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Review

Steroidal influences on anxiety disorders in childhood and their animal models

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ABSTRACT

We are interested in the interaction of steroids and behavior, including psychiatric disorders, as revealed by animal models. Here we use a small slice of steroid - psychopathology interactions as a heuristic template for research in the broader field. Specifically, the review examines anxiety in relation to the sex steroidal changes prior to puberty. Ontogeny of psychopathology is understudied in children and has been largely neglected by animal researchers. This is likely due to the common belief that psychiatric disorders are the exclusive domain of humans after achieving puberty. Yet, most major psychiatric disorders have been documented in children, and anxiety is the most commonly occurring disorder in children. Many of the same factors that predispose adults to psychopathology have their antecedents in childhood. This review is to examine the literature on steroids and the emergence of anxiety in children and prepubertal laboratory animals. The literature on hormonal relations to anxiety in children or in young animal models is small and requires tying together disparate studies to reveal patterns that suggest topics most in need of experimental attention. The primary conclusions are that there are sex differences in most forms of anxiety in children, most commonly with girls expressing the disorders at an earlier age and in greater number than boys. Findings from the animal literature largely support that conclusion. Endocrine factors underlying these sex differences may include fetal organization from androgens or estrogen, or subtle effects of current gonadal sex steroids during prepuberty. However, the low circulating levels of the gonadal hormones in juvenile animals and children recommended that we look elsewhere for an endocrine mechanism. By contrast, adrenal steroids androstenedione, dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) increase well before the pubertal rise in gonadal hormones. We suggest that this event, the adrenarche, is the most likely endocrine factor contributing to anxiety in youngsters.

KEYWORDS: prepuberty, juveniles, children, dehydroepiandrosterone (DHEA), psychopathology

INTRODUCTION

Traditional views have held that children are incapable of suffering from psychiatric disorders, and a diagnosis and pharmacological treatment of mood or anxiety disorders in a child is often met with skepticism by the lay public. Even for many clinicians and basic researchers in psychiatry, it comes as a surprise to learn the frequency of childhood psychopathologies. Indeed, in very young children from 2-5 years of age prevalence of mood and anxiety disorders has been estimated as high as 2.1% and 9.4%, respectively [1].

There is more acceptance, albeit begrudgingly, for adult-like behavioral disorders emerging with the onset of puberty. And, incidences of psychopathology in adolescents are typically

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higher than in prepubertal youngsters. The dramatic increase in depression with the rise in gonadal hormones, especially in girls, is well documented [2, 3]. Yet, anxiety disorders are one of the most common psychiatric disturbances of children 5 years and over with estimates ranging from 5-18% [4, 5]. Retrospective studies of adults being treated for anxiety recall the age of onset was often during the prepubertal years [6]. Early anxiety is the highest risk factor for later comorbidity with debilitating depression [7], as well as for substance abuse and suicide [8].

Similar to the traditional views of childhood psychopathology, the prepuberty stage often is described as a period of endocrine quiescence in lab animals and humans [9]. Prepubertal individuals were believed to have low levels of circulating sex steroids with minimal morphological and functional effects and no observable differences between young males and females. Today, it is clear that a child undergoes significant hormonal influences well before puberty.

The influences of sex hormones on behaviors of children, including psychopathologies, are best illustrated with correlational studies of human sex differences assessing the incidences, development and expression of clinically relevant disorders in children. The role of research with animal models is to extend our knowledge beyond simple correlations. For studies of sex differences, it means hormone manipulations and observing physiological and behavioral outcomes [10, 11].

Unfortunately, that role has not been adequately appreciated in studies of anxiety using lab animals. There are relatively few reports in the animal literature on the normal postnatal ontogeny of anxiety, much less sex differences or steroid hormones before the onset of puberty. It is our hope that this review will be a useful heuristic for research questions that deserve further study with animal models.

Other factors influencing sex differences in endocrinology and behavior

It is necessary at this point for us to acknowledge that there are many factors other than sex steroids that influence normal and pathological behaviors of young animals and certainly of children. There is now wide acceptance of environmental influences and epigenetic effects on function, including endocrine function, at all ontogenetic stages. Moreover, other endocrine and neural changes during infancy and prepubescence surely contribute to sex differences in psychopathology, including anxiety [12, 13].

Study of the role of neuropeptides in prepubertal individuals on non-reproductive behaviors is still in its infancy [14]. Better studied are the hypothalamic peptides and reproductive related functions. For example, gonadotropins likely have direct influence on the behaviors of youngsters. Although hypothalamic gonadotropin releasing hormone (GnRH) is closely related to the rise and fall of sex hormones in adults, it is not a direct coupling in youngsters. The characteristic pattern of GnRH secretion in young rats is sexually dimorphic. While GnRH levels and pituitary sensitivity to the gonadotropin is low in prepubertal males, their sisters experience high levels of GnRH and hypersensitivity of the pituitary. As puberty approaches, the patterns switch, with 30 day-old males showing greater sensitivity to GnRH along with an increase in pituitary GnRH receptors [15].

Sexual dimorphism of circuits during the organizational stage underlying anxiety is unmistakable. The neural response originates with activation of the amygdala and orbitomedial prefrontal cortex assigning emotional value to the environmental situation. Both brain regions project to a variety of brain systems responsible for the physiological and behavioral components of the emotional response [16].

Further, serotonergic, dopaminergic, opioidergic, and noradrenergic regulation of GnRH and the hypothalamic-pituitary-gonadal (HPG) axis show sex differences as a function of ontogenetic stage. As but one example, capacity for synthesis of serotonin in young children is more than 200% of adult values until the age of 5 years and then decline toward adult values [17]. Serotonin synthesis capacity values decline at an earlier age in girls than in boys.

Sexual dimorphism is also found in young animal models in neurotransmitter systems related to mood. In the developing female rat serotonin

induces the release of pituitary luteinizing hormone (LH) from 12 days of age onward. The maximal response is observed between 15-18 postnatal days old (PND 15-18), and then decreases gradually as the rat nears sexual maturity. On the other hand, serotonin in male rats does not appear to induce the release of LH during prepubertal stages [15].

