at 4,000 BC. However, fear, misunderstanding, discrimination, and social stigma have surrounded epilepsy for centuries. In fact, this stigma continues in many countries today and can impact on the quality of life for people with the disease and their families and the disease also has a high impact in the medical burden globally and in each country. It is necessary to have an adequate epidemiological survey to have an early diagnostic of epilepsy and give as early as possible to provide the patients with the adequate treatments.

It is important to emphasize that epilepsy is one of the leading causes of chronic morbidity worldwide, and that receive an epilepsy diagnosis is associated with a certain degree of stigma, with patient experiencing restrictions related to driving, swimming, school, or job performances. Epilepsy is the result of different structural or metabolic etiologies so called acquired epilepsy and they represent half of all cases of this neurological disease and could be due to neurotoxicity, brain injury, anoxia, metabolic imbalances, stroke, drug withdrawal, tumors, or encephalitis (Berg et al., 2010). It is important to emphasize that women exhibit epilepsies and seizures syndromes that are much more complex and often intractable, including menstrual cycle-linked catamenial epilepsy due to different levels of neurosteroids and other signaling molecules in the brain that could influence seizure protection or susceptibility in both sexes differently. There is a lot of scientific data of the hormonal effects on seizures; many women report that their seizures pattern changes at puberty, menstrual cycle, and

menopause being the hormonal changes the main cause (Reddy, 2016; Herzog et al., 1997).

It is important to emphasize that an estimated of 100 million women worldwide use hormonal contraceptives, and these steroid hormones analogs are associated with some risks, including drug interactions and seizure exacerbations (Reddy, 2014). However, the mechanisms underlying sex differences in epilepsy unknown, and these differences in seizure susceptibility between men and women may arise from a myriad of factors, including microsomal enzyme activity, steroids hormones, and sexual dimorphisms in the different networks of the brain. Sex-related differences in neurosteroids levels, neuroendocrine regulation, steroid hormones, and different inhibitory and excitatory balance in the brain could influence seizure susceptibility in males and females (Reddy, 2014; Reddy, 2009).

Sexual differences in the susceptibility and incidence of different types of epilepsy.

From epidemiological and genetic studies, it has been demonstrated that some epileptic syndromes are more common in females, including those which are X chromosome-linked such as Aicardi syndrome, Rett syndrome and protocadherin 19-related infantile epileptic encephalopathy, as well as juvenile myoclonic epilepsy, childhood absence epilepsy, and photosensitive epilepsy (Qureshi and Mehler, 2014). However, Ohtahara syndrome, infantile spasms, Lennox-Gastaut, West's, Dravet's, and Landau-Kleffner's syndromes, as well as myoclonic atomic seizure and febrile seizures that are more common in males (Qureshi and Mehler, 2014).

Most of the patients with epilepsy have an inherited basis with complex, polygenic inheritance. The study of monogenic epilepsy syndromes offers the opportunity to uncover the effects of sex on the phenotypes produced by the alteration of specific genes. Thus far, mutations in many of the epilepsy genes that confer generalized epilepsy syndromes, such as the genes that encode the $\alpha 1$, $\beta 3$, and $\gamma 2$ gamma aminobutyric acid (GABA) type A receptor subunits, have not been identified in enough patients to be able to determine if there are statistically-significant sex–gene interactions (Cossette et al., 2002; Lachance-Touchette et al., 2011; Maljevic et al., 2006; Tanaka et al., 2008; Wallace et al., 2001). In contrast, in the case of Lennox–Gastaut or Angelman syndromes the have a female predominance (Trevathan et al., 1997). In the case of Angelman syndrome they are typically reported without mention of sex effects because the syndrome is

very rare and with less epidemiological studies (Buntinx et al., 1995). In fact, in most of the studies few attentions are takin into account about sex predominance, changes in the susceptibility or incidence of seizures along development or in different phases of the menstrual cycle.

