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Sexual Differentiation of the Brain and ADHD: What Is a Sex Difference in Prevalence Telling Us?

Jaylyn Waddell and Margaret M. McCarthy

Department of Physiology, School of Medicine, University of Maryland, Baltimore, MD 21201, USA

Jaylyn Waddell: jwadd001@umaryland.edu

Abstract

Sexual differentiation of the brain is a function of various processes that prepare the organism for successful reproduction in adulthood. Release of gonadal steroids during both the perinatal and the pubertal stages of development organizes many sex differences, producing changes in brain excitability and morphology that endure across the lifespan. To achieve these sexual dimorphisms, gonadal steroids capitalize on a number of distinct mechanisms across brain regions. Comparison of the developing male and female brain provides insight into the mechanisms through which synaptic connections are made, and circuits are organized that mediate sexually dimorphic behaviors. The prevalence of most psychiatric and neurological disorders differ in males versus females, including disorders of attention, activity and impulse control. While there is a strong male bias in incidence of attention deficit and hyperactivity disorders, the source of that bias remains controversial. By elucidating the biological underpinnings of male versus female brain development, we gain a greater understanding of how hormones and genes do and do not contribute to the differential vulnerability in one sex versus the other.

Keywords

Development; Estradiol; Gonadal steroids; Sensitive periods; Sexual differentiation

1 Hormone-Defined Critical Periods of Development and Pathology

Three basic subtypes of Attention-Deficit/Hyperactivity Disorder (ADHD) describe the two basic dimensions of the disorder: inattention and hyperactivity/impulsivity (American Psychiatric Association 2000). The predominantly inattentive type of ADHD is associated with academic impairment, and is the most common (American Psychiatric Association 2000). The predominantly hyperactive/impulsive and combined subtypes are strongly predictive of externalizing behaviors, characterized by aggression and disruptive behavior (Graetz et al. 2001, 2005). In clinically referred populations, males are estimated to suffer from ADHD 2:1 to 9:1 compared to females (Anderson et al. 1987; Bird et al. 1988). However, sampling of nonreferred populations suggests the incidence may not differ, but rather reflects lower clinical referrals in females (Rucklidge 2010). More generally,

pathologies including symptoms of hypermobility, impulsivity, aggression, and disruptive behaviors are more common in boys, thus increasing comorbidity of disruptive behavior disorders in males significantly above that in females (Abikoff et al. 2002; Gaub and Carlson 1997; Rucklidge 2010). Overall, males are more likely to suffer from disorders that manifest early in development, such as ADHD and learning disabilities (American Psychiatric Association 2000), whereas females are more likely to develop mood disorders with a later onset, such as depression (American Psychiatric Association 2000; see Martel et al. 2009 for review). Critical periods of gonadal steroid release correspond to this divergence at the onset of psychiatric disorders in males and females. It is not possible to effectively separate biological variables from societal and cultural influences on human brain development and behavior. Conversely, discerning the impact of experience and external influence on brain development in animal models is modestly successful, at best, but biological variables are readily explored and provide insight into the cellular basis for both variability and vulnerability.

Comparison of the developing male and female brain presents an opportunity to assess potential mechanisms of developmental differences between the sexes. As the brain prepares itself for successful reproduction, gonadal steroids can contribute to pathology by increasing vulnerability, and exacerbating symptoms in males earlier in development. ADHD is a complex developmental disorder characterized by a high degree of heritability (Faraone et al. 2005). Despite the reported sex bias in incidence, none of the genetic loci identified to date as potential contributors to the disorder are located on the X or Y chromosome (Sharp et al. 2009). The genetic contribution to ADHD is multifactorial, with each gene making small but significant contributions to overall risk. The sex bias in prevalence suggests a point of convergence of genetic and hormonal influences. Thus, high circulating concentrations of gonadal steroids in the developing male may be an additional risk factor, interacting with genetic predisposition.