There are other neuroendocrine changes during development that contribute to psychopathological outcomes. Of particular importance are corticosteroids and the peptides of the hypothalamic-pituitary-adrenal (HPA) axis in mood disorders. There are already several fine reviews of those relations across ontogenetic stages [18-20]. However, the role of corticosteroids in prepuberty may not be a factor. No statistically significant sex differences in cortisol were observed in children and adolescents aged 8-16 yrs old suffering from anxiety, including the typical rise in cortisol after awakening observed in adults. Although the girls had baseline values of cortisol that appeared higher than the boys, the variability of values in the girls prevented the differences from achieving statistical significance [21].

We maintain that examination of sex steroid effects is always a good starting point upon observing sex differences in function, including behaviors. And there are definite gender differences in psychopathology, including anxiety, in children, adolescents and adults. Here our focus is on sex steroids and anxiety in children prior to puberty, an ontogenetic stage often mistakenly considered one of endocrine quiescence.

Sex hormones in prepubertal individuals

Gonadal steroids in pre-pubertal children

Endocrine-based sex differences in behavior arise at distinct stages of development. The fetal stage is an active endocrine period, as described in the organizational - activational model [22]. Sex hormones, most importantly testosterone, organize as masculine the brain and bodies of fetal males. The absence of testosterone, and likely the presence of estrogen, feminizes the fetal female. Later, dramatic surges of circulating androgens, estrogens and progesterone at the pubertal stage activate the previously organized brain circuitry and peripheral structures.

The organizational - activational model suggests that the period between birth and puberty is a time of endocrine quiescence. Indeed, measurements of circulating gonadal steroids in childhood and young lab animals show low levels of gonadal hormones.

As depicted in Figure 1 (adapted from [23]), the concentrations of estrogen (E2), progesterone (PROG) and testosterone (TS) hormones in boys and girls undergo modest changes before the age of 9-10 yrs [24]. Nonetheless, the values for first few months of life are unexpected. This “neonatal surge”, the term used to describe the relatively high circulating titers of TS in newborns, remains elevated for weeks or months [25, 26]. Cells isolated from the postnatal human testis produce more TS and have better responsiveness to LH during the early months than during later childhood [27]. The downstream consequence is that estradiol, as a TS metabolite, is also higher than expected in the newborn. It is notable that a similar surge is observed in neonatal rodents [28].

The function of the neonatal surge is not known, but one suggestion is that postnatal androgen secretion prior to PND10 permanently alters

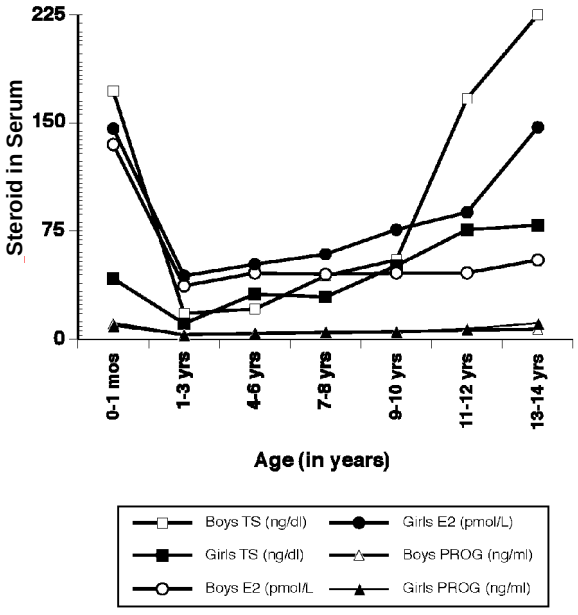

Figure 1. Circulating levels of estrogen (E2), progesterone (PROG) and testosterone (TS) in boys and girls from birth to adolescence (adapted from [23]).

patterns of estrogen receptor (ER) expression in the brain [29]. Neonatal males castrated immediately after birth exhibited higher ER levels at PND 28 in the preoptic area and the ventromedial and arcuate nuclei of the hypothalamus than either control males or males castrated on day 10. Females injected with TS at birth exhibited reduced ER binding in the same brain regions.

After the neonatal surge, sex hormone titers are low during the remaining prepubertal period. Yet, there is evidence that their gonads are surprisingly active. Testicular volume of boys increases 3-fold from birth to the onset of puberty and, perhaps most important, the prepubertal testis synthesizes steroidogenic enzymes capable of converting precursors to bioactive steroids [30]. Studies with lab animals demonstrate that gonadally intact males and males castrated during the juvenile period show differences in neuroanatomy, physiology and behavior [10, 31-33]. A resolution may be to conceptualize an endocrine organizational stage that extends into the prepubertal period [34].

To summarize, upon recognizing that circulating gonadal hormones prior to puberty are low and unlikely to activate functional changes, research attention often turns to fetal hormonal effects. It may be misleading, however, to attribute all gender differences observed in youngsters prior to puberty to the legacy of organizational effects of sex hormones during fetal life [35]. Not only are the prepubertal gonads likely functional, other steroids also may play a biologically significant role. Examination of behavior - endocrine relations in prepubertal individuals indicates more complexity than might appear on the surface.

Gonadal hormones in young animal models and the problem of translation

It is clear that rodents are a defensible choice as an animal model of steroid - behavior relations for prepuberty. Similarly to humans, gonadal steroids remain at relatively low circulating levels in prepubertal rats and mice [36, 37]. The initial issue facing the translation of research findings from the animal lab to the clinic is selecting the appropriate ages that model pre-adult humans. That issue points out the absence of agreement on terminology defining ontogenetic stages in the rodent models.

In common parlance for humans, childhood refers to the pre-puberty period following infancy, both being the domain of pediatric medicine. Terms for the pubertal and post-pubertal human include adolescent, teenager or, sometimes, juvenile. There is no designation in the rodent literature similar to childhood or children. Instead, adolescent and juvenile are terms used interchangeably to describe a young animal during several distinct ontogenetic stages. Researchers often use adolescents or juveniles to identify both pre- and post-pubescent rodents [38, 39]. The result is rats and mice being designated as adolescents or juveniles whose ages range from weaning (circa PND 21) through the onset of early adulthood (PND 60) [40].