In general, such different sex susceptibilities in different types of epilepsy are through to arise from the effects of sex hormones. The steroids are capable to modify the neural substrate of the brain through the so called "organizational" effects during development that are capable of programming specific genes in fetal and perinatal period that modify neural circuits. There are also "activational" effects due to exposition of sex steroids including androgens, estrogens, or progesterone (see Figure 1).

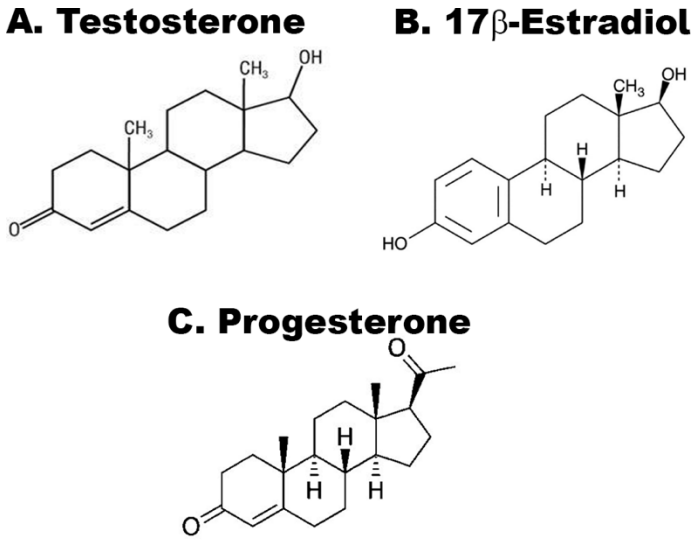

Figure 1. Molecular structure of testosterone, 17β-estradiol and progesterone.

Sex steroids usually happen later in life from puberty to later which are capable to modulate the neuronal circuits, neurogenesis and even cell death (Frye, 2008).

These effects of sex steroids include canonical genomic functions as well as non-genomic activities. Importantly can even be synthesized locally within nervous system the so called neurosteroids highlighting their critical role in neuromodulation activities within specific nuclei in the central nervous system (Reddy, 2014). In fact, recent evidence shown in different cell type

and neural circuits with specific gene expression profiles and associated behavioral phenotypes can be sexually dimorphic independent from the effects of sex steroids. For example, sex determining region Y (SRY) gene, mediate sex differences directly (McCarthy and Arnold, 2011); but a modern view must include epigenetic regulatory mechanisms as a main mechanism for the integration of hormonal and genetic influences that interact with different environmental stimuli that are capable to modify at the molecular, cellular, and neural network levels in the central nervous system (McCarthy and Nugent, 2013; Qureshi and Mehler, 2014).

Among epigenetic factors the non-coding RNAs (ncRNAs) are novel factors with key regulatory roles that can be functionally linked with DNA methylation and chromatin modifications (Qureshi and Mehler, 2012). Recent experimental data showed that nuclear genome harbors tens of thousands of these ncRNAs genes, which are expressed in highly tissue-specific patterns and particularly abundant within neural cells that are conformed either by short (ncRNAs) or long ncRNAs (ln RNAs) based on their size, with ln RNAs being greater than 200 nucleotides in length. It is relevant that among short ncRNAs are microRNAs (miRNAs), small nucleolar RNAs (snoRNAs) and PIWI-interacting RNAs (piRNAs). On the other hand, ln RNAs are more heterogenous and versatile because long RNAs can engage in sequence-specific interactions with other DNA and RNA molecules (Mercer and Mattick, 2013). Accordingly, ln RNAs have a complex and increasingly broad spectrum of activities including chromatin remodeling complexes, sequestering miRNAs, modulating nuclear-cytoplasmatic transport of proteins and controlling local protein synthesis at synapse level (Mercer and Mattick, 2013).