Although controversial, Geschwind proposed that males are at a higher risk for learning disabilities and hyperactivity because testosterone slows development of the brain in early development, rendering males vulnerable to insult for longer periods of time, allowing a wider range of behavioral outcomes (Geschwind and Galaburda 1985; Morris et al. 2004). Consistent with this view, high levels of testosterone in the perinatal period leads to increased neural lateralization, by promoting cell death in the right hemisphere of the brain, and slowing development of the left hemisphere (Geschwind and Galaburda 1985; Goodman 1991). Functional brain imaging of ADHD-diagnosed children reveals reduced volume of the right hemisphere as well as abnormalities of the corpus callosum (reviewed in Seidman et al. 2005). Smaller brain volumes appear to be fixed in ADHD patients across childhood and adolescence suggesting that genetic and early life experiences that contribute to ADHD induce seemingly permanent changes in the brain (Castellanos et al. 2002). Thus, enhanced lateralization of the brain may render cognitive abilities less flexible and permit some of the paramount symptoms of ADHD, such as impulsivity. Indeed, adult females exhibit flexibility in learning strategy in response to fluctuations in estrogens that is not evident in males (Korol and Kolo 2002). This flexibility relies on the absence of perinatal gonadal steroids. In humans, the menstrual cycle drives performance differences in sexually dimorphic tasks, particularly those related to motor and spatial ability (Hampson and

Kimura 1998). Gender-specific patterns of lateralized activity have been demonstrated in language processing tasks as well, with females exhibiting greater hemispheric connectivity (Bitan et al. 2010). Although the relationship between hemispheric connectivity and cognitive ability is not completely clear, these results highlight sex differences in nonreproductive areas involved in cognition (Bitan et al. 2010).

Gonadal steroids define developmental sensitive periods of plasticity that shape reproductive success in adulthood (e.g., Phoenix et al. 1959; Rhee et al. 1990a, b). Organization of the male brain is achieved, in part, by two critical periods of elevated testosterone. In rodents, testosterone surges during the last few days of gestation in male fetuses and remains elevated until shortly after birth (Fig. 1; Corbier et al. 1992; Weisz and Ward 1980). In humans, testosterone is elevated in the fetus during the latter portion of the second trimester and remains high for several months after birth but subsequently declines to undetectable levels. During this hormonally defined sensitive window, testosterone, and its metabolites, estradiol and dihydrotestosterone (DHT), evoke permanent changes in brain structure and function (MacLusky and Naftolin 1981; Phoenix et al. 1959). A second postnatal critical period for sexual differentiation of the brain occurs in both sexes during the peripubertal phase. This is characterized again by a surge in testosterone in the male and the onset of the estrous (animal) or menstrual (human) cycle in the female (Schulz and Sisk 2006; Schulz et al. 2004). At this time, social and mating behaviors are shaped by both hormones and experience, refining further the neural circuitry involved in reproductive success.

Most of our current understanding of sexual differentiation of the brain comes from the study of animal models, especially laboratory rats and mice. Sex differences in the brain are most robust and reliable in brain regions directly involved in sex behavior, such as specific nuclei of the hypothalamus. These differences can be volumetric, meaning a region is larger in one sex. Alternatively, or in addition to volume differences, sex difference may be connective, such that the type or amount of synapses, or size of a particular projection, differs between males and females. Sex differences are also found in the amount of neurochemicals, or neurotransmitters, or the intrinsic excitability of particular classes of neurons (e.g., Nunez and McCarthy 2007; Perrot-Sinal et al. 2003; Davis et al. 1999). In rodents, estradiol is synthesized, by the enzyme aromatase cytochrome P450, from testicular derived androgens, and mediates many of the sex-specific brain differences that endure through the lifespan (for review, see McCarthy 2008). Male rats castrated shortly after birth exhibit adult sex behaviors characteristic of females following estrogen and progesterone administration. If castration occurs later, after the first few days of life, males do not exhibit female sex behaviors (Fig. 2). In parallel, females exposed to high levels of testosterone or estrogen any time between late gestation and the first 10 days or so of life will not exhibit typical female sex behavior as adults. Instead, the hormonally masculinized female exhibits male sex behaviors when treated with testosterone in adulthood. Testosterone or estradiol exposure after this sensitive developmental window does not change adult female sex behavior (Gerall et al. 1992; Weisz and Ward 1980). Thus, as outlined by Phoenix et al. (1959), gonadal steroids act within critical windows of development to change the brain permanently to support male or female sex behavior in adulthood.