This leads to translational confusion because the endocrine changes signaling puberty that distinguishes childhood from adolescence in humans is approximately half way into the PND 21 - PND 60 period in rodents. The pubertal rises in gonadotropins and resulting sex steroids in rats begin circa 40 days of age. Females experience the beginning of the hormonal surge a few days earlier and males a few days later than PND 40 [36, 41]. As a result, it is common to employ PND 35 animals identified as adolescents that likely have undetectable levels of gonadal hormones. Another potential problem of translation is that a PND 60 rat is almost universally designated in the literature as an adult despite physiological evidence of full maturity being reached at PND 100 or later [42]. It is surely more accurate to identify PND 60 rats as young adults.

In this review, we will use “juvenile” to describe prepubertal rodents 20-39 days old. We will reserve the “adolescent” designation for peri- and post-pubertal animals from 40-59 days of age [43]. Nonetheless, the reader is urged caution. In the animal literature, ages of animals at the time of testing often are not explicit.

A brief introduction to anxiety

Physiological and behavioral systems that promote vigilance to potential dangers, naturally, have survival value for human and non-human animals. Anxiety is the maladaptive expression of excessive, chronic vigilance that spills over into events confronted in everyday life.

Because anxiety-related behaviors can be viewed from a continuum of normal attention to pathological hypervigilance, diagnosing individuals who qualify as having crossed the excessive line to pathology is not easy. As is characteristic of most psychiatric conditions, there are no unique diagnostic biomarkers. Anxiety involves sympathetic activation with symptoms characteristic of fear, e.g., palpitations, trembling, and sweating [44], but these are not specific to anxiety disorders. The result is reliance on the standard resource for diagnosticians in the mental health field, the DSM [45]. Diagnoses in the DSM are based on behaviors, often self reported behaviors [46].

The DSM lists a dizzying array of behaviors under anxiety disorders, yet all anxieties have in common feelings of fear, worry and vigilant apprehension [47]. Another key feature linking the anxiety diagnostic categories is their comorbidity [48], often overlapping with each other and with major depression. Still, it is likely that each anxiety disorder has a distinctive neural profile [49, 50] and, perhaps, endocrine profile. A largely unmet goal in psychiatry is to verify the physiological differences and include the biomarkers alongside behaviors in the DSM.

For the most part, diagnostic criteria for psychiatric disorders were established for adults. Assessment of children and adolescents, consequently, are limited. As an example, the adult symptoms of PTSD include re-experiencing the traumatic experience, nightmares and excessive sympathetic arousal. Application to children may lead to over-diagnosis [51, 52]. Still, tracking of adult-defined anxiety and mood disorders from childhood to adolescence to adulthood is informative [6]. Indeed, one interesting hypothesis is that the same underlying neural dysfunction is expressed as anxiety during childhood and as depression during adolescence [53].

Clinically defined categories of anxious behaviors

There are at least five categories of anxiety disorders. **Generalized anxiety disorder** (**GAD**) is the most fundamental anxiety, and because GAD includes the basics of anxiety, its diagnosis is often difficult to distinguish from other anxiety disorders. GAD also has the highest comorbidity of the anxiety reactions to other mood disorders. Nonetheless, a distinguishing feature of GAD is a chronic background of worry which the individual finds difficult to control or ignore.

Panic disorder (PD) is the experience of bouts with extreme sympathetic nervous system arousal. PD is characterized by the person being unable to identify a particular triggering stimulus. Panic attacks are often observed in the other anxiety diagnostic categories.

Phobias, of which social phobia are a common diagnosis, often emerging early in life. Also known as **social anxiety disorder** (**SAD**) [54], social phobia has been one of the least understood and investigated anxiety disorders in children [55, 56]. Ironically, it is probably the most easily adapted for animal research.

SAD is a distinct disorder involving a marked fear or anxiety of behaving in an embarrassing manner while under the gaze of other people, leading to avoidance of social settings. The first of two SAD subtypes, non-generalized, is distinguished by being specific to a performance situation, public speaking for instance, while generalized SAD includes fear of a multitude of social and performance situations. Interestingly, often people expressing SAD will report being eager to make connections and attachments with others but are fearful of the adverse consequences of social interactions.

The reader should be aware that one will find in the psychiatric literature the use of the acronym SAD for other disorders, separation anxiety disorder and seasonal affective disorder. Here, SAD will refer exclusively to social anxiety disorder.

Post-traumatic stress disorder (**PTSD**) is marked by intrusive, anxiety-provoking memories of trauma. A diagnosis of PTSD requires both trauma exposure and symptom duration components. The trauma is one in which there is serious physical injury or the perception of a life threatening event. Symptomology of re-experiencing the trauma with frequent intrusive recollections, flashbacks or nightmares must remain for several months. Only a minority of individuals who have had a death threatening experience meet the duration criteria for a PTSD diagnosis [57]. This finding has led to intensive search for the risk factors that distinguish between the two groups, for example, prior trauma or childhood abuse [58, 59].

Finally, **obsessive-compulsive disorder**, (**OCD**) is characterized by unwanted, intrusive thoughts and images (obsessions) and repetitive, ritualistic behaviors (behavioral compulsions). The latter presumably serve to reduce the distress caused by the obsessions. The most common form of OCD is compulsive checking, involving the performance of routines related to security, orderliness, and accuracy but without resolution. In many ways OCD is one of the most disruptive psychopathologies to everyday function and, unlike many other anxiety disorders, untreated OCD usually fails to remit with the passage of time [60, 61].

Modeling anxiety

Most studies of anxiety with lab animals do not identify the specific form of the psychopathology that is being modeled. Likely, most could be considered related to GAD. Paradigms most often used to evaluate anxiety in rodents are the open field and the elevated plus maze (EPM). Both paradigms measure locomotor activity, and both are used to assess existing differences in temperament or experimentally induced timidity to novel situations [62].