Several studies have shown that mutations in genes encoding factors from across the spectrum of different epigenetic mechanisms are responsible for human epilepsy disorder. From point mutations, deletions, and insertions in the MECP2 gene represent the primary causes of Rett syndrome, a dominant X-linked disorder associated with infantile spasms in girls (Qureshi and Mehler, 2014). Mutations in the nuclear receptor-binding SET domain containing protein (NSD1) gene, which also encodes a histone methyltransferase, are diagnostic of Sotos syndrome, an overgrowth syndrome associated with febrile seizures and adult epilepsy (Nicita et al., 2012). These are several X-linked intellectual disabilities disorders associated with epilepsy that are linked to mutations in histone modification and chromatin remodeling genes. It is necessary to continue analyzing human beings as well as animal model to fully characterized all the possible role of epigenetic mechanisms in epileptogenic mechanisms. These data can

be very useful to design novel therapeutical approaches including gene therapy.

Catamenial epilepsy

Catamenial epilepsy refers to cyclic seizure exacerbation in relation to menstrual cycle (Herzog et al., 1997). Instead that since 1881 was described that most of the women reported worsening of seizures perimenstrual by William Gowers, there are a limited studies that deeply analyzed the causes of seizure oscillations. previously proposed the existence and common occurrence of three distinct patterns of catamenial seizure exacerbation: perimenstrual and preovulatory in women with normal ovulatory cycles, and entire luteal phase in women with anovulatory cycles (Herzog et al., 1997). It is important to emphasize that data pathophysiology is common to record seizure clustering at certain times of the menstrual cycle supporting that sex steroid, estrogens, and progesterone, modulate neuronal excitability through changing the gene expression or changing GABAa or glutamate receptors affecting neurotransmission (Voinescu, 2019). It appears that progesterone and its metabolite allopregnelone (3α-hydroxy-5α-pregnan-20-one) fluctuations affect inhibitory GABAergic neurotransmission (see Figure 2).

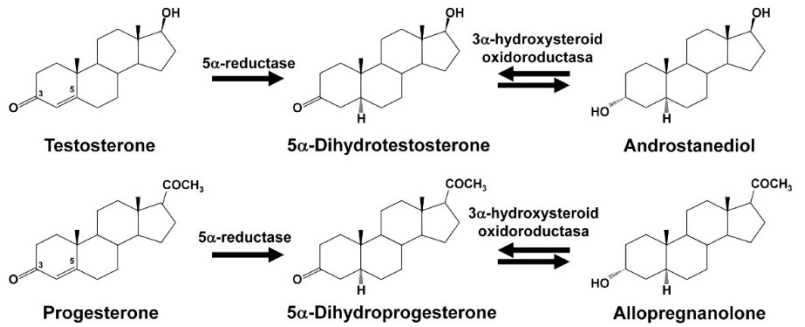

Figure 2. Metabolism of progesterone

Additionally, within the central nervous system there are the enzymes that converted cholesterol to steroids named neurosteroids. These neurosteroids rapidly modulate GABAa receptors mainly in the cerebral cortex and hippocampus (Reddy, 2013). The GABAa receptor is a pentamer consisting of five subunits that from chloride channel. They have sixteen subunits (α1-6, β1-3, γ1-3, δ, ε, θ and π subunits) have been identified so far. The binding

sites for neurosteroids are distinct from the recognition sites for GABA, benzodiazepines, and barbiturates. The GABAa receptors mediates two types of GABAergic inhibition divided into phasic (synaptic) through γ2-containing, or tonic (extrasynaptic) through δ-containing subunits. Neurosteroids act on all GABAa receptor isoforms, but they have large effects of extrasynaptic δ subunits mediate tonic currents. Tonic current causes a steady inhibition of neurons and reduces their excitability and then play role in setting the level of their excitability by potentiation of tonic inhibition (Reddy, 2013). The biosynthesis of neurosteroids oscillates with the menstrual cycle and can be a key factor in catamenial epilepsy.