2 The Hypothalamus: A Model of Many Mechanisms

The mechanisms by which the brain achieves both the male and the female patterns of sex behavior are diverse. Many of these mechanisms are exemplified in the developing hypothalamus (McCarthy 2008; Schwarz and McCarthy 2008). Within the hypothalamus there are dissociable zones with distinct patterns of connectivity. The periventricular zone of the hypothalamus is situated along the medial ventricular wall, where sensitivity to circulating peptides and gonadal steroids is maximal and most direct (Simerly 2002). From this vantage point, the periventricular zone regulates steroid secretion from the anterior pituitary and is characterized by dense, internally reciprocal connections (Simerly and Swanson 1988; Simerly 2002). This region is also connected to extrahypothalamic structures involved in olfaction, pheromone processing, and social behavior (see Swanson and Petrovich 1998 for review). The medial zone of the hypothalamus innervates the periventricular zone and is reciprocally connected to limbic pathways (Canteras and Swanson 1992; Risold and Swanson 1987). Through these diverse connections, the hypothalamus integrates neuroendocrine responses to environmental cues and helps to guide motivated behavior. The hypothalamus is critically involved in circuits that regulate the response to stress and reward and is a critical mediator of sex behavior. It is not surprising that this brain region is characterized by sexual dimorphisms in morphology and connectivity.

The preoptic area (POA) lies just rostral to, and is closely associated with, the hypothalamus. Neurons located here mediate both male and female specific behaviors that are critical for successful reproduction and rearing of offspring (Numan and Numan 1995; Numan and Callahan 1980; Simerly 2002). In the male, the POA is critical for copulatory behavior, including mounting, intromission, and ejaculation (Christensen et al. 1977). In the adult female, this area supports maternal behaviors, such as nest-building, pup retrieval and protection of offspring (for review, see: Numan 2006). To support these sex-specific behaviors, the POA and other subregions of the hypothalamus undergo critical periods of plasticity, driven largely by exposure to gonadal steroids in the same manner as seen for the hypothalamus and both regions are nexus points for the integration of social behaviors that involve motivation and reward.

In rodents, aromatization of testosterone into estradiol initiates complex interactions between cells to build the circuitry necessary for reproduction. The arcuate nucleus (ARC) lies within the periventricular zone of the hypothalamus, where it regulates release of the gonadotropin hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), from the pituitary (Ojeda and Urbanski 1994; Simerly 2002). Astrocytes in the ARC of neonatal rats exhibit sexually dimorphic morphology. Astrocytes provide metabolic substrates to neighboring neurons and maintain homeostasis in the extrasynaptic space through regulation of glutamate recycling (Brown and Ransom 2007; He and Sun 2007; Nave and Trapp 2008). Beyond these roles, accruing evidence suggests that astrocytes secrete trophic factors as well as neurotransmitters that shape the number and strength of neuronal synapses or spines (Barker and Ullian 2010; Christopherson et al. 2005; Hamilton and Attwell 2010; Kozlov et al. 2006; Ullian et al. 2001). Dendritic spines are the direct source of excitatory signals to neurons; differences in spine number and shape determine

electrical and chemical activity in the neuron (Hayashi and Majewski 2005; Bourne and Harris 2008; Sorra and Harris 2000). The ARC of neonatal male rats has more fully differentiated astrocytes, characterized by long, thin processes and many branches (Mong et al. 1996). This sex difference is the direct result of higher testosterone in developing males (Mong et al. 1996) and is coupled with decreased neuronal spine density within the ARC (Mong et al. 1999, 2001). These data suggest that astrocytes participate in synaptic patterning by guiding or blocking the path of neurites. Thus, modulation of astrocyte differentiation affects circuit structure and activity. Sexual differentiation of such mechanisms may contribute to sex differences in motivated behavior (e.g., Becker 2009).

Astrocyte morphology in the male POA is also more complex than in females. Male astrocytes have more branches and longer primary processes and like other subregions of the hypothalamus, astrocyte complexity can be masculinized by estradiol, as can the higher density of dendritic spine synapses (Amateau and McCarthy 2002). Estradiol enhances astrocyte complexity in the POA but it is not known whether this is necessary for the action of estradiol on neuronal spines. Estradiol increases neuronal spine density in the neonatal male POA through upregulation of cyclooxygenase-2 (COX-2; Amateau and McCarthy 2002, 2004), the rate-limiting enzyme that converts arachidonic acid to prostaglandins (Brock et al. 1999; Yang and Chen 2008). Prostaglandins mediate inflammation and fever, but are also emerging as regulators of synaptic plasticity in both the developing and the adult brain (Vidensky et al. 2003; Yang and Chen 2008).