The open field test is the oldest paradigm to examine locomotor activity, with the presumption of movement across checkerboard squares being inversely related to level of anxiety [63]. The EPM has four arms that are either covered or open. Time spent in the closed arms relative to the open arms is the primary measure of anxiety [64]. Sometimes crossings between closed and open arms are recorded as an additional indicator of anxiety. Although not without its critics [65], the EPM has become the standard paradigm in the research field.

Various forms of a light-dark box share features of both the open field and the EPM. The basic apparatus is divided into brightly lit or darkened compartments in which the animal is allowed to move freely. As with the EPM, the index of anxiety is time spent in the light relative to the dark compartment [66].

A unique but infrequently used animal model is the discovery that anxious rats will bury novel objects. Marble burying with or without an aversive stimulus is tested in a cage with thick bedding in which marbles have been introduced. Numbers of marbles buried are believed to be a behavioral marker of anxiety [67, 68].

Another is the acoustic startle response paradigm in which the intensity and latency of a startle response to a loud sound is measured [69, 70]. Although appearing to confound fear and anxiety, justification is that startle reflects a response to an existing, chronic state of anxiety that shows individual differences among animals.

Because of the subjective nature of the response, panic disorder is the least available to animal models. By contrast, SAD is easily adapted for study with lab animals either by genetic selection, drug administration, experimental induction or simply selection for individual differences [71, 72].

The typical paradigm used in preclinical studies of SAD is a test developed 30 years ago by File and her colleagues [73]. The social interaction (SI) paradigm was designed as a measure of anxiety in which environmental conditions can be altered to either suppress or facilitate social interaction depending on current anxiety levels of the animal. The amount of time the test animal spends interacting with another animal is measured over several encounters. The social interaction test has been used extensively as an ecologically valid assessment for new pharmacological compounds for social anxiety [74]. This social interaction test of anxiety has been validated extensively in adult male rats and is also suitable for use with adolescent males and females.

Methodologies to minimize direct physical contact between the animals have been developed to evaluate interest in social contact without the potential confounds of direct interactions, for example aggression. A “stimulus” animal is placed behind a mesh screen to provide visual, auditory and olfactory sensory information while preventing the confounds of aggression between same-sex animals and of sexual contacts between males and females [75]. An alternative is to eliminate the stimulus animal altogether by using, instead, only a Petri dish containing the soiled bedding of another animal and measuring olfactory investigation of the bedding [71].

In summary, there are a number of animal models targeted to mimic symptomology of specific anxiety categories. Still, the EPM is the most

commonly used apparatus today for the study of anxiety. That may be unfortunate when comparing adult male with female rodents and also may be problematic for assessing sex differences in young animals [76, 77].

The basic problem is that the EPM loses face validity in tests of sex differences. The adult human literature is unambiguous in reporting women having higher rates of anxiety than men. In animal paradigms using locomotor activity as the sole marker of anxiety, adult male rats are most often observed to have lower locomotion scores than females following an experimental manipulation. The suggestion is that males are more anxious than female rats [78, 79]. There are exceptions, for instance in gerbils [80] or with transgenic mice [81]. Nonetheless, the gender differences in the most common laboratory animal, rats, have repeatedly found females spending more time in the open arm of the EPM [35, 82, 83].

Closer scrutiny of these behaviors suggests several explanations. One is that baseline locomotor activity of female rats and mice is generally higher than the males [84], and the same pattern has been observed in prepubertal and peripubertal rats [85-87], [cf. 88]. Moreover, males show higher levels of freezing behavior [77] thereby reducing time in the open arm. A revealing study that included an endocrine measure of stress and anxiety, baseline corticosteroid, suggests that even in the presence of high anxiety, females spent more time in the open arm than males [89].

These observations suggest the EPM may be a valid test of anxiety within a gender. The validity of comparisons between males and females is more questionable. In those cases, other anxiety paradigms would seem preferable.

Anxiety in prepubertal individuals

Children and anxiety

It has been known since the early studies of community samplings [5] that pre-pubertal children experience clinically relevant anxiety disorders, and that there are gender differences. Childhood and adolescence are the core risk phase for the development of anxiety with the range from temporary, mild symptoms to a full-blown, chronic disorder [55]. As with adults, there is high comorbidity with other conditions. Rather than the comorbid depression observed in adults, attention deficit hyperactivity disorder (ADHD) and conduct disorder are most often comorbid with anxiety in children [90].

Most often 5 yrs is identified as the earliest age of onset for most anxieties. Studies of prevalence indicate that GAD and SAD are the most common forms in children [91]. Adults diagnosed with GAD and SAD often describe themselves as being anxious most of their lives with onset in childhood. By contrast, panic disorder is quite rare in children under 12 yrs old [48].

The initial signs of general anxiety can appear years before the onset of puberty. Researchers examining shy or behaviorally inhibited children suggest that it may begin in toddlers. For example, some parents of children 1.5 to 5 yrs old already described their children as nervous, high strung or tense, appearing to be worrying and anxious [8].

The classic findings of very early onset of a form of social anxiety are the longitudinal studies by Kagan and his colleagues of children exhibiting “behavioral inhibition” [92]. About 20 percent of children appear behaviorally inhibited by 2 yrs of age, defined by fear and withdrawal in unfamiliar situations. Perhaps the most remarkable discoveries among their many remarkable findings are that shyness detected at such an early age often remains stable through adolescence and into adulthood and that regional brain correlates of behavioral inhibition are observable throughout the entire period [93].

The importance of early detection and treatment is highlighted by psychiatric disorders in childhood predicting both adolescent and adult disorders [4]. Behavioral inhibition in childhood appears to be an enduring trait that may persist into adulthood, when it becomes one of the features that predispose persons to SAD and panic disorder [94]. The results of these and other studies suggest that early childhood shyness is related to the SAD found in the general population of older children and adults [95, 96]. Of equal interest are the findings that disorders such as anxiety at 7 yrs old predict adult pathology, principally in girls [97].

Although formerly thought to be rare in children [98], renewed interest has led to confirmation that

OCD can develop prior to puberty. The condition, however, often does not come to clinical attention until years later [90]. Early onset OCD is now one of the most studied disorders in pediatric psychopharmacology literature [90].