Variations in seizures in men

The clinical evidence supported that sex steroids affect seizure occurrence in men has been sparse, although animal experimental studies have shown that certain testosterone metabolites affect neuronal excitability, kindling susceptibility, and seizure occurrence. Aromatization of testosterone produces estradiol that is highly epileptogenic in male rodents (Saberi et al., 2001). The 5α reduction and subsequent 3α-hydroxylation produced androstanediol (5α-androstan-3α,17β-diol), an androstane that has potent GABA modulation and inhibits seizures (see Figure 2; Rhodes and Frye, 2004). In a recent investigation Herzog et al. (2004) showed that different serum levels of neuroactive steroids could affect neuronal excitability and seizure occurrence. Specifically, they showed that antiepileptic drugs are associated with lower bioavailable testosterone levels than controls or men who take lamotrigine and that this was likely related, in part, to induction of testosterone metabolism to neuroactive steroids such as estradiol and androstanediol. While the reduction of testosterone may be viewed as an undesirable outcome, the resulting increase in androstanediol, a potent positive allosteric modulator of the GABA receptor, and androstanediol/estradiol ratio would tend to have a net antiseizure effect as might the substantial decrease in dehydroepiandrosterone sulfate, a neuroexcitatory steroid. These results clearly showed that metabolism in the brain impact seizure susceptibility in male brain.

Absence seizures in human patients

Absence seizures are characterized by a sudden loss of awareness without aura or postictal state and are accompanied by synchronous, bi-hemispheric spike-wave discharges (SWDs) on EEG recordings. Absence seizures are classified as typical or atypical (Nolan et al., 2005; Snead, 1995; Stefan et

al., 2008; Onat et al., 2013; Duron et al., 2005). Typical absence seizures are accompanied by very rhythmic and synchronous spike-wave discharges (SWDs) on EEG recordings with a mean frequency around 3 Hz using the Fast Fourier Transformation analysis. When compared with typical absence seizures with atypical pattern are usually longer in duration, and more often associated with changes in postural tone, and less likely to be associated with automatisms, and the discharges are less rhythmic, exhibit some bi-hemispheric asymmetry, and occur with frequencies of less than 3 Hz (Panayiotopoulos, 2008).

Many of the genetic generalized epilepsy syndromes such as childhood absence epilepsy, juvenile absence epilepsy, and juvenile myoclonic epilepsy are associated with typical absence seizures. Epilepsy syndromes with typical absence seizures are medically controlled in most cases and are usually associated with minimal or no long-term cognitive impairment (Callenbach et al., 2009; Brouwer, 2009).

In contrast, epilepsy syndromes with atypical absence seizures are seen in epileptic encephalopathy syndromes such as Lennox–Gastaut Angelman or Dravet syndromes and myoclonic atonic epilepsy. They are associated with atypical absence seizures and less common to be controlled by the classical antiabsence drugs such as lamotrigine, valproic acid and ethosuximide, and they are associated with a severe impairment in cognition and other neurodevelopment alterations (Nolan et al., 2005; Gulhan et al., 2011). It is imperative to identify novel therapies, for patients with syndromes conferring atypical absence seizures and neurosteroids could be the base of these new therapies.

In the case of childhood absence seizures typically begins at 4 to 9 years of age with multiple daily absence seizures and is usually associated with normal cognition and seizure remission during adolescence (Janz, 1997; Chaix et al., 2003; Trinka et al., 2004; Callenbach et al., 2005, 2009). However, in juvenile absence epilepsy and juvenile myoclonic epilepsy with a later seizure onset around 10 to 12 years of age with longer duration, and a greater potential for developing other seizure types compared to childhood absence epilepsy (Jallon and Latour, 2005; Gulhan et al., 2011). Although there are some exceptions, childhood and juvenile absence epilepsies have been reported to show prominent sex differences because they are much more prevalent in females than in males (Nicolson et al., 2004; Trinka et al., 2004; Asadi-Pooya et al., 2012a; Asadi-Pooya et al., 2012b).