A current working model proposes that estradiol acts first in neurons to increase COX-2 concentration and activity, which increases PGE2 release from neurons to act on neighboring astrocytes. This, in turn, increases glutamate release from astrocytes and increases neuronal spine density (Bezzi et al. 1998; McCarthy 2008). Estradiol administration to neonatal females increases COX-2 and PGE2 levels to that of males in the developing POA, and is sufficient to enhance astrocyte complexity and neuronal spine density (Amateau and McCarthy 2002, 2004). Further, female pups, treated with PGE2 within the first few postnatal days, exhibit male-typic sex behavior in adulthood. Likewise, inhibition of COX-2 in the neonatal male brain results in a profound disruption of sex behavior (Amateau and McCarthy 2004). These results predict that males are more vulnerable to insult from commonly used COX inhibitors (nonsteroidal anti-inflammatory drugs), such as aspirin. In brain areas not directly involved in reproduction, such as the hippocampus, COX-2 also modulates excitatory glutamatergic transmission, although estradiol does not appear to change COX-2 activity in the neonatal hippocampus (Amateau and McCarthy 2004). Nevertheless, the action of COX-2 is modulated by a number of stimuli, including seizures, brain injury and NMDA receptor activation (Yang and Chen 2008), but the role of this ubiquitous signaling system in normal brain development remains poorly understood.

Estradiol establishes higher excitatory synapse density in the male mediobasal hypothalamus through mechanisms that differ from those of the POA. Estradiol increases dendrite length and the number of excitatory synapses in the mediobasal hypothalamus via protein synthesis and protein synthesis-independent pathways (Schwarz et al. 2008). In presynaptic neurons, estradiol increases glutamate release which, in turn, activates MAP kinase pathways through

NMDA and AMPA receptors in the postsynaptic neuron. This sequence of pre- and postsynaptic modulation of excitation induces a protein synthesis-dependent increase in spine number (Schwarz et al. 2008). Direct pharmacological enhancement of glutamate transmission in the medial basal hypothalamus is sufficient to masculinize spine density. Thus, estradiol establishes masculine levels of spines by modulating glutamate release between neurons (Schwarz et al. 2008).

Administration of testosterone in the neonate also changes astrocyte and neuronal sensitivity to gonadal steroids in the adult. The adult female hypothalamus undergoes synaptic remodeling in response to changing estrogen levels across the phases of the estrus cycle (Garcia-Segura et al. 1994a). The intact male or masculinized female brain does not exhibit this plasticity in adulthood (Garcia-Segura et al. 1994b). Thus, neonatal differentiation of ARC astrocytes by testosterone or estradiol may suppress sensitivity to circulating hormones in the adult. Similar changes in hormone sensitivity in the adult brain induced by neonatal testosterone administration have been demonstrated in areas not directly involved in sex behavior, such as the hippocampus (Garcia-Segura et al. 1998; Gould et al. 1990; Leraneth et al. 2003; MacLusky et al. 2006; Woolley and McEwen 1992). Administration of estrogens does not change neuronal morphology in the male hippocampus, but can increase neuronal spine density in the female hippocampus (MacLusky et al. 2006). Thus, the perinatal period is a sensitive period during which gonadal steroids determine sensitivity to the adult hormonal milieu demonstrating the impact gonadal steroids have on the brain throughout the lifespan.