OCD in childhood also shows symptoms similar to those that occur in adult patients [50]. Both are characterized by recurrent thoughts and behavioral compulsions that are distressing and interfering with everyday life. And, both are treated effectively with similar cognitive therapies and serotonin-related medications.

Controversy remains over the diagnosis of PTSD in children before the age of 10. Underreporting in children relative to adults may be related to younger children being unable to describe their reactions to the traumatic event necessary for the PTSD diagnosis. On the other hand, children have been reported to be more vulnerable to developing PTSD after experiencing trauma from natural disasters than their adult counterparts [52]. It also is possible that PTSD is expressed differently in children. For example, there are data suggesting differences in the endocrine response to PTSD. Youngsters may have chronically higher than normal levels of circulating cortisol, while at least a significant portion of PTSD adults show chronically reduced titers [52, 99]. The suggestion is that severe trauma earlier in life may alter normal neurodevelopmental processes and permanently change the brain, especially in youngsters. Support for neurological changes comes from findings in lab animals [100].

Boys, girls and anxiety

The literature is not in complete agreement on the existence of sex differences in childhood anxiety [101]. Nevertheless, most researchers conclude that there are sex differences. And most agree that, with the likely exception of OCD, anxiety problems are more common in girls than in boys beginning at the tender ages of 5-6 yrs [3, 48, 102].

Estimates of the incidences of GAD and SAD are up to twice as prevalent in girls. Indeed, the sole reliable risk factor for incidences of anxiety in children is gender [7]. This relation is in marked contrast to adolescent and adult differences in mood disorders and other psychopathologies with their multiple risk factors.

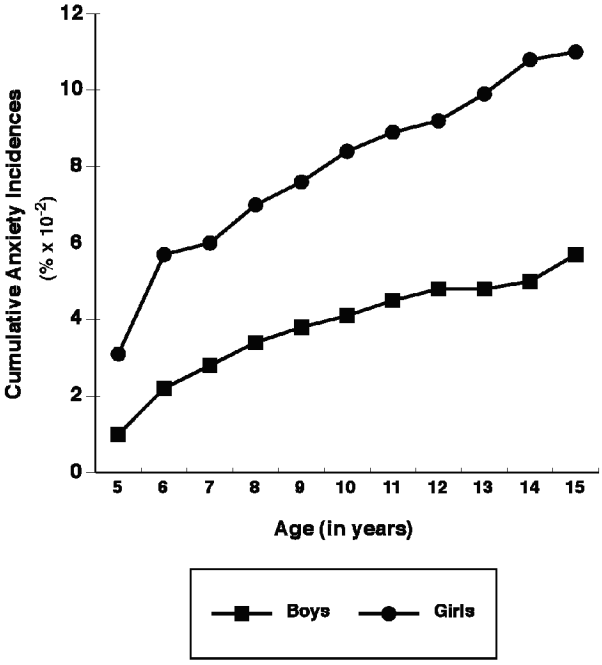

Figure 2. The cumulative incidences of anxiety disorders during childhood and early adolescence (adapted from [104]).

Several surveys have indicated there is a steady rise in SAD beginning at 5 yrs old, with girls showing a higher rate of incidences than boys [55, 103]. Indeed, Figure 2 (adapted from [104]) depicts a cumulative graph of anxiety data from prospective studies revealing a continuously increasing slope in girls from childhood through adolescence. Although rates of anxiety among boys also increase during the same ontogenetic stages, the rise is far more gradual than that of the girls.

This conclusion is not unequivocal, however. Another recent study confirmed that girls displayed a gradually increasing risk for developing an anxiety disorder, but suggested the risk in boys remained fairly stable throughout childhood [105]. Also, although accepting the more rapid increase in anxiety disorders with age in girls than in boys, Merikangas [48] suggested there may be no gender differences in the mean age at onset or in the duration of anxiety disorders.

A closer look at specific anxiety disorders suggests reasons for the discrepancies in the literature. PTSD is one example. Sex differences in PTSD are reported in adults, with women experiencing higher incidences as men, even to the same

trauma [59]. The same sex dimorphism is reported with children and teenagers diagnosed with PTSD but the sex differences are not so great [106]. There is evidence that girls are more likely to develop PTSD despite boys being exposed to more traumatic events [90]. On the other hand, abused boys with PTSD had evidence of greater abnormal brain development than girls with PTSD [107].

Sex differences in the prevalence of OCD in prepubertal children have been described [108]. As noted earlier, OCD appears to be the exception to the rule for elevated risks of girls developing anxiety. Although no sex differences have been reported, most epidemiologists have found boys to be more likely to display OCD [109, 110]. Estimates of rate of OCD occurrences in boys to girls are almost 3:1. This ratio reverses in those diagnosed with the disorder during or after puberty, with a male-to-female ratio of 1:1.35 [50].

Only recently have genetic and neural correlates of childhood OCD been explored systematically, and the results also reveal sex differences [90]. Mono- and dizygotic twin studies have provided evidence for a greater genetic contribution in boys with childhood-onset OCD [50]. Moreover, the boys may show more neuroanatomical pathology than the girls [49].

Juvenile animals and anxiety

Most experiments in the animal literature on developmental biology of anxiety have been designed to compare responses by groups representing peripubertal and adult animals. There are a few reports comparing anxiety of the same animal as a youngster and again as an adult [69]. Most also have used only males, and only a handful included both genders to examine sex differences.

The basic question addressed in these studies is the relative anxiety levels of prepubertal versus adult animals. There are conflicting reports on an answer to the question, likely a result of different methodologies and ages employed [66]. Still, the common findings are that, in the absence of experimentally induced stress, juveniles are less anxious than adults.

The elevated plus maze is used to model overall anxiety, and GAD specifically, and the EPM has been applied to assess age differences in anxiety [111]. Prepubertal rats (PND 33-35) are generally less anxious than adult rats. In a departure from the male-centric paradigms, Genn and collaborators [112] compared adult and juvenile female rats. They reported that the juvenile females showed less anxiety than adult females in the EPM. Another experiment [38] used the EPM paradigm to compare adult rats of both genders and juvenile females (PND 23-28) administered progesterone or its metabolite allopregnanolone. Juvenile males were not included. Yet, both hormones were more anxiogenic for the juvenile females than for the adults of either gender.