Animal models of absence seizures

In the XX century, researchers from Strasbourg, France and Rijswijk, Nijmegen, The Netherlands described two well-known rat models of absence epilepsy. First in 1982, the Genetic Absence Epilepsy Rats (GAERS) was discovered (Vergnes et al., 1982); and four years later, the Wistar Albino Glaxo Rijswijk rats (WAG/Rij) reported (van Luijtelaar and Coenen, 1986). The GAERS originated from a Wistar colony, in which about 38% of the rats showed SWDs (Vergnes et al., 1982). In the WAG/Rij strain was already fully inbred when it was discovered that all subjects at an age of six months had several hundred SWDs per 24 h. Several studies have since characterized the behavioral, electrophysiological, pathophysiological, and pharmacological features of these models, which closely resemble human typical absence epilepsy. Both strains are now considered well-validated rat models for absence seizures (Avanzini, 1995; Danober et al., 1998; Crunelli and Leresche, 2002, Coenen and van Luijtelaar, 2003; van Luijtelaar and Sitnikova, 2006; Depaulis and van Luijtelaar, 2006; Onat et al., 2013).

In the case of WAG/Rij rats, there were no sex differences in the incidence of rats showing SWDs including frequency, mean and cumulative duration of the spontaneous occurring SWDs at postnatal day (PND) 75 that is in young adolescent, and between 125 and 175 PND in adulthood (Coenen and van Luijtelaar, 1987). The presence of strain-dependent sex difference is not uncommon, and therefore it is very reasonable to investigate putative sex differences in GAERS. In young adult male and female GAERS were compared and no differences in number, mean and cumulative duration of SWDs in an EEG in a morning session between 09:00 to 12:00. It is important to emphasize that the prevalence of spike-wave discharges in WAG/Rij and GAERS rats is 100% in both males and females. The difference in prevalence in the genetic rodent models and the clinical population can be due to inbreeding or selecting animals so that a putative sex difference present in the original population might have disappeared. However, GAERS were not developed to study sex differences but were developed with the purpose to isolate the epileptic phenotype and to contrast them with the non-epileptic rats. Interestingly, a higher female prevalence can be inferred from the first paper of GAERS (Vergnes et al., 1982), when the rats were not fully inbred and not genetically homozygous for all autosomal genes. On the other hand, WAG/Rij's were already fully inbred when the epileptic phenotype was discovered and, indeed, all animals of this strain show the age dependent SWDs. In contrast, in humans there is no known genetic mutation that confers a 100% penetrance for absence

seizures; perhaps this variable penetrance allows sex-associated factors to influence the incidence of SWDs.

The presence of SWDs is not unique for GAERS and WAG/Rij rats; other outbred and inbred strains are endowed with SWDs, including some Wistar, Long-Evans, Sprague–Dawley, Fisher 344, G/Cpb and Brown Norway (BN) rats, although the incidence is generally lower than in the two established models and fewer studies are available (Kleinlogel, 1995; Robinson and Gilmore, 1980; Willoughby and Mackenzie, 1992; Inoue et al., 1990; Jandó et al., 1995).

Atypical models are highly important from a clinical perspective, although they were less commonly studied. In a well-described animal model of atypical absence seizures. The early postnatal treatment of Long-Evans hooded rats with the cholesterol synthesis inhibitor, AY9944, confers a lifelong atypical recurrent spontaneous between 5 to 6 Hz spike-wave discharges that are associated with behavioral, pharmacological, and cognitive features similar to human atypical absence seizures (Onat et al., 2013). These atypical absence seizures lasted longer in females than in males and this sex difference emerged before the onset of puberty (Cortez et al., 2002; Persad et al., 2002). The fact that these sex differences were prepubertal, suggested that the sex-related structural and functional changes developed earlier, perhaps during the critical period that occurs in rats' late pregnancy and early postnatal period in which sex hormones shape and organize the brain and body involved in reproductive behavior.

Sex differences in spike-wave discharges duration in the AY9944 model are consistent with clinical data reporting that absence seizures are more common in female, with some studies showing 2 to 1 female to male ratio. These sex differences could be caused by potential alterations of the cholesterol-derived steroid including neurosteroids pool during development (table 2).

Previous studies suggest that a reduction in progesterone and its metabolites result in an increase in convulsive seizure threshold. whereas increases in testosterone and estradiol enhances seizure development. In fact, both progesterone and allopregnanolone exacerbate AY9944-induced atypical absence seizure probably due to GABAa receptor.