3 Hormones, Neurogenesis, and Apoptosis

Hormones can promote cell survival or cell death, depending on the brain region and sex of the animal (Forger 2006). Testosterone and its metabolites inhibit cell death in the perinatal POA, the bed nucleus of the stria terminalis (BNST) and the spinal nucleus of the bulbocavernosus (SNB), all of which are larger in the gonadally intact male (Arai et al. 1996; Breedlove and Arnold 1983; Gotsiridze et al. 2007; Guillaumon et al. 1988; Nordeen et al. 1985; Murakami and Arai 1989). In the rat, sex differences in cell death contribute to sexual dimorphisms in brain circuitry (Forger et al. 2004). The most striking example is the sexually dimorphic nucleus (SDN) of the POA (Gorski et al. 1978). The male SDN is 3–5 times larger than that of the female: this difference is driven by higher rates of cell death in females, postnatally which are due to a lack of testosterone and its metabolite, estradiol (Davis et al. 1996). In males, testosterone decreases cell death in the SDN, as well as other brain regions involved in sex behavior (Dodson and Gorski 1992; Gotsiridze et al. 2007; Nordeen et al. 1985; Murakami and Arai 1989; Davis et al. 1996). Castration of male rats at birth causes a reduction in SDN size. Conversely, administration of testosterone or estradiol increases SDN size in the newborn female rat (Dohler et al. 1982; Gorski 1984).

In most areas that have been studied, gonadal steroids modulate cell death through two proteins, Bcl2 and Bax (Forger et al. 2004; Holmes et al. 2009; Zup and Forger 2002; Zup et al. 2003). The Bcl2 protein family regulates apoptotic programs in many cell types (Merry and Korsmeyer 1997). Testosterone and its metabolites can increase Bcl2, which inhibits the apoptotic action of Bax, which is a second class of proteins in this family (Garcia-Segura et

al. 1998; Pike 1999; Zup and Forger 2002). Specific subpopulations of cells are vulnerable to cell death during specific critical phases and the impact of exogenous hormone administration depends on the brain region and sex of the animal. For instance, the anteroventral periventricular nucleus of the hypothalamus (AVPV) contains a greater number of dopaminergic neurons in females, which can be reduced to that in males by administration of testosterone or estradiol (Simerly et al. 1985; Waters and Simerly 2009; Krishnan et al. 2009). Deletion of Bax eliminates volumetric sex differences in the BNST and the AVPV (Forger et al. 2004).

Puberty is a second developmental period of brain organization that refines circuits that have sexually differentiated during early neural development (Schulz et al. 2009). During puberty, gonadal steroids profoundly shape social behaviors in both sexes (Schulz and Sisk 2006). At this time, both testicular and ovarian steroids mediate the addition of new cells in sexually dimorphic brain regions. The sexually dimorphic pattern of cell genesis parallels that observed perinatally: new cells are added in the brain regions of the sex that exhibits the larger volume in adulthood. Thus, more proliferating cells are detected in the pubertal female AVPV and, likewise, more proliferating cells are detected in the male SDN (Ahmed et al. 2006). The addition of new cells during the prepubertal period has not been directly tied to behavior or hormones but hormones during the pubertal transition further differentiate sexual behavior, aggression, and territoriality (Schulz et al. 2004; Schulz and Sisk 2006). Thus, gonadal steroids also define, in part, this sensitive period during which social behavior is shaped to support reproductive success in adulthood.

In rats, a distinct period of rough and tumble play begins to emerge around 19 days of age and continues to develop and peak between 25 and 40 days of age (Panksepp 1981). This play occurs at higher frequencies in male rats (Olioff and Stewart 1978; Panksepp 1981; Pellis et al. 1994). A complex repertoire of defensive and aggressive behaviors is shaped through social interaction. Deprivation of play fighting produces long-lasting deficits in social behavior, and increases displays of anxiety and aggression (Bell et al. 2010; Bock et al. 2008; Panksepp and Beatty 1980; van den Berg et al. 1999). This reflects a second critical period in plasticity, during which overabundant synaptic connections are pruned as development of the brain progresses (Zehr et al. 2006). The lack of permanence of some neural connections is highlighted by experience-evoked changes, through which stabilization of synapses and dendritic arborizations is refined (e.g., Rakic et al. 1986; Bock et al. 2005, 2008). In prefrontal cortical regions, which are extensively connected to the limbic system, neuronal morphology is shaped by stress and social isolation in the neonatal and adolescent rat (Helmeke et al. 2001; Ovtscharoff and Braun 2001; Poeggel et al. 2003; Bock et al. 2005). Stressors experienced in early development can be reversed or ameliorated through socialization during puberty (Bock et al. 2008). Interestingly, many children diagnosed with ADHD experience an amelioration of symptoms at puberty (Gittelman et al. 1985). This may relate to the maturation of the frontal cortex which modulates response inhibition, impulsivity, and dopaminergic reward circuits (Bell et al. 2010; Bock et al. 2008).