Findings with non-EPM paradigms mostly have confirmed the lesser anxiety in non-stressed prepubertal animals. As a measure of anxiety, latencies to enter a brightly lit open field were assessed for changes from juvenile (PND 25) to adolescence (PND 45) to young adulthood in male rats [113]. The animals were least anxious at PND 25.

A marble burying paradigm was used to compare groups of males aged either PND 30 or PND 50 with adult males [114]. Duration of burying was longer in the juvenile and adolescent groups than in adulthood, suggesting less anxiety in the males prior to full adulthood.

A series of studies used the social interaction (SI) test to model SAD in humans. PND 28 males were more interactive, suggesting low anxiety, than adults in an unfamiliar apparatus. There were additional differences in juvenile and adult anxiety. Adult male rats experienced more social anxiety in an unfamiliar environment than a familiar one. By contrast, PND 28 males showed similar SI behaviors in the two environments [115].

That the endocrine factor responsible for those results is testosterone was implicated by subsequent findings. Castrations on PND 19 rendered the rats at 60 days old behaving similarly in the SI test to the intact PND 28 males [33]. It appears to be binding of the estrogen receptor in the males that is necessary. This conclusion was reached with an experiment in which an aromatase inhibitor or a 5-alpha reductase inhibitor was used in the SI paradigm [116] to block the conversion of TS to its metabolites, E2 and dihydrotestosterone (DHT), respectively.

In the presence of aversive conditions, juveniles appear to be relatively more reactive and exhibit heightened anxiety [117]. This conclusion is reached using paradigms subjecting the animals to stress prior to testing for anxiety. Although stress and anxiety are not interchangeable physiological states, they are surely related. In those conditions, juveniles appear more disturbed than adults by experimental manipulations [118].

For example, PND 28-32 rats displayed greater anxiety-like behavior than adults under very bright lights when tested in the light-dark apparatus [66]. Juvenile rats have also been reported to be more sensitive to restraint stress. Prepubertal male rats experienced prolonged corticosterone elevations relative to adults, suggesting a greater level of anxiety [19].

There is the suggestion that chronic stress may produce different results. First, an experiment using food deprivation as the stressor reported the EPM performance of adult female rats was not affected [112]. However, juvenile (PND 35) female rats significantly increased time in open arms of the EPM, suggesting less anxiety with the chronic stressor. By contrast, after chronic mild stress, juveniles (PND 32-38) showed a greater startle response than they did as adults [69], suggesting elevated anxiety to chronic stress in the juvenile period that waned with achieving adulthood.

Regardless of the direction of results in experiments comparing anxiety between juvenile and adult animals, the distinct implication is that juveniles are not small adults [119]. Prior to puberty brain regions underlying affect are immature with the outcome that juvenile anxiety responses are different before and after the sex steroid rise with puberty [111, 117]. Timing of the change to adult-like status is not yet clear, however. For example, PND 35 days old males were more similar to the young adults than PND 28 days old animals in the SI paradigm [116], suggesting brains were undergoing changes prior to the pubertal surge of TS.

Juvenile animal sex differences in anxiety

The rare comparisons of anxiety in male and female juveniles have pointed to likely sex differences with female lab animals displaying more anxiety than their male counterparts. An experiment using a modification of the marble burying task to model GAD tested both genders of juvenile (PND 30), adolescent (PND 50) and adult rats. Females showed shorter durations to bury the objects, a marker of greater anxiety, than males in both the juvenile and adolescent stages [114].

Spontaneous alternation behavior (SAB) was recommended as an animal model of OCD [120]. The spontaneous alternation paradigm takes advantage of rats favoring to alternate between two arm choices in a maze that have been equally baited or non-baited. The suggestion is that fewer alternations would reflect anxiety. SAB was said to emerge in pre-weanling rats and increases with age to reach a plateau above 80% around PND 40.

In a test of the SAB of rats from 17-41 days old, males alternated their arm choice from PND 32 onwards, while females perseverated in the chosen arm until PND 38. The mean number of repetitive choices remained close to 1 in males from PND 23 onwards, but females showed a mean number of repetitive choices higher than 1.5 until the end of the test.

Other experiments in the literature on gender and anxiety were designed primarily to test various independent variables, but their control groups prove informative. For example, in a study of the effects of sexually dimorphic anxiety responses to nicotine [121], examination of the control groups tested at PND 35-39 suggested that the females were more anxious in the SI paradigm than were their male counterparts.

Sex differences were observed in lab rats exposed to stress from being isolated PND 30-35. The animals were tested over the next few days in various apparatus. Compared to their control animals housed in groups, only the stressed females showed greater anxiety. The conclusion was that peripubertal females, but not males, were more anxious after the stressful prepubertal experience [122]. Neurophysiological comparisons confirmed the greater influence of isolation stress on female rats.

The general conclusion from these studies of sex differences of lab animals in non-EPM paradigms show face validity to the gender differences in anxiety in children. In non-EPM settings and in the absence of stressful conditions, female juveniles are more anxious than males.

Reliance on the EPM to evaluate sex differences in young animals, however, seems to ensure

conflicting data. For example, groups of male and female rats representing ages from prepubertal to adolescence to young adulthood to older adulthood were tested in the EPM [123, 124]. Results indicated sex differences in anxiety at every age tested with females spending more time in open arms of the maze. However, only in the two oldest groups, PND 65-69 and PND 104-109, did the difference reach statistical significance. Not surprisingly, activity in the open field was higher in females at all ages tested.

Among the several puzzling findings reported by Macri and his colleagues [43], there were no sex differences in anxiety in mice at any age tested. This conclusion was reached in a comparison of juveniles (PND 35), adolescents (PND 48) and young adults (PND 61) in the EPM. They also found that the PND 35 mice spent similar time to the adults in the open arms and much more time in the open than closed arms, suggesting low anxiety at both ages. The PND 48 mice showed the greatest anxiety, spending significantly less time in the open arm than the other groups.