Model	Steroid	Convulsions	Reference
PTX - induced	Estradiol silastic implant	- -	Schwartz - Gibblin et al., 1989
PTX – induced	Testosterone	+ +	Schwartz - Gibblin et al., 1989
Kindling	Estradiol	- -	Hom an Buterbaugh 1986
Absence Seizures	Progesterone Through pregnancy	- -	Tolmacheva et al., 2004
Kindling In – vitro	Androstandiol	- -	Reddy 2004 and Jian, 2010
PTZ – induced	Testosterone	- -	Reddy, 2004
PTZ – induced	Estradiol	+ +	Reddy, 2004
Absence Seizures	Estradiol	+ +	Van Luijtelaar et al., 2001
Absence Seizures	Progesterone	+ +	Van Luijtelaar et al., 2001
Absence Seizures	Testosterone	- -	Van Luijtelaar et al., 1996

Table 2. Effect of sex steroids in animal models of epilepsy.

The development of sexual dimorphic synaptic organization may reflect sex steroid–modulating synaptogenesis in the hypothalamus and limbic system. These natural processes of differentiation also may help explain the emergence of the increased seizure severity seen during the proestrus stage in female AY9944-treated rats. Although, we demonstrated that progesterone and estrogen have opposing effects on atypical absence seizures, both hormones peak during the proestrus stage. This fact could be reconciled when considering that progesterone blood plasma concentrations are 1,000-fold higher than estrogen concentrations, suggesting that progesterone's proabsence effects override estrogen's antiabsence effects in this stage. Estrogen can act as a neurotropic factor stimulating axonal and dendritic growth and synapse formation. Estrogen decreases the functional coupling of the GABAb receptor to the inwardly rectifying potassium channel possibly through an action on the G protein and can activate both protein kinase A and protein kinase C to cause a heterologous desensitization of

GABAb receptors. This evidence may help explain precisely how estradiol is acting to suppress the AY9944-induced seizure. The observed estradiol reduction in spike-wave discharges appears to be specific for the atypical form of absence seizures, because this attenuation was not observed in a previous study using a genetic model of typical. The observed estradiol reduction in SWDs appears to be specific for the atypical form of absence seizures, because this attenuation was not observed in a previous study using a genetic model of typical absence seizures.

Additionally, there were no reports of sex differences in a Wistar derived tremor rat (tm/tm). This model develops tremor of the whole body at 2 weeks of age. With 5–7 Hz spike-wave discharges that appeared synchronously in the cerebral cortex and hippocampus accompanied by absence-like seizures. The tremor gradually disappeared between 6 and 8 weeks of age (Serikawa et al., 1987). The disappearance at puberty is interesting, considering that absences also tend to disappear, or evolve in children with absence seizures. However, the mechanisms responsible for the remission of this phenotype remain unknown but may be related to changes in the hormone milieu.

Sex differences have been observed in pharmacological models of absence epilepsy in rats. One such model is produced from a systemic injection of a low dose of bicuculline, a GABAa receptor competitive antagonist. Because electrographic seizures were found in the hippocampus as well as in the cortex, it is thought that this model represents atypical absence seizures (Matejovska et al., 1998). Female rats with natural or exogenous estrogens exhibited these atypical absence seizures with a higher incidence than male rats. A proepileptic effect of estrogens is classically assumed (Velísková, 2007), although estrogens have also antiepileptic effects as was established on clonic seizures in a kainic acid induced status epilepticus model (Velísková et al., 2000). In contrast to all this, estradiol had either no effects in a typical absence model or anti-absence effects.

As with rats, most of the mouse models of absence epilepsy do not exhibit sex-dependent phenotypes. Experiments in commonly used spontaneous inbred genetic mouse models of absence seizures such as tottering, lethargic, and stargazer were performed in both males and females without any reported sex differences in the frequency of absence seizures (Aizawa et al., 1997; Noebels and Sidman, 1979; Lacey et al., 2012; Eguibar and Cortes, 2010). Moreover, studies of atypical absence epilepsy phenotypes generated in mice transgenically overexpressing GABAb receptor subunits did not report sex-dependent effects (Aizawa et al., 1997; Lacey et al., 2012;

Noebels and Sidman, 1979; Stewart et al., 2009). Pharmacologically evoked absence seizures in mice have been produced through the systemic administration of GABAa receptor antagonists and GABAb receptor agonists. None of the experiments that used these drugs suggested a sex-dependent phenotype; the studies were either reported without specifying mouse sex or were performed using only males or females without a stated rationale (Aizawa et al., 1997; IIshige et al., 1996; Weiergraber et al., 2008; Zaman et al., 2011; Choi et al., 2010).