4 Hormonal Modulation of Excitation Via Effects on GABA

Males are more vulnerable than females to disorders of excitability and movement, such as epilepsy, Tourette's syndrome, ADHD, and Parkinson's disease (Haaxma et al. 2007; McHugh and Delanty 2008; Shulman 2007). An additional contributing variable to insult in males could be the enhanced neuronal excitation induced by steroids and manifest through multiple systems as highlighted above. Processes that lead to higher cellular excitation are inherently fraught with greater risk for disruption, either by inadvertently triggering cell death programs, or over-exuberant innervation and inappropriate pruning of extraneous synapses. Hormone-mediated sex differences in the developmental maturation of neurons in brain areas involved in cognition, movement, and motivation produce sex differences in the timing of sensitive periods such that events can differentially alter excitation between the sexes, in both direction and magnitude (Auger et al. 2001; Nunez and McCarthy 2007; Galanopoulou 2006, 2008a, b; Perrot-Sinal et al. 2003).

Excitation in the developing brain is mediated through both GABAergic and glutamatergic systems. In early cell development, GABA induces cell membrane depolarization and is the primary excitatory neurotransmitter in immature neurons. GABA also functions as a trophic factor, regulating neurite outgrowth and spine formation (Cancedda et al. 2007; Cherubini et al. 1991; Owens and Kriegstein 2002; Pfeffer et al. 2009; Plotkin et al. 1997; Sipila et al. 2006; Spritzer 2006). As cells mature, GABA gradually becomes inhibitory and glutamate emerges as the primary excitatory neurotransmitter (e.g., Ben Ari et al. 1997; Tyzio et al. 1999). Gonadal steroids play a complex role in this developmental progression. Here, we discuss the mechanisms of this "developmental switch" and differences in its developmental progression between the sexes. Across seemingly disparate brain regions, males switch from depolarizing GABA to hyperpolarizing GABA more slowly than females (Nunez and McCarthy 2007, 2008; Galanopoulou 2006, 2008a; Kyrozis et al. 2006; Perrot-Sinal et al. 2003, 2007).

The developmental progression, from excitatory to inhibitory GABA, is driven by the balance between two cation-chloride cotransporters: the $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransporter, NKCC1, and the K^+-Cl^- cotransporter, KCC2 (Riviera et al. 1999). Males have higher levels of NKCC1 protein in the first week of life relative to females in both the hypothalamus and the hippocampus (Nunez and McCarthy 2007; Perrot-Sinal et al. 2007). The sex difference in magnitude and duration of NKCC1 expression is robust in early postnatal life, but dissipates by 2 weeks of age (Galanopoulou 2008a; Nunez and McCarthy 2007; Perrot-Sinal et al. 2007). Sex differences in chloride transporter expression correspond to robust GABA-mediated sex differences in measures of Ca^{2+} -dependent cell excitability (Auger et al. 2001; Nunez and McCarthy 2008; Perrot-Sinal et al. 2003). Female hippocampal neurons exhibit desensitization to GABA stimulation and so do not respond to a second exposure to the GABA-A receptor agonist muscimol (Nunez and McCarthy 2008). This sex difference represents not only a difference in the magnitude of excitation, but also cellular adaption to GABAergic excitation. Application of gonadal steroids can reverse this, suggesting that androgens promote excitability in response to GABA by attenuating desensitization (Nunez and McCarthy 2007, 2008). Estradiol similarly promotes depolarizing responses to GABA

by extending the duration of expression of NKCC1 in the developing hippocampus (Fig. 3; Nunez and McCarthy 2007).

In the developing hippocampus, GABA-mediated excitation is critically involved in the incorporation of new neurons into circuits, followed by promotion of neurite outgrowth and synaptogenesis (Estrada et al. 2006; Ge et al. 2006). This influence of GABA on cell maturation and incorporation into mature circuitry is also important in adult hippocampal neurogenesis (Esposito et al. 2005; Tozuka et al. 2005). The sequential contribution of GABA and glutamate corresponds to the development of the dendrite and increase in soma size (Tyzio et al. 1999; Demarque et al. 2002). Perturbation of the temporal progression from GABA to glutamate-mediated excitation can induce profound changes in cell morphology (Barbin et al. 1993; Cancedda et al. 2007). Depolarizing GABA may promote cell development in synaptically silent, immature cells, through paracrine modulation of cell morphology (Barbin et al. 1993; Demarque et al. 2002). Excitability in the developing substantia nigra (SN) is similarly modulated by gonadal steroids (Galanopoulou and Moshe 2003; Galanopoulou 2006). The SN plays an established role in seizure control and movement (Giorgi et al. 2007; Iadarola and Gale 1992). Males are more likely to suffer from both seizure and movement disorders, suggesting a sexual dimorphism in this brain region (McHugh and Delanty 2008).