In a stark reminder of the precariousness of the EPM results compared to non-EPM paradigms, a pair of experiments was conducted to assess sex differences in juveniles and adults in both the EPM and the SI paradigms [39]. Juvenile females exposed to restraint stress, social isolation or no stress showed a similar pattern to adult females in time in the open arm of the EPM. Juvenile males, however, were observed to have a dissimilar pattern to adult males. The prepubertal males, especially after social isolation, showed increased anxiety by their lesser time in the open arm.

In the second paradigm, the SI test, it was the juvenile females who were more anxious after stress, especially restraint. The conclusion of the authors was that different experiences reveal different patterns of anxiety sexual dimorphism in the EPM vs. SI tests [39]. Our interpretation is that it is preferable to use non-EPM animal paradigms to model the behaviors of children.

Adrenarche

Children, by definition, have the low circulating levels of gonadal hormones characteristic of prepubertal animals. Yet, there is a significant endocrine event taking place that may be important for steroid - psychopathology relations in children and adolescents [125]. That event is the adrenarche when the adrenal glands begin synthesizing and releasing androstenedione (4A), dehydroepiandrosterone (DHEA), and its sulfate form DHEA-S. Although the two steroids may have slightly different properties [126], levels of DHEA and DHEA-S are highly correlated in circulation and in the brain. Moreover, their effects are similar [127]. Indeed, DHEA-S appears to be a backup source for DHEA.

These adrenal steroids pose a problem for the development of animal models because adrenarche does not occur in rodents. Rats have DHEA levels that are too low to be measured until sexual maturation and even adult rodents have low levels [128]. These observations would seem to make rodents a poor choice to model adrenal steroid functions. It can be argued, however, that rodents are a reasonable model in which to explore the effects of high levels of DHEA that are seen in humans without interference from changes in endogenous levels. Moreover, the pattern of circulating DHEA in rodents is similar to humans. DHEA increases in late adolescence into adulthood (2 - 6 mos), then declines [129].

The rise in DHEA and DHEA-S begins in early childhood with the adrenarche as the zona reticularis of the adrenal cortex matures and changes in size, cell distribution and function [130]. Central to the changes is the expression of enzymes necessary for synthesizing the adrenal steroids [131, 132]. Those steroids rise rapidly as puberty nears, and peaks between the ages of 20 and 30 yrs of age. With the approach of middle age, the levels of both DHEA and DHEA-S undergo linear decreases, and by 70 to 80 yrs old, levels reach approximately 10-20% of those of a 20 yr old [133, 134]. The patterns of change also are sexually dimorphic [125, 135]. See Figure 3 (adapted from [23, 24]).

Of particular interest is that these highly predictable lifetime changes in DHEA and DHEA-S closely parallel increases in human maladies associated with different ontogenetic stages [136, 137]. Thus, any discussion of sex steroidal effects on psychopathology would seem to require a consideration of the adrenal steroids holding for Barrett-Conner [138].

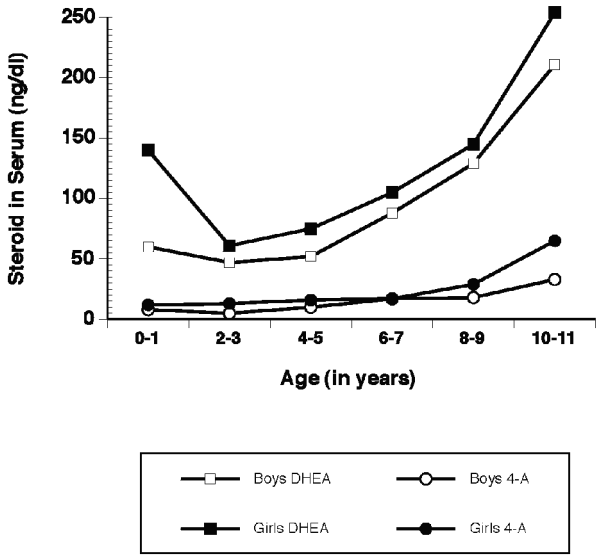

Figure 3. Circulating levels of dehydroepiandrosterone (DHEA) and androstenedione (4A) in boys and girls during childhood and early adolescence (adapted from [23, 24]).

Adrenal steroids are classically defined by their role as precursors of androgens and estrogens. Yet, both DHEA and 4A likely serve other functions than simply as precursors of androgens and estrogens [139]. Most research has focused on DHEA and DHEA-S. Less is known about 4A other than that it is synthesized and released from the adrenal cortex and post-pubertal ovary. However, by its location in a strategic spot in the metabolic cascade of the steroids, 4A has the potential to be converted via a primary pathway into androgen and estradiol or into various estrogens via a secondary or "backdoor" pathway [140, 141]. By contrast, DHEA is positioned only for conversion to androgens and estradiol. During the peripubertal stage, however, there is an inexact correlation between DHEA-S and TS [142, 143], suggesting that the adrenarche steroids play a unique functional role.

DHEA and DHEA-S are among a handful of neurosteroids identified that influence normal brain function in adults, and likely youngsters as well. No unique receptor appears to exist in the brain for either form of DHEA or of 4A [144, 145]. Although they may bind the androgen and estrogen receptors, affinity is certainly low [128]. It now seems likely that the adrenal steroids influence brain function by modulating excitation and inhibition of neuronal membranes [146]. Of particular importance is that a primary target for neurosteroids, including DHEA, is the GABA neurotransmitter that plays a critical role in anxiety [147, 148], and that the effects may be sexually dimorphic [149].

Adrenarche is confined to great apes and a few other primates. The suggestion is of the adrenarche being a relatively recent evolutionary development, and that its functional significance remains unknown [150]. Here, we propose that one area of significance is in the normal brain development of children prior to puberty. Further, a premature rise in the adrenal androgens may influence the development of psychiatric conditions, including anxiety, in childhood and the transition into puberty [151].