Mice have also been produced to contain human epilepsy gene deletions or mutations. Angelman syndrome usually results from a de novo maternal deletion or mutation involving chromosome 15q11.2–q13, a region that contains the ubiquitin ligase 3A (UBE3A) gene as well as the genes that encode the β3, α5, and γ3 GABAa receptor subunits (Bird, 2014). Mice with a maternally inherited heterozygous UBE3A deletion (hybrid C57BL/6/129SvEv) or a 1.6Mb chromosomal deletion from Ube3a to GABA receptor subunit beta experience atypical absence-like seizures and other behavioral abnormalities, but do not demonstrate sex-dependent phenotypes (Jiang et al., 1998, 2010). Three other genetic models, the succinate semialdehyde dehydrogenase deletion mouse, the Efhc1 deletion mouse, and the GABAa receptor γ2(R43Q) subunit knock-in mouse also exhibit seizures without reported sex-selective effects (Matejovska et al., 1998; Suzuki et al., 2009; Errington et al., 2011; Reid et al., 2013; Tan et al., 2007).

Mutations in two other epilepsy genes, Gabrb3 and Gabra1, do produce sex-selective changes in behavior and seizures. The GABA receptor subunit β3 subunit is associated with childhood absence epilepsy and Angelman syndrome. The human GABAb receptor in childhood absence epilepsy missense mutations did not exhibit a female predominance in patients (Tanaka et al., 2008) and have not yet been reported in mice. However, the phenotype of a GABA receptor subunit β3 subunit deletion mouse has been described and shown to experience both sex-dependent and parent of origin-dependent effects. Male mice with maternally transmitted GABA receptor subunit β3 subunit deletion exhibited an increased abnormal synchronous theta activity that correlate with atypical absence seizure and impaired contextual memory compared with female mice with maternally inherited GABA receptor subunit β3 subunit deletions (Liljelund et al., 2005). In addition, male mice with a paternal deletion had more fast activity from 12 to16 Hz than male mice with a maternal deletion (Liljelund et al., 2005).

Several loss-of-function mutations in the GABAa receptor α1 subunit have also been associated with genetic generalized epilepsy syndromes in human patients (Cossette et al., 2002; Lachance-Touchette et al., 2011; Maljevic et al., 2006). Arain et al. 2012 reported that heterozygous α1 subunit deletion caused electrographic and behavioral absence-like seizures in both the DBA/2J and C57BL/6J strains of mice. In the DBA/2J strain, heterozygous α1 subunit deletion did not reduce viability in either sex and produced the same frequency of spike-wave discharges in male and female mice. However, in the C57BL/6J strain, female, but not male. Moreover, female heterozygous knockout mice had a significantly greater incidence of SWDs than male heterozygous knockout mice. These experiments were performed in mice of age postnatal days from 33 to 37 PND their first estrus, they do typically exhibit several signs of sexual maturity. Even though changes in GABAa receptor composition, physiology, and cell surface endocytosis have been observed in female heterozygous α1 subunit deletion mouse (Zhou et al., 2013) the corresponding changes have not yet been explored in male mice and thus the mechanisms that underlie these sex differences remain unknown.

The *taiep* rat as a model of absence seizures

In 1989 Holmgren et al., at the Institute of Physiology, Benemérita Universidad Autónoma de Puebla described a spontaneous mutant from Sprague-Dawley rat during the inbreeding process to obtain a subline with high-yawning frequency (Urbá-Holmgren et al., 1990). The rats are named *taiep* in base of the acronym of the neurological signs that appears along the first year of life such as tremor at weaning, ataxia at 2 to 3 months of age, immobility episodes with a peak at 8 to 9 months age, epilepsy and finally hindlimb paralysis after one year of life (Holmgren et al., 1989; Cortes et al, 1995). The pathology had an autosomal recessive inheritance.