Developmental GABA responsivity in the SN is sexually dimorphic (Galanopoulou 2006; Kyrozis et al. 2006). For instance, 2 weeks after birth, the GABA-A agonist, muscimol, has a proconvulsive effect when injected into the male rat SN, and increases Ca^{2+} influx (Galanopoulou et al. 2003; Galanopoulou 2006; Sperber et al. 1987). The same treatment does not induce convulsions (i.e., seizures) or Ca^{2+} entry in female rat pups of the same age (Galanopoulou 2006; Veliskova and Moshe 2001). This difference is dependent upon testicular derived steroids (Veliskova and Moshe 2001). As in the hippocampus, expression of KCC2 is slower to develop in males than females (Galanopoulou et al. 2003). Both androgen and estrogen receptor expression differ between the sexes, and also differ across development within the SN (Ravizza et al. 2003). At birth, the male SN contains more androgen and estrogen receptors, and this pattern reverses by the first day of life (Ravizza et al. 2002, 2003). Administration of testosterone to females abolishes this sex difference (Ravizza et al. 2003). Testosterone or DHT, a potent androgen receptor agonist, upregulates KCC2 mRNA in both sexes, whereas estradiol downregulates KCC2 mRNA in males, but not females (Galanopoulou and Moshe 2003; Galanopoulou 2006). This opposing effect of androgen relative to estradiol suggests a complex steroid sensitivity that is dependent on the sex of the animal.

5 Hormonal Modulation of Excitation Via Effects on Dopamine

Gonadal steroids contribute to sex differences in dopaminergic circuits and transmission. These differences may be particularly relevant to sex differences in prevalence of ADHD as dopamine-modulating drugs are most widely used to treat ADHD. Because of their established role in reward, motivation, decision making and cognition, dopaminergic pathways and the prefrontal cortex have been the subject of intense study in regard to the neurobiology of ADHD (e.g., Seidman et al. 2005). Ascending dopaminergic projections

from the ventral tegmental area (VTA), which have a key role in reward pathways, guide motivated behaviors that differ between the sexes, such as rearing of offspring (Becker 2009). These circuits are modulated by gonadal steroids during both perinatal development and puberty. Exposure to steroids at these critical phases of development induces sex differences in the effects of hormones in the adult (Kritzer 1997, 1998, 2003; Stewart and Rajabi 1994). In the neonate, testicular derived steroids are necessary for masculine neuronal architecture and neuronal survival in both the VTA and the SN (Becker 2009). Projections from these dopaminergic midbrain structures to prefrontal cortex and other association cortices are sexually dimorphic (Kritzer and Cruetz 2008). Castration at birth reduces catecholamine activity in the prefrontal cortex and cortical regions regulating motor control and attention (Kritzer 1998; Stewart and Rajabi 1994). Furthermore, removal of perinatal steroids through castration also produces hemispheric differences in dopamine projections to cortical circuits, suggesting that these pathways are lateralized in a sex-specific manner (Kritzer 1998). The BNST, a sexually differentiated area of the limbic system, sends excitatory projections to the VTA (Hines et al. 1992; Jalabert et al. 2009), which have been directly tied to impulsive behavior in animal models of drug-seeking behavior (Aston-Jones and Harris 2004). Precisely how gonadal steroids might contribute to sex differences in this projection remains largely unexplored.

Sex differences have also been noted in the distribution and density of dopamine receptors in the striatum, the nucleus accumbens, and the prefrontal cortex during juvenile development in rats, with males exhibiting a much higher increase in receptor expression during puberty than females (Andersen and Teicher 2000). Symptoms of ADHD often attenuate during and after puberty, suggesting that gonadal steroids may shape the disorder across development (Gittelman et al. 1985). As noted above, social experience during puberty can attenuate some deleterious effects of early-life stress (Bock et al. 2008). It is possible that those children receiving treatment, whether psychotherapeutic or pharmacological, achieve healthy social interactions during this second critical window.