Several features of the adrenarche are central to that proposal. **a)** An increase in circulating adrenal androgens can be detected around 6 years of age. **b)** The subsequent pattern of increasing serum amounts is sexually dimorphic with girls showing a rise a year or more before boys. **c)** The initial clinical features of adrenarche are the increase in body hair growth occurring at circa 7-8 years of age, thus defining individual differences described by the Tanner stages [152]. **d)** The initiation and progression of adrenarche is independent of maturation of the hypothalamic-pituitary-axes, e.g., prior to the rise in gonadal hormones and without changes in cortisol [128, 131, 132, 153, 154]. **e)** Finally, evidence that the adrenarche is associated with adult-like behavioral effects is suggested by data that a significant percentage of girls begin masturbating up to two yrs before the onset of puberty [143]. Also, 4A levels are a better predictor of aggression than the usual suspect, testosterone [155]. The suggestion is that DHEA, DHEA-S and/ or 4A are responsible for the behaviors that, similar to psychopathologies, are commonly thought to appear only after puberty.

Sex steroids, prepuberty and anxiety

There are a few reports in the literature relating to adrenal steroids and anxiety in children. Almost all are correlational with the measurement of adrenal and gonadal steroids and other biological products [156, 157]. The following is a brief overview of those experiments.

The most direct test of a relation of adrenal steroids and childhood anxiety was reported by Dorn and associates [151]. Comparisons of girls 6-8 yrs old with normal or premature adrenarche indicated that the latter had higher levels of various psychiatric conditions, including all forms of anxiety disorders. The implication is that an unusually rapid and early rise in DHEA and 4A can contribute to the emergence of anxiety in children. However, it is notable that the premature adrenarche girls also had higher TS titers than the controls.

A more subtle role for the gonadal steroids is suggested in other analyses of prepubertal development. Hair follicles are dihydrotestosterone (DHT) sensitive, and some of the childhood adrenal steroid must be converted to DHT to produce the body hair growth of adrenarche. Because circulating TS and DHT are not increasing during this period, the suggestion is that it is the local metabolism of DHEA to DHT responsible for the axillary and pubic hair growth [131]. This observation raises the possibility for the local metabolism of adrenal steroids to gonadal hormones in the brains of children.

We were unable to find a literature with juvenile lab animals that directly test for the effects of prepubertal DHEA, DHEA-S or 4A on anxiety. The prepubertal animal studies reported have included physiological features but typically no behaviors were measured. An early experiment administered DHEA and 4A to juvenile rats for 3 wks beginning at PND 22 [158]. There was clear evidence at sacrifice that the steroids prevented the normal development of sex steroid sensitive organ systems. The suggestion is that the adrenal steroids can suppress the HPG axis during the prepubertal stage. Similarly, pharmacologic doses of DHEA administered to PND 25 female rats blocked the typical involution of the thymus gland with age, although not as effectively as DHEA supplements to adult females [159]. The suggestion is that DHEA has different influences on the juvenile vs. adult immune system.

Although using an animal model of childhood depression, experiments were reported on the correlation of DHEA in juvenile rats (PND 30-35) with measures that may reflect anxiety as well as depression [160]. The most affected animals had the lowest levels of DHEA-S and, in a subsequent experiment supplements of DHEA-S improved their behavioral scores to control levels [161].

It should be noted that there is a larger literature on the long term influences on anxiety of prepubertal, often neonatal, animals exposed to stress and HPA activation. The reliable findings are that the experience increases the signs of anxiety when the animals are tested as adults [19, 100, 113]. Yet studies are sorely needed that measure anxiety in juvenile animals after adrenal steroids are experimentally manipulated.

Inclusion of other neurosteroids in prepubertal studies also may prove informative. Direct measurement of the progesterone metabolite, the neurosteroid allopregnanolone, in the hippocampus was higher in PND 25 rats than in young adulthood [162]. These data suggest that the anxiolytic properties of allopregnanolone may be particularly active in juveniles in a brain region subserving HPA axis activation and anxiety.

Finally, progesterone and epitestosterone are other steroids that have received little experimental attention in prepubertal animals. In one experiment, adrenal progesterone was suggested as responsible for juvenile female rats (PND 28) displaying a significantly higher and longer plasma progesterone response, and likely anxiety, than adults to a stressor [163]. Gonadal sources were not responsible because the females had been ovariectomized, leaving the adrenal as the likely source of progesterone in prepubertal animals. Also, epitestosterone can function as an endogenous anti-androgen. Concentrations of epitestosterone were reported to be surprisingly high in both prepubertal boys and girls [164].

CONCLUSIONS

Major conclusions to take home from this review include incidences and gender differences in anxiety that are observed in adulthood emerge surprisingly early in children that can be modeled in lab animal models. Moreover, the rise in adrenal steroids well before puberty plays an underappreciated role in behavior, and that hormone release patterns during the prepubertal period correspond with the development of many of the clinically diagnosed anxiety dysfunctions. Also, the rise in gonadal steroids with the onset of

puberty tracks changes in anxiety. By comparison with the clear correlation of sex steroids with substantial increases in mood disorders accompanying puberty, we conclude there appears to be a lesser influence of sex steroids on the higher incidences of anxiety at puberty. Finally, steroid - anxiety relations in young animal models have received short-shrift attention from behavioral researchers. It is hoped that this review has pointed the way to a subsequent burst of research in this important field of endocrinology.

The most immediate reason to recommend further studies with juvenile animal models is the surprising occurrences of psychiatric disorders in children. However, this importance is magnified with the recognition that most adults diagnosed with psychopathology met diagnostic criteria during childhood and adolescence [94].

The mention of animal models for anxiety or other psychopathologies is apt to be of some concern to some readers. Granted, it would seem on the surface to be folly to believe that animals can model disorders that are defined in terms of human cognitive and emotional processes, the delusions of schizophrenia, the worry accompanying anxiety and the obsessions in obsessive-compulsive disorder [165]. Most animal models rest upon assumptions that the observable behaviors reflect underlying pathology. Certainly, researchers should continuously reevaluate the assumptions upon which animal models are based. Modeling symptoms rather than syndromes has provided the ability to adapt animal models to the study of anxiety disorders. The fruits of those efforts are in evidence here in demonstrating the potential influence of the sex steroids on the many and varied facets of childhood anxiety. There is unquestioned value for expanding the animal research to search for childhood mechanisms and pharmacological therapies.

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