At the ultrastructural level taiep rats had an accumulation of microtubules in the cytoplasm and its processes (Duncan et al., 1992), particularly microtubules are physical bounded to endoplasmic reticulum suggesting an alteration of the transporting mechanism in this glial cell (Couve et al., 1997). In fact, all the major proteins of myelin are present but with lower levels including myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG), myelin-associated oligodendrocyte basic protein (MOBP) and the 2´,3´-cyclic nucleotide 3´-phosphodiesterase (CNP) and the decrease with the age of *taiep* rats (Möller et al., 1997). The total lipid component of myelin also decreases with the age of *taiep* rats

(Eguibar et al., 2012). This decrease correlated with the progressive demyelination in the central nervous tracts such as corticospinal and dorsal columns in the spinal cord (Lunn et al., 1997).

Recently, our research group demonstrated that *taiep* rats is the only available model of the human leukodystrophy named hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC), because using magnetic resonance imaging (MRI) had a hypomyelination in the corpus callosum associated with ventriculomegaly and atrophy of the cerebellum and the caudate nucleus (Garduno-Robles et al., 2021). In fact, *taiep* rats had mutation in exon 4B of the tubulin β 4A with a Guanine to Adenine point mutation (g.6337G>A). At the amino acid level, the *taiep* mutation changes an Alanine by a Threonine at position 302 (Garduno-Robles et al., 2021; Lopez-Juarez et al., 2021; Alata et al., 2021). Until now, there are 40 different mutations of TUBB4A have been found, being the p.Asp249Asn the most frequent in humans' patients with H-ABC (Curiel et al., 2017); and this is also the mutation that our group identified in the first Mexican patient diagnosed with H-ABC (Lopez-Juarez, 2021). The auditory brainstem evoked potentials are delayed in the central components and are similar to a Mexican human patient (Lopez-Juarez et al., 2021; Roncagliolo et al., 2000), and recently we already demonstrated that somatosensory evoked potentials showed similar altered pattern in human patient and in male taiep rats (Eguibar et al., submitted for publication).

In the first report of Holmgren et al. (1989) reported that taiep rats had audiogenic seizures induced by a loud buzzer of 103 dB applied by 30 seconds and tonic-clonic discharges appeared that increased with the age of subjects from 6.25% in *taiep* rats between the ages 2 to 6 months of age to 62.5% in *taiep* rats between 12 and 13 months of age (Holmgren et al., 1989). Further long-term electroencephalographic studies shown spike-wave discharges (SWDs) in the cerebral cortices that are similar to that already reported in WAJ/Rij and GAERS rat models (Eguibar and Cortes, 2010). Importantly the main frequencies of the SWDs had a main frequency at 6.25 Hz that is like both absence rat models and are also similar to absence discharges in human subjects (Eguibar and Cortes, 2010). Then we observed that male *taiep* rats had more SWDs with respect to female counterparts (Ibarra et al., submitted for publication). In fact, at 3 months of age male rats had SWDs but nor the female counterparts, while in *taiep* rats of 9 months or older both sexes had SWDs (Ibarra et al., submitted for publication). In base of that our research group analyze the possible organizational or activational role of androgens in SWDs using orchiectomy after birth, at 5 PND, or in adulthood, at 90 PND. Neonatal orchiectomy

significantly decreased the incidence of SWDs along the circadian cycle and the opposite happen with adult orchiectomy that significantly increased the frequency of SWDs, with marginal effect in the mean duration of SWDs in both groups of spayed male *taiep* rats (Cortes et al., 2022). In preliminary studies systemic administration of testosterone propionate in orchiectomized adult male rats did not significantly increase the frequency of SWDs, but further experiments are need it to fully address the role of different androgens in the incidence and mean duration of SWDs.

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