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When is a sex difference not a sex difference?

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Abstract

Brain sexual differentiation in mammals requires activity of gonadal hormones; organizational effects of these steroids on brain development occur early in life while activational ones in adulthood ensure appropriate and timely sex-specific behaviors. This traditional view has long served as a reliable model for sexual differentiation of reproductively relevant brain structures. Here, we take a fresh look at this model but refocused in the context of sexual differentiation of non-reproductive parameters and with an emphasis on the hippocampus, a telencephalic brain structure predominantly involved in cognition and stress regulation. We explore sex differences in morphology, neurochemistry, and hippocampal-dependent behaviors to propose a new prototype that can be used to explain and further investigate the effects of steroid hormones, those synthesized gonadally or intracerebrally, on hippocampal development and function. We also propose that a new vernacular be employed, one that distinguishes hormonally modulated responses from sex differences, and argue these are mechanistically and functionally distinct. Understanding when and how the sexes are different is as important as understanding when and how they are the same, at the biological, social, and cultural level.

Keywords: Sex similarities; Sex difference; Hippocampus; Cognition; Stress; Development; Estradiol

1. Introduction

At the time of this writing the 21st Century has dawned, it is the Age of Aquarius and a brave new world. One would think that the idea of something as insignificant as gender determining intelligence would be a quaint old-fashioned notion long since dispensed with in this enlightened era. Yet, the controversy rages on. Males have bigger brains than females, so they must be smarter. Or are they just better at spatial ability while females are good talkers? Females can read body language but males can solve quadratic equations while riding a unicycle. Facts and anecdotes abound, but what is the truth? Is there a truth and if there is, do we know it? This review does not presume to reveal the truth, but rather aims to further the dialog that is already ongoing by focusing in specifically on the basic science research in rodent animal models and examine what these studies tell us, and in many cases more importantly, what they do not. We also propose there is a need for a clarification in the literature regarding what is a sex difference, meaning fundamentally and permanently different between males and females, versus what is a hormonally modulated response, meaning a parameter that varies in meaningful ways in response to changes in adult circulating hormones.

The study of biological sex differences in the brain has been an active field of investigation for close to 50 years, and by some accounts the field has ironically been reinvigorated in the last decade or so by the influx of women scientists. For neuroendocrinologists, there is no debate on whether there are sex differences in the brain, including humans. The obvious sex differences in reproductive physiology; females ovulate on a periodic and regular basis, get pregnant, deliver, and lactate, and males do

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not, necessitate that the brain regions regulating these diverse profiles be different. Extensive studies have provided a rich description of the relative size, neurochemistry, and connectivity of subnuclei within the preoptic area and hypothalamus of a variety of species, ranging from amphibians and reptiles through birds and mammals, including higher primates. The magnitude of the sex difference in various morphometric parameters tends to be large, in the range of 2- to 7-fold, leading easily to the moniker "sexually dimorphic." Dimorphic means of two forms, and therefore is a term best kept for those differences that are big and have little overlap. But terminology has a way of slipping and frequent use leads to further imprecision. As a result, there has been an increasing tendency to equate "sex differences" with "sex dimorphisms" which simultaneously leads to a loss of attention to the all important question of magnitude and reliability. A central theme of this review will be that this process has occurred in the conflating of sex dimorphisms in reproductively relevant physiology and behavior and the attendant neurological substrates, with sex differences in cognition. This has occurred not only in the arena of basic science investigation but in the popular press as well.

Sex differences in learning appears in the literature by at least the 1950s and studies on humans generally preceded those in animals [22,23] which appeared in the late 1960s [43], although the first reports of hormonal influences on learning in the rat date to 1926 [7]. By the mid-1970s, the notion that males have superior spatial abilities to females was well entrenched in both the human [100,62] and animal literature [77,67]. The need for standardization across studies gradually led to the use of the mental matching of rotated three-dimensional images as the gold-standard task for spatial ability in humans, and the Morris Water Maze as a hippocampal-dependent task for testing spatial learning in rodents. Males located the submerged platform in the Morris Water Maze with a shorter latency than females. Morphometric and physiological differences in the hippocampus or other cognitive brain regions were predicted to underlay the sex difference in performance, and the perception that this is true pervades the collective view of both the expert and casually interested lay person. But what is the evidence for sex differences in brain regions mediating cognition, and more importantly, what is the nature of the evidence for sex differences in cognition? The goal of this review will be to take a fresh look at some old ideas and challenge researchers to re-examine the data that have led us to the current understanding. Are there sex differences in cognition? Certainly, but what are they and how do they arise? Moreover, what can we, as neuroendocrinologists, contribute to the discussion that will shed light on this issue that impacts on us all culturally, socially, and biologically.

Many of the ideas reviewed here are neither new nor original, but they bear repeating. Arguments that sex differences in cognition are not related to learning but to strategy [163,126], that classic views on sexual differentiation of the brain do not generalize to all brain regions or endpoints [3], and that the sexes often try to be similar rather than different [32] have all been artfully and convincingly made. We strive to synthesize each of these arguments by reexamining long established findings that have cemented into tenets, with recent and unexpected results that may assist us in furthering our understanding of how the brain develops, and how it develops specifically in males and females.

2. Brief overview of the study of sex differences in the brain

2.1. Sex differences are determined developmentally by gonadal steroids

The brain begins life as a generic, neither male nor female, but instead waits to be impacted by the circulating hormonal profile that will be determined by the gonads. The best characterized example of this is the laboratory rat from which we know that at a predetermined developmental time point late in gestation the fetal testis secretes copious amounts of testosterone into the circulation. From the blood stream, the testosterone gains access to the brain where it is locally aromatized to estradiol and it is this hormone that then differentiates the neural substrate into a male phenotype (Fig. 1). Testosterone and its 5α-reduced product, dihydrotestosterone, also exert differentiating effects on the nervous system, the best example being the spinal nucleus of the bulbocavernosus. In other species, in particular primates, the majority of differentiation deemed masculinization is mediated by androgens as opposed to estrogens.