6 Conclusion

Although it is clear that perinatal exposure to testosterone and its metabolites influence neural development through a myriad of mechanisms, the contribution of steroids to sex differences in prevalence of pathology is not well understood. ADHD is characterized by inattentiveness, impulsivity, and hyperactivity (American Psychiatric Association 2000). If sex is a predictor of pathologies such as ADHD, then we predict that aberrant developmental processes will occur during periods of dynamic changes in exposure to gonadal steroids. In males, there is a perinatal sensitive period of elevated androgens and estrogens that females do not experience, and this is a dynamic period for cell birth, death, differentiation, and synaptogenesis. Puberty is a second period of dynamic steroid hormone profiles, with females experiencing dramatic increases in estrogens and progesterone, which are themselves dynamic due to the cyclical nature of female reproduction, while males have a resurgence in androgen production which remains elevated throughout adulthood. The onset of puberty, and its attendant hormonal changes, is considerably earlier in females: as much as 5 years is normal in humans. This, combined with different maturation rates for distinct portions of the brain in males and females, creates a complex and variable developing brain

that is further influenced by experience, environment, and behavioral expression itself. Understanding the relative contribution of each variable to normal brain development in males versus females, and to then translate this into clinically meaningful predictors or indicators of pathology, is a goal best achieved by studying each in isolation as well as an integrated whole.

Abbreviations

ARC	Arcuate nucleus (of the hypothalamus)
AVPV	Anteroventral periventricular nucleus of the hypothalamus
BNST	Bed nucleus of the stria terminalis
COX-2	Cyclooxygenase-2
FSH	Follicle-stimulating hormone
KCC2	K ⁺ -Cl ⁻ cotransporter
LH	Luteinizing hormone
MAP	Mitogen-activated protein (kinase)
NKCC1	Na ⁺ -K ⁺ -2Cl ⁻ cotransporter
POA	Preoptic area
SDN	Sexually dimorphic nucleus
SN	Substantia nigra
SNB	Spinal nucleus of the bulbocavernosus
VTA	Ventral tegmental area

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Fig. 1.

Sexual differentiation of the brain is a consequence of sex differences in gonadal steroid synthesis during both perinatal and peripubertal sensitive periods. In male rats, the production of testicular androgens begins prenatally, around embryonic day 18, and defines the beginning of the perinatal sensitive period. During this time, the female ovary is quiescent. Sex differences in brain and behavior in adulthood are largely determined by the actions of steroids during the perinatal period, with an additional organizational effect at puberty.

Fig. 2.

Testosterone and its aromatized product, estradiol, in the perinatal period are necessary for expression of masculine sex behavior in the adult. Testicularly derived steroids organize the hypothalamus and other brain areas to support mounting of the female, intromission and ejaculation in adulthood. The absence of androgens and estradiol is necessary for normal development of the female brain. This absence of steroids is necessary for the sexually receptive posture in the adult female, termed lordosis. Administration of exogenous testosterone to the neonatal female induces masculinization of adult brain and behavior. Likewise, removal of male gonadal steroids by castration in the neonatal male induces feminized sex behavior in the adult male

Fig. 3.

GABA is largely excitatory in the developing brain. This is due to a shift in the reversal potential for Cl^- . Administration of the GABA-A agonist, muscimol, opens Cl^- channels, resulting in Cl^- efflux and depolarization of the cell. This depolarization is sufficient to activate voltage-gated Ca^{2+} channels and allow influx of Ca^{2+} . Gonadal steroids enhance the excitatory actions of GABA in immature cells manifesting as increased Ca^{2+} entry, increased numbers of cells exhibiting excitation in response to GABA-A agonists and extension of the developmental expression of depolarizing GABA. Gonadal steroids increase the amount and activity of the $Na^+-K^+-Cl^-$ cotransporter, NKCC1, possibly through its phosphorylation. Depolarizing action of GABA is more protracted in the developing male brain relative to females, suggesting that the absence of gonadal steroids allows this earlier switch in females