The concept of sexual differentiation of the brain was codified in a seminal paper by Phoenix et al. [129] which postulated the organizational/activational hypothesis of steroid hormone action on the brain. This conceptual framework was built on extensive evidence in a variety of species but was empirically established by hormonal treatment of pregnant Guinea pigs and behavioral observation of the adult offspring. Put quite simply, if the fetuses were exposed to androgens they would behave like males as adults if again provided with androgens, but if they did not have the androgen developmentally, they did not. Other studies conducted around the same time in rats noted that newborn female pups exposed to androgen resulted in infertile adults due to defects in ovulation. It seems fairly obvious in retrospect that there must have been a permanent change in the neural architecture regulating control of gonadotropin secretion and sexual behavior and that ergo there must be naturally occurring sex differences in the brain, but at the time it was not clear that changes had not simply

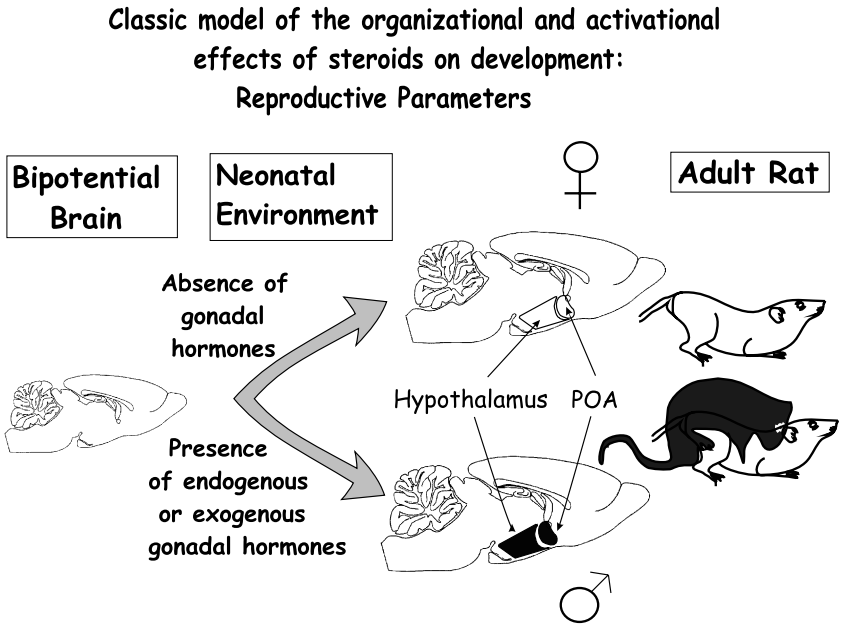

Fig. 1. Sexual differentiation of reproductively-relevant hypothalamic nuclei. The embryonic rat brain is bipotential. The adult male brain phenotype results from exposure of the late gestational/early neonatal brain to estradiol (E_2), aromatized from testicular testosterone (T). Exogenous administration of testosterone or estradiol to the genetic female results in a complete male phenotype for hypothalamically determined reproductive responses. Absence of gonadal hormone in the newborn rat will yield a feminized brain and associated behaviors with appropriate hormonal priming in adulthood.

been limited to the ovary or pituitary. Once it was determined the brain was responsible for the androgen-induced infertility, a flurry of studies examined a variety of neural chemical endpoints, with reports of sex differences in histamine acetyltransferase activity, cholinergic, and serotoninergic parameters and synthesis and metabolism of steroids, mostly estradiol. Surprisingly little attention was paid to anatomy, although there was some. Early castration was found to induce changes in the adult male preoptic area [128], which is logically consistent, but the notion of sex differences in the brain did not genuinely catch fire until the report of a sexually dimorphic nucleus in the mammalian brain [39] and equally if not more dramatic sex differences in the brains of canaries [116]. These high profile reports were followed by an explosion of interest in morphometric sex differences that continues to this day. It seems wherever you look, there is a sex difference in the size of a region, the density of the neurons or the pattern of connectivity. Or is there?

2.2. Common mechanistic principles in establishment of brain sex differences

Some simple generalizations can be made regarding what is considered hormonally mediated sexual differentiation of the brain. One is that steroid hormones, be they androgens or estrogens, do not seem to affect the genesis of cells in brain regions destined to be sexually dimorphic, instead they determine whether pre-existing cells will survive or die. For example, the sexually dimorphic nucleus of the preoptic area (SDN-POA) is 5–7 times larger in volume in adult males than females. Newborn males and females have the same number of neurons in the region destined to become the SDN and the adult sex difference is due to cells dying within the first week of life in females. These cells can be rescued by treating newborn females with testosterone or with estradiol directly, but if treatment is delayed past the first week, the female will permanently have a smaller SDN than the male. A variant on this theme is the anteroventral periventricular nucleus in which the opposite occurs, the cells die in the males in response to estradiol action, resulting in a smaller nucleus than that found in adult females. These sex differences in volume are robust and permanently established during the perinatal sensitive period, they are also directly relevant to reproduction, as opposed to volumetric changes in other brain regions that are responsive to circulating gonadal steroids in adulthood.

A second generalization involves the establishment of sexually dimorphic synaptic connections which are equally robust to those identified volumetrically, but are generally less investigated due to the inherently greater technical challenges. Plasticity in synaptic connectivity until the closing of a sensitive period is a well-established parameter of brain regions influenced by external sensory stimuli, such as components of the visual system that receive direct or indirect input from the retina or the barrel cortex that maps the input from specific whiskers in the rat. Over exuberant synaptic connections are appropriately pruned and thereby tuned in response to

specific sensory stimuli that will then determine the adult response profile. By contrast, sexually dimorphic projections and/or connectivity involve the active recruitment of innervation by the target and the addition of new synapses in response to hormonal signals, most notably estradiol. Examples of such have been carefully characterized for the preoptic area, the arcuate nucleus, the ventromedial nucleus, and the innervation of the AVPV by the principle subdivision of the bed nucleus of the stria terminalis. Again, each of these is directly relevant to reproductive physiology and/or behavior. Recent and excellent reviews provide additional details on advances made in the cellular mechanisms by which these sexual dimorphisms in the brain are established [149,113].

3. Sex differences in non-reproduction related brain regions—the hippocampus as a model

While many brain regions contribute to a process we refer to rather broadly as "cognition," in the field of sex differences the lion's share of attention has been given to the hippocampus. Excellent and provocative studies on the cortex of rodents and humans are also of considerable interest [95,96,167,168] but for illustrative purposes the present discussion will focus on the hippocampus. Many if not all studies on sex differences in cognition are predicated on the fundamental principles of sexual differentiation: elevated testosterone in neonatal males is locally aromatized to estradiol in the brain to induce masculinization. Neuroendocrinologists have made the reasonable assumption that the observed relationship between serum levels of testosterone correlated with elevated estradiol levels in the hypothalamus within hours of birth [161] generalizes to any brain region possessing aromatase activity. The neonatal telencephalon has low but detectable aromatase activity [91], and the highest level of estrogen receptor expression in both the cortex and hippocampus appears to be within the first few days of life, thereafter declining to adult levels [110,123,124]. That estradiol sexually differentiates the developing telencephalon into a masculine phenotype seems self evident, and it is generally assumed that this is the basis for a male advantage in spatial learning. This statement rests on at least three fundamental assumptions; (1) estradiol levels are higher in developing male telencephalon compared to female, (2) ergo the telencephalon is sexually differentiated, and (3) males have an inherent advantage in spatial learning. Each assumption warrants independent verification both empirically and in the existing knowledge of hippocampal development and its function.

3.1. Hippocampal development

The hippocampus is a telencephalic brain region centrally involved in a variety of critical life functions including learning/memory and stress responding. It is a structure celebrated for its laminated appearance, organized synaptic inputs and clear functional significance. The characteristic folds of the adult hippocampus originate from a slight curvature in the developing telencephalon [12]. The laminated appearance is generated by a tightly packed pyramidal neuron cell body layer, their apical and basal dendrites and a thin layer formed by the exiting axons of the pyramidal neurons. The hippocampus proper is divided into fields, CA1–CA4 that are distinguished by their cytoarchitectural connectivity. CA3 projects prominently to CA1 via the Schaffer collateral and the paired synaptic connections between these two regions form the foundation for much research into synaptic plasticity. Intrinsic Basket neurons provide inhibitory control at the level of pyramidal neuron output that is powerful, long lasting, diffuse and largely of a feedback nature. The dentate gyrus is a component of the hippocampal formation and consists of granule cells that give rise to the mossy fibers. Together the hippocampus and dentate gyrus form two interlocking C-shaped structures. A primary route of information into the hippocampus is via the perforant path, which originates with neurons in the entorhinal cortex that project to the granule cells of the dentate gyrus. The mossy fibers of the granule cells then project to the pyramidal neurons of CA3. Information flows out of the hippocampus via axons of the pyramidal neurons forming the fimbria.

Hippocampal development is a complex process that cannot be assessed with a single level of analysis. The majority of neurogenesis in the rat hippocampus proper is complete by birth although a low but significant number of new neurons continue to be born in the adult hippocampus [41]. The dentate gyrus continues high levels of neurogenesis until well into postnatal life, with up to 10% of cells being born after postnatal (PN) day 18 [13]. Synaptogenesis is largely postnatal, with the density of dendritic spines doubling from the juvenile period to adulthood [48]. Electrophysiological approaches to hippocampal development indicate an increase in the number of synaptic contacts between the CA3 and CA1 regions from the neonatal period to adulthood, and this appears to be due to increased strength in the coupling between individual CA3 and CA1 pairs rather than a change in the reliability or efficacy of individual synapses [56]. Increased AMPA receptors contribute to the maturational change, but conclusions are partially confounded by the concurrent increases in dendritic arborization. Analysis of hippocampal development at the molecular level reveals varying expression patterns for calcium-dependent kinases considered central to establishment of long term potentiation (LTP). CAMKIIa expression is high as early as PN1 in CA3 and not expressed at appreciable levels in CA1 until afferent input from CA3 increases to this region. PKCγ mRNA expression on the other hand is independent of afferent

input and delayed in expression relative to CAM-KIIa, which may be limiting to long term plasticity [49,50].

The developmental period from PN7 to PN14 is particularly dynamic in regards to excitatory amino acid neurotransmission. This is a time of maximum sensitivity to exogenously administered glutamate, kainic acid or NMDA [105–108]. CAM-KIIa is transiently overexpressed at this time [49] and the kinetics of the NMDA receptor are markedly altered compared to both earlier and later time points [56]. Concurrent with the elevation in glutamate mediated excitation is a decline in depolarizing GABA responses [16]; the two events may be causally linked.

From a neuroendocrine perspective, the first 2 weeks of postnatal life constitute the hyporesponsive period of hippocampal development. The hippocampus is a central component of the stress axis and mediates negative feedback control of glucocorticoid secretion from the adrenal glands (see discussion below). Prior to PN1 and after ~ PN14, the hippocampus is exquisitely sensitive to circulating levels of corticosterone but becomes markedly unresponsive from PN 2 until completion of the second week of postnatal life [142]. Functional significance of this includes avoidance of the catabolic effects of glucocorticoids during a developmental period of pronounced anabolic activity. An additional and previously unconsidered advantage of low glucocorticoids during this developmental time could be to allow for maximal responsiveness to estradiol. The receptors for corticosterone and estradiol can heterodimerize, resulting in a decrease in estrogen action [152]. If estradiol action is required for normal hippocampal development as postulated below, removing a potential endogenous antagonist has clear benefits. Neonatal stress severe enough to induce corticosterone release impairs cognitive function in adulthood and interference with estrogen action could be one causal basis for this effect. There are no apparent sex differences in the duration or magnitude of the hyporesponsive period in neonatal rats. Moreover, the hippocampal formation exhibits extensive postnatal neurogenesis and synaptogenesis in the first few weeks of life but the potential for sex differences or hormonal modulation of either of these endpoints has not been explored.

3.2. Hormonal modulation and sex differences in the hippocampus

Despite having little to no role in the control of reproductive function, the hippocampus is nonetheless a sensitive target for gonadal steroids. Considerable attention has been given to estradiol modulation of synaptic plasticity in the adult [171,33] and effects on cognitive functioning [163,71,145,88,90,28,29,141]. Interest in estradiol action on the mature and aging hippocampus has been further increased by controversy surrounding the use of hormone replacement therapy in women. There is irrefutable evidence that estradiol potently enhances synaptic efficacy in the hippocampus by increasing the density and functionality of dendritic spine synapses in the female rat [172,139]. Behavioral tests designed to temporally correlate physiological changes with spatial learning have provided equally strong evidence of enhanced cognitive ability in response to estradiol action in the hippocampus [141]. The considerable body of work on estradiol and the hippocampus leads towards the logical assumption that there are substantial sex differences in the hippocampus as well. Indeed, the effect of estradiol on spatial learning tasks in rats and verbal recall tasks in humans is often interpreted as a sex difference in cognitive abilities. However, when questions are specifically framed to ask whether there are sex differences in the hippocampus and hippocampally regulated functions, the evidence is often in favor of either no sex difference, very subtle sex differences or differences in which hormonal modulation in the female results in a mean response that varies, but remains within the range of the male response. In our view, this illustrates the point that there has been a general conflating of the two distinct endpoints of a sex difference and hormonal modulation. The use of the term "sex difference" should be restricted to a parameter or outcome that is independent of circulating gonadal steroids. This may sound overly restrictive to some, but it does not mean that the endpoint is not modulated by or even dependent upon gonadal steroids, but rather that there exists a sex difference that is not solely a function of hormonal modulation. In many ways this harkens back to the original organizational/activational hypothesis of steroid-induced sex differentiation. Sex differences are organized early in development, perhaps by steroids but perhaps also genetically, and then these organized sex differences may or may not be activated in adulthood. If, however, a parameter is only influenced by steroids in adulthood, then this does not constitute a sex difference per se. This distinction is more than just semantics as it seems self evident that the mechanisms and cellular underpinnings dictating a sex difference versus hormonal modulation are likely to be unique. Demonstrating this empirically requires that experiments be appropriately constructed to make such a distinction.

3.2.1. Morphometric sex differences in the hippocampus

By definition one would assume that a sex difference in the size and/or shape of the hippocampus would meet the criteria of being robust, permanent, and not subject to change by gonadal steroids in adulthood. But surprisingly, this is generally not the case for sex differences in the morphometry of the hippocampus. Volumetrically, males have a larger hippocampus than females [93,117], with the CA1 region being 16% larger in males in one study [61] and only 10% larger in another [118]. A sex

difference is evident within the first week of life, and appears to be due to differential cell death between males and females [118,51] but in our experience these differences are subtle and inconsistent, we see the sex difference in one group of animals but not necessarily in another. In mice, males have more granule cells in the dentate gyrus than females, but only in three of six strains examined [166]. A sex difference in favor of females in number of dendritic segments on the granule neurons of the dentate gyrus at weaning was found to disappear by adulthood [68]. Moreover, sex differences in dendrite morphology in CA3 pyramidal neurons as well as dentate granule cells were markedly altered by the complexity of the rearing environment [69,70]. It is important to compare this to the robust volumetric sex differences observed in subnuclei of the reproductively important preoptic area where differences are in the range of 300–700% [149,150,40]. These observations are also in marked contrast to sex differences in reproductively relevant brain regions where sex differences are at their most robust in adulthood and appear relatively resistant to experience. Quantification of the entire apical dendritic tree in CA1 revealed more branches in males than females, but this sex difference was eliminated with aging due to a loss of branches in males [97]. The same study found no sex difference in the density of dendritic spines at any age. Taken together, these studies suggest that there are either no or only minor sex differences in the morphometry of the hippocampus and what the small differences that are observed might have to do with hippocampal function remains unknown.

If there is no sex difference in the hippocampus, how about hormonal modulation? It has been known for some time that estradiol increases the density of dendritic spines and attendant excitatory synapses in the distal dendrites of CA1 pyramidal neurons of female rats [173]. Examination of males was slower to come and while gonadectomy dramatically reduces spine density, by up to 50%, it is only restored by androgen treatment, with relatively little effect of estradiol [83]. Thus, adult males and females with appropriate levels of androgen or estradiol, respectively, have relatively equivalent amounts of synaptic input onto the dendrites of CA1 pyramidal neurons but apparently achieve this via different mechanistic routes. There is not a sex difference per se in the morphometry, precluding the use of the term sexual "dimorphism," but there does seem to be a sex "difference" in the hormonal strategy used to achieve the same goal. In other words, males and females exploit their unique hormonal milieu, of androgens versus estrogens, respectively, to achieve the same endpoint of increased dendritic spine synapses. Whether or not the endpoints are truly the same is unknown as there is much about the functionality of these new synapses that remains to be explored. That males and females achieve similar ends by different means is a theme that will begin repeating itself as we review other aspects of sex differences in the hippocampus.

3.2.2. Sex differences in stress responsiveness

The hippocampus is a major site for negative feedback control of the hypothalamic–pituitary adrenal axis or stress response system. Pyramidal neurons express high levels of glucocorticoid receptors, Type I and Type II, and projections via the subicullum regulate the output of the paraventricular nucleus (PVN) which projects directly to the anterior pituitary to regulate ACTH release into the circulation. Increased circulating levels of glucocorticoids act on the hippocampus in a negative feedback loop to decrease the output of the PVN and return the system to homeostasis (see for review [153]). Dysfunction of this feedback loop is observed in a significant portion of patients suffering debilitating depression [169] and reduced hippocampal volume is associated with a number of neuropsychiatric disorders including post-traumatic stress syndrome and schizophrenia [115].

There are sex differences in stress responding in adults, with females exhibiting a more robust and longer lasting increase in glucocorticoids and behavioral impairment following exposure to a stressor [6,146] and this sex difference appears to be organizationally determined [104]; however, experimental designs previously employed would preclude definitively concluding that any sex differences observed in stress responding is the result of organizational steroid influences on the hippocampus. Sex differences in the hippocampus have been speculated to underlie the variance observed in adult stress responding but this connection has not been definitively established. The stress axis is highly complex with multiple components and involves several extra-hippocampal sites of negative feedback including the highly sexually dimorphic preoptic area [154]. At this juncture it is premature to make strong conclusions about sex differences versus hormonal modulation of sex differences in stress responding but this would be an excellent framework for future investigations.

Measuring stress sensitivity in the adult can be done behaviorally or physiologically. However, there are at least two crucial considerations when assessing sex differences in stress reactivity through these means. The first is related to the characteristics of the stressor to which the test subject is exposed; these include the period of life during which the subject is exposed and the duration and type of stressor. Stress exposure in adulthood is associated with a variety of physiological and behavioral impairments. However, these effects and recovery from their impact are highly dependent on the length of stressor exposure as well as the psychogenic versus neurogenic nature of the stressors involved. Perinatal stressors on the other hand are well known to disrupt HPA axis development and function, thus often

resulting in more permanent consequences. Of particular interest are the prominent sex differences associated with perinatal stress exposure. Whereas prenatal stress exposure in rats appears to impair performance on most cognitive tasks similarly in males and females [44], an increase in measures of anxiety is observed only in females [20]. Additionally, prenatal stress masculinizes the neurochemical profiles of females in the prefrontal cortex, amygdala, and hippocampus [19,57,74,112,114,138,159]. In contrast, neonatal stressors, such as maternal deprivation, have a more drastic impact in male rats, resulting in increased anxiety and fear behavior, as well as hyper-responsiveness of the HPA axis to stressors [8,81,162]. Thus, we cannot make blanket statements about the impact of perinatal stress on males versus females as the result appears to depend on the nature of the stressor and the response parameter being measured. That experience affects males and females differently we can say with confidence, but is that a sex difference or more of a variance in sensitivity? Arguments can be made either way, anything that varies at all between males and females can be called a sex difference, or we can revise our wording to more appropriately reflect what is a biologically relevant variance in sensitivity that is partly explained by sex but retains an enormous amount of overlap in responsiveness in males and females.

The type of stressor to which a subject is exposed is also of importance. While inter-species differences in the types of treatments that are considered stressful are more obvious, sex-specific stressful manipulations have been less studied. One manipulation that has received attention over the years is the subject's social environment. A strong sex difference in reactivity to social stress has been documented; male rodents exhibit impaired stress responses following crowded housing rather than isolation [21], presumably due to increased aggression. In females, social instability and isolation appear to be stressful in non-human primates and rodents, respectively [47]. These findings are akin to those in the human literature, particularly those dealing with sex differences in psychopathologies, suggesting that stress associated with social isolation and instability has a greater impact in women than men, and might be related to depression in this population.

A second issue to consider when assessing sex differences in stress reactivity is the behavioral test employed. Over the years, a plethora of stress-related behavioral tasks have emerged, from tests that evaluate the effects of previous stressor exposure in a rather neutral environment, for example, the open field, elevated plus maze, and social interaction test in rodents, to those behavioral paradigms that incorporate a stress component within the task, including the forced swimming test and stress conditioning paradigms. Basal sex differences in many of these tests have been reported. For example, males typically spend more time than females interacting with a same-sex conspecific in a social interaction test [63]. In terms of the open field task, female rats typically show shorter latencies to enter the center of the field and greater active exploration as indexed by increased ambulation, rearing, and sniffing novel objects (reviewed in [2]), suggesting that basal anxiety levels are lower in females than males. However, further exploration of this sex difference indicated that increased ambulation is more specific to females in estrus; females in diestrus showed similar locomotor activity as males [133]. Basal differences are also apparent in the elevated plus maze, with females spending more time in the open arms than males [64] but similar to results reported for the open field, these are dependent on the phase of the estrous cycle; females in diestrus do not differ from males [94]. Moreover, in males prior stress reduces the latency to explore in the open field. Unfortunately, the reproductive status of females was not reported [14]. Females typically show increased activity levels in the forced swimming test, a task that inherently incorporates a stress component (reviewed in [10]). Again, a sensitivity to prior stress was found exclusively in males [18]. Thus, two broad factors, characteristics of the stressor and nature of the behavioral test, interact to produce different effects in males versus females. These variables may play an important role in the outcome of any particular cognitive task. The influence of secondary parameters on primary cognitive outcomes needs to become routinely incorporated into interpretation of studies on sex differences in which the primary effect might be on the secondary parameter.

3.2.3. Sex differences in long term potentiation and long term depression

A potential physiological underpinning of learning that is easily measured, generally accepted and well understood is Long Term Potentiation (LTP), a physiological phenomenon in which the strength of synapses is enhanced by high frequency stimulation [79]. The opposite is known as Long Term Depression (LTD), which occurs in response to low frequency stimulation of long duration. Both LTP and LTD are modulated by estradiol.

When examined at the level of physiology, estradiol enhances LTP in ovariectomized females [27] and in naturally cycling females [158]. Unfortunately, few studies have compared LTP induction across the sexes and in the one that has, the magnitude of LTP induced in females on the afternoon of proestrus (high estradiol) was the same as that seen in males during the morning [158]. There was also no sex difference in the input/output curves generated by evoked excitatory field potentials. Hippocampal-dependent fear conditioning was similarly examined for sex differences using cycling females and intact males. In this instance, males and

estrous females performed similarly, whereas females in proestrus showed less spatial–contextual conditioning than both males and estrus females [98]. In contrast to estradiol's well-established ability to enhance LTP in CA1 induced by Schaffer collateral stimulation, it reduces LTP at perforant path synapses, an effect believed to be functionally related to the impaired contextual (hippocampal-dependent) fear conditioning also observed in estrogen-replaced ovariectomized rats [45]. This was again one of the few studies that compared sexes and interestingly a different pattern appears in that intact males and ovariectomized females show identical fear conditioning but now both differ significantly from estradiol-treated females. Induction of LTD at CA3-CA1 synapses is also enhanced by estradiol treatment of females. A low-frequency (2 Hz), asynchronous conditioning stimulation protocol does not produce LTD in slices prepared from ovariectomized females but does so readily in slices from estradiol replaced females [176] . Unfortunately there was no sex comparison in this study so it is unknown if the threshold for LTD induction varies between males and females. Changes in the density of dendritic spines on CA1 pyramidal neurons are correlated with LTP and again, there is a sex difference but the direction of the sex difference varies with the phase of the female estrous cycle, being higher than males in the proestrus female but lower than males in diestrous females [146]. Thus in regards to LTP, dendritic spine density and fear conditioning, it appears there is a hormonal-dependent modulation of the response in females, but this response varies around the mean in males and therefore does not constitute a sex difference per se. This principle is illustrated in Fig. 2.

3.2.4. Sex differences in hippocampal-dependent spatial learning

At the behavioral level, males learn spatial tasks, like the Radial Arm Maze or Morris Water Maze, faster than females, but with practice they both perform the task equally well and empirical evidence indicates no difference in memory capacity between males and females. Sex differences are limited to acquisition of the task, not steady-state performance. In fact, when the cues used for acquisition are altered, females outperform males. Systematic control of environmental variables reveals that males use the geometrical cues, such as the shape of the room or location of large objects as guides, whereas females attend to local small scale details [163]. Thus both sexes learn, they just use different strategies to do it. Even more importantly, if males and females are familiarized with the spatial task in advance, resulting in a reduction in the natural tendency of females to show greater thigmotaxis than males, females actually outperform males on finding the hidden platform in the water maze, and there is no sex difference during the probe trial in which the platform is removed [126]. These studies illustrate the potential for non-learning related parameters, such as response to novelty, to skew results to appear as if there are sex differences in spatial ability when in fact there are none. This is not to say there is not

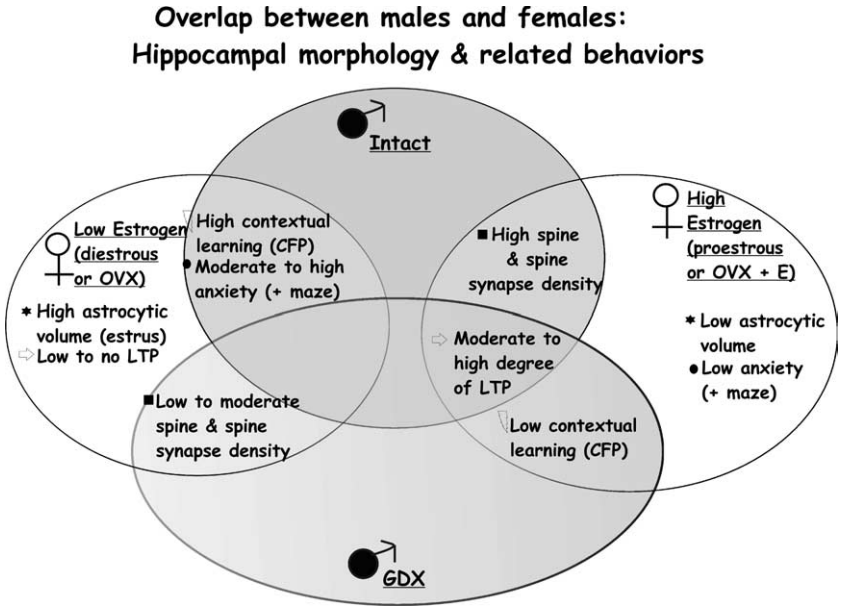

Fig. 2. A schematic depiction of the relationship between gonadectomized or gonadally intact males and females on measures of hippocampal morphology and hippocampal-related behaviors. Note that the female response typically varies around that of the male; the direction of variability depends on hormone status. This serves to show the importance of distinguishing between hormonally modulated variance and intrinsic sex differences. These are based on a collection of results by [94,158,46,140,34,30,38,73,174,84,76,27,98,85,175,87,42,92]. *Abbreviations:* CFP, conditioned fear paradigm; GDX, gonadectomized; LTP, long term potentiation; OVX, ovariectomized; OVX + E_2, ovariectomized with estradiol administration; + maze, elevated plus-maze.

a sex difference in anxiety, it appears that there is, but this is entirely different than saying there is a sex difference in cognitive ability.

A recent meta-analysis examined 39 experiments from 24 studies on sex differences in spatial learning in rats or mice that used either the Morris Water Maze or the Radial Arm Maze [65]. Among the selection criteria for inclusion was a requirement that all animals be free of prior surgery, therefore by definition not controlling for the variable endocrine status in females. This seems puzzling given the evidence for variation in performance across the estrus cycle of females, although the author refutes this as being inconsistent across studies. The major conclusion is of "large reliable male advantages for rats in radial maze and water maze protocols." However, there is a substantial impact of rearing condition, being reared alone reduced sex differences, and the magnitude of the sex difference varies considerably across rat strains. Pre-training, and thereby reducing stress associated with the tests, also substantially reduces any sex differences. The author rightly points out that it is hard to accept an evolutionary basis, the "hunter–gatherer" theory of spatial abilities, for a sex difference in spatial ability when it is so markedly affected by strain, rearing condition, and prior testing. In the case of the Radial Arm Maze, whether the protocol included tests for working and reference memory or just working memory, substantially altered the magnitude of any sex differences. In the end the author is left with the tentative conclusion that sex differences in "strategy," as suggested by others, is the primary basis for the sex difference in spatial ability. This conclusion is further supported by a recent study on the Morris Water Maze in which the path to the platform was assessed in male and female rats on the initial trial in which the platform was visible. Males and females could see the platform equally well, but rather than swim directly to it through the open water, as males do, females chose to swim along the walls of the enclosure before cutting across to the platform [15]. Since performance on the task is based on latency in seconds, the data would say that females have a poorer performance than males. Yet no one would argue that females are somehow too dumb to swim to a perfectly visible platform, in fact may be they are the smart ones not risking plunging out into open water. This illustrates a basic principle in the study of sex differences, the importance of understanding the meaning of the end points. What is really being measured, is it spatial ability or is it strategy? (Fig. 3). Ironically, many earlier tests of spatial ability that reported male advantages, such as the Lashley III, Hebb-Williams, and T-Mazes, were discarded due to the confound of sex differences in locomotor and exploratory activity [65], only to be replaced by the apparently equally confounded Morris Water Maze.

Sex differences in spatial navigation tasks have been examined in the context of organizational versus activational hormone actions. The traditional strictures all apply in that removal of the male testis early in life feminizes learning in adulthood and treating newborn females with testosterone or estradiol masculinizes their responses as adults [164,165,137]. Thus, as with the majority of other sex differences in the brain, estradiol has been implicated as the masculinizing factor. Females treated with estradiol for the first 10 days of life show masculine learning patterns as adults, and males castrated neonatally are predictably female-like in their responses [164]. However, note that females were not treated with an aromatase inhibitor and removing the testis in males would remove both androgens and estrogens. In a separate study, the effects of hormonal manipulation during late gestation included treatment with the non-aromatizable androgen, dihydrotestosterone, and the anti-androgen flutamide. Animals were tested on a water maze as adults and at the behavioral level the findings are unambiguously interpretable as an *androgen*-mediated effect on spatial learning, with there being no effect of exogenously administered estradiol [61]. This study is not without its caveats, but raises the intriguing possibility that androgens, not estrogens, mediate sex differences in performance on a task of spatial ability. Interestingly, these same authors performed a morphometric analysis of adult brains from neonatally

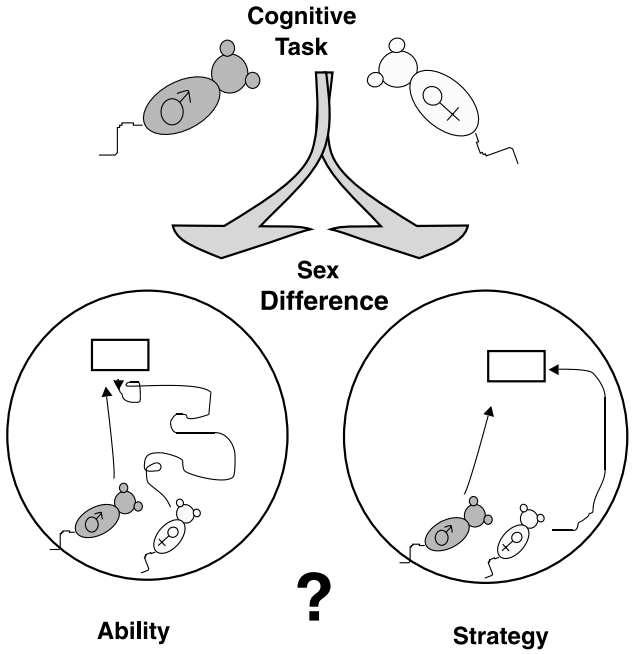

Fig. 3. Stylized representation of the sex difference in latency to reach a platform in the Morris Water Maze (based on [15]). It has been proposed that these differences are not due to impaired cognitive ability in the female but rather a difference in the strategy used to find the platform; the first few test trials consist of males venturing out into the open water whereas thigmotactic females make their way around the arena by following its edge. Since the parameter measured is time in seconds to reach the platform, females are de facto scored as having a "poor" performance compared to males.

treated animals and found androgen effects on neuron number in CA3 that were consistent with enhanced spatial performance, but in CA1 cell number was increased by estradiol. We have also noted an effect of increased cell number by postnatal estradiol administration to females that is selective to the CA1 field [51], suggesting that there may be considerable regional heterogeneity of steroid action and/or availability within the developing hippocampus. Estradiol effects restricted to the CA1 field may be the functional basis for the simplifying of associational–perceptual processes that improve acquisition of spatial tasks as proposed by Williams and Meck [165].

An additional approach for assessing the role of estradiol on hippocampal function is to knock out its synthesis. This has been achieved with transgenic technology in the form of the aromatase deficient mouse (ArKO). Male ArKO mice have severely disrupted male sexual behavior [55], consistent with the effects of perinatal aromatase inhibition in the rat. However, both males and females are equally impaired in a spatial reference test, the Y-maze [99], which negates the premise that an organizational action of estradiol has selectively acted in one sex to distinguish it from the other. Adult female mice lacking functional ERα (ERαKO) show retention deficits on a hippocampal-dependent inhibitory avoidance task, a form of trace conditioning [35], but unfortunately this parameter does not appear to have been examined in males in this strain of mouse.

Learning and memory and stress intersect in the form of the Yerkes–Dodson law which states that learning is enhanced by stress at one level, but impaired if that stress is increased or unaffected if the stress is decreased (see for review [163,31]). This centuries old tenet has been recast recently in light of sex differences. Exposure to acute stress in male rats enhances learning, but has the opposite effect in females, impairing their performance in an eye-blink conditioned reflex experiment. Treatment of neonatal females with testosterone results in a male-like performance as adults, whereas castration of newborn males leads to a female-like response. Antagonizing the androgen receptor during late gestation blocks the enhanced learning induced by stress in adult males [147], suggesting these effects are mediated by androgens, not estrogens.

Though sex differences in physiological and behavioral measures of stress are observed in many species, due to inconsistencies in the results associated with the differing tasks, how are these sex differences meaningful? To make sense of them, an ethological perspective must be employed when evaluating these data, taking into account ecologically relevant sex-specific behaviors and how these may translate into sex differences on performance in behavioral tests. This "confound" is no more evident than when evaluating behavioral measures of cognition in many mammalian species. Sex differences in cognitive ability are apparent in tests such as the mental rotation test and spatial perception task in humans [155], object reversal, and concurrent discrimination task in monkeys [5,37] as well as a variety of learning paradigms in rodents [26,170]. However, the best known and most studied sex difference in cognition is likely the reported difference in spatial ability described in rodents and humans, typically favoring males. While a variety of evolutionary theories have been postulated for explaining these differences (for review see [66]) none has been overwhelmingly accepted. Recent findings suggest that these sex differences may instead be simple methodological artifacts. In some of our own preliminary work [75], we found that sex differences in the Morris Water Maze may in fact be seasonal (see Fig. 4). While this explanation appears to fit with some of the evolutionary theories, it is important to note that these data were collected from rats, which being commissural with man, are non-seasonal breeders. Additionally, these are pre-pubescent animals so the reported changes do not appear to be related to alterations in gonadal hormone levels. Further support for sex differences in spatial ability being partly methodological is the finding that female and male latencies in the water maze are similar when animals are first pre-trained [126]. This eliminates differences that may in fact have been related to novelty stress rather than spatial ability or sex differences in the interaction of stressor exposure and cognitive ability. In fact, stress exposure on other cognitive tasks such as the hippocampal-dependent trace conditioning paradigm has been shown to facilitate male performance and reduce the female's whereas unstressed proestrous females learn the conditioned response more quickly than males [170]. These data also seem to parallel dendritic spine density in the

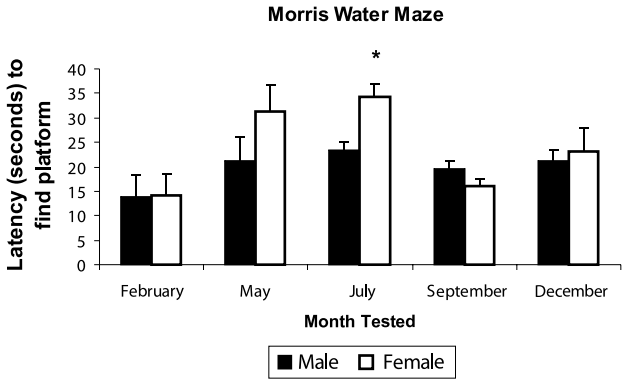

Fig. 4. Seasonal variation in latencies to find a submerged platform in the Morris Water Maze; note that the subjects are pre-pubertal rats, thus hormonal status is not a contributing variable. Sex differences are only observed in the summer months. These seasonal variations represent an important methodological source of variation that may contribute to perceiving sex differences in hippocampal-dependent learning tasks.

CA1 of the hippocampus; females in proestrous have a greater density of spines than males, however, with stressor exposure, spine density decreases in females and increases in male rats [148]. These same investigators have now taken the story even one step further with the fascinating revelation that if controllability over the stressor is introduced, the detrimental effects of stress in females and the beneficial effects on learning in males are reversed, resulting in no sex difference [86].

The previously mentioned meta-analysis on sex differences in spatial learning in rodents concluded that sex differences in the spatial learning of mice are far less robust than those in rats, and are biased towards females, raising questions about their utility for understanding sex differences in humans [65]. Primates, on the other hand, are of obvious utility. A recent study in the Rhesus monkey used a Delayed Recognition Span Test and showed young males did better than young females, but that this sex difference was eliminated by prior training, which had no effect on males but improved females to the level of males [78]. One of the reasons we justify research on cognition in animals is that there is no bias due to culture, and experience can be controlled for, but can it really? Gender is one of the primary variables by which an individual of any species is codified by other members of the same species, with age, size, and general health being the other non-behavioral measures used to assess conspecifics. Juvenile males of almost all species engage in more rough and tumble play than females, and they do this with other males, thereby assuring that the rearing experience of males and females differ. Even in rodents, the dam grooms male newborns more frequently than females. In humans, our perception of an infants gender will predict the manner in which we interact with that child, even if the gender does not match our perception. Thus, we cannot say even in our simplest animal models that the early life experiences of males and females are the same and that sex differences in adulthood are due solely to an innate biological difference. This may seem like stating the obvious, but sometimes the obvious gets lost in the quest to report significant differences on a particular variable.

So are there sex differences in the hippocampus? Yes, but they are by and large of a much smaller magnitude than generally assumed, and whether they are established by the same mechanisms leading to sexual differentiation of the diencephalon, brain regions directly relevant to reproduction, seems less and less likely upon closer investigation. In particular, if the hippocampus is undergoing sexual differentiation there should be a sex difference in gonadal steroid exposure during the perinatal sensitive period. And if the hippocampus follows the same rules as the hypothalamus, then estradiol, aromatized from testicular androgens in males, should be several fold higher in males than females. This has been assumed but never examined until recently when our laboratory made the serendipitous discovery that females have as much estradiol in the developing hippocampus as males ([1] and see below). This has led us to propose a reinterpretation of estradiol action in the developing hippocampus that postulates this hormone in fact acts to *reduce not produce* sex differences selectively in this brain region.

4. Does the developing hippocampus make its own estradiol?

The primary focus of this laboratory has been delineating the mechanisms establishing sex differences in the brain during perinatal development. These studies have ranged from sex differences in astrocyte morphology and SDN volume to depolarizing GABA action and synthesis of prostaglandins. In each of these mechanistic studies a recurrent theme has been the role of estradiol. In virtually every instance, treatment of newborn female rats with sufficient estradiol produced a masculine phenotype, regardless of the endpoint (see for review [101,102]). As a result of these experiences, which corroborated the same principle found in many published studies, we found ourselves equating estradiol exposure with maleness. When attempting to interpret our findings in the context of normal development, we discovered there was a dearth of data on brain region specific levels of estradiol. Most data on the hormonal milieu of the perinatal rodent are measures of circulating plasma levels or whole body content as opposed to content within brain [161,80,134,89,11,111]. To fill this gap in our collective knowledge, we used radioimmunoassay (RIA) to measure estradiol content of six brain regions of newborn male and female rat pups and made the surprising discovery of substantial levels of estradiol in the developing male and female hippocampus. In the female, hippocampal estradiol levels were significantly higher than any other region of the brain at 2 h after birth and were still elevated at 36 h of life [1]. Estradiol levels were also elevated in the female cortex. This prompted a further examination using treatment of neonates with an aromatase inhibitor given directly into the brain. This classic approach has been used to disrupt masculinization of sexual behavior and regulation of gonadotropin secretion, but has not been extensively employed for examining non-reproduction-related brain regions. We found that intracerebroventricular administration of formestane, a potent aromatase inhibitor, significantly reduced estradiol levels in the traditionally sexually dimorphic brain regions, the hypothalamus and preoptic area of males, but also unexpectedly in the female hippocampus and frontal cortex. Given there is no obvious peripheral source of estradiol in females, we have been developing the hypothesis of de novo estradiol synthesis by hippocampal and cortical neurons in the developing brain.

4.1. Are the necessary steroidogenic enzymes present in the neonatal rat brain?

There is precedent for de novo synthesis of estradiol in the avian brain which has significance to the sexual differentiation of the song system [143,54]. To our knowledge, there had been no previous reports of de novo estradiol synthesis in the mammalian brain until two very recent ones involving the hippocampus [132,53]. This is surprising given convergent evidence generated from studies focusing on opposite ends of the steroidogenic pathway; synthesis of neurosteroids known to modulate the $GABA_A$ receptor and studies of aromatase (CYP19) in the context of sexual differentiation of the brain. Steroidogenesis requires the transport of cholesterol into the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR) and the subsequent conversion to pregnenolone by the P450scc enzyme, CYP11. Both of these have been detected at high levels in hippocampal pyramidal neurons, along with picomolar levels of pregnenolone and its sulfated derivative [72]. Conversion of pregnenolone to progesterone, as well as dehydroepiandrosterone (DHEA), requires 3β-hydroxysteroid dehydrogenase (3β-HSD). Developmentally the expression of 3β-HSD is at its highest level in newborn hippocampus and declines with increasing age. Concentrations of hippocampal pregnenolone and progesterone measured by gas chromatography/mass spectrometry were also at their highest on the day of birth and exceeded those found in plasma, suggesting local synthesis [59]. Other studies confirm the presence of 3β-HSD as well as 5α-reductase and 21-hydroxylase [59,144,151,109]. Allopregnanolone, a 5α-reduced metabolite of dihydroprogesterone, as well as DHEA have also been detected at low levels in the brains of adult adrenalectomized and gonadectomized male rats [24,136]. Cultured astrocytes and neurons from neonatal rodent cerebral cortex are capable of synthesizing DHEA and subsequently converting the steroid to testosterone and ultimately estradiol [177]. In situ hybridization detection of CYP19 mRNA reveals high levels in POA, hypothalamus, and amygdala, with moderate levels in the hippocampus [156,157]. Activity assays reveal the same pattern in explants or cultured neurons of neonatal rat and mouse brain. The highest levels of estradiol production from [^{3}H]androstenedione are found in the diencephalic brain regions, but the neonatal hippocampus appears to make up to half that seen in these sexually dimorphic brain regions [91]. Interestingly the hippocampus and cortex are distinct from sexually dimorphic brain regions in not exhibiting aromatase activity until shortly after parturition [91]. A critical enzyme, CYP17, required for the conversion of pregnenolone to DHEA, had long eluded detection in the adult brain, despite measurable levels of DHEA, until a recent report detecting it in adult male hippocampus [53]. Previous reports of this enzyme had been limited to the developing brain [109,177]. Thus, all of the requisite machinery appears to be in place for the de novo synthesis of estradiol by the brain, and particularly in the hippocampus.

4.2. What is the function of estradiol in the developing hippocampus?

4.2.1. Estradiol enhances depolarizing GABA action in developing hippocampal neurons

Views on GABA action have changed considerably over the past decade. Once considered the primary source of inhibition in the brain, we now know it to be a principle source of excitation via depolarization-induced calcium influx through voltage sensitive calcium channels. This action is most prominent developmentally and appears to be present throughout the brain (see for review [103]). The calcium influx induced consequent to depolarizing GABA action places it squarely in the camp of being a trophic factor and it appears to contribute to the maturation of synapses [36]. The excitatory effects of GABA are mediated by the $GABA_A$ receptor, a chloride ionophore, and the relative transmembrane chloride gradient. Whether $GABA_A$ receptor activation results in chloride influx or efflux is determined by the transmembrane chloride concentration gradient which is in turn determined by the activity and expression of chloride co-transporters [36,130,131,135]. During the neonatal period, the reversal potential for chloride (E_{Cl-}) is positive relative to the resting membrane potential [9] resulting in a net outward driving force upon chloride when $GABA_A$ receptors open and membrane depolarization sufficient to open voltage-sensitive calcium channels, primarily of the L-type [82,122,60,125]. As development progresses, E_{Cl-} becomes negative relative to the resting membrane potential, thus shifting the driving force on chloride to inward and leading to $GABA_A$ receptor-mediated hyperpolarization, the primary basis for synaptic inhibition in the mature brain.

We have previously reported that estradiol enhances the depolarizing action of GABA in developing hypothalamic neurons [127] and have recently extended this to hippocampal neurons [119]. Our findings corroborate and extend the elegant work by Ben-Ari and colleagues [82,17] showing that GABA-mediated excitation is observed by the day of birth and persists at least through the end of the first postnatal week in the hippocampus. We found that $GABA_A$ receptor activation by the selective $GABA_A$ receptor agonist, muscimol, significantly enhanced intracellular calcium levels in over 80% of all cultured hippocampal neurons, supporting the claim that GABA is the major excitatory neurotransmitter during the late embryonic and early postnatal period [82,25]. We also found that pretreatment for 2 days with nanomolar concentrations of estradiol significantly increased the magnitude of

the calcium influx induced by $GABA_A$ receptor activation in individual neurons (Fig. 5). On a population level, the percentage of cells responding to GABA as depolarizing gradually diminishes with increasing age. However, chronic estradiol treatment essentially prevented this decline and maintained up to 80% of cells responding to GABA as depolarizing up to 11 days in culture, the developmental equivalent of postnatal day 9. Of interest is that the cellular mechanisms by which estradiol increases the magnitude of the calcium influx and the probability of responding to GABA as depolarizing seem to be independent from each other as they did not change in tandem. More specifically, older neurons pretreated with estradiol continued to respond to GABA as depolarizing with a higher frequency, but the magnitude of the calcium influx was only marginally different (14%) from that of control neurons that still responded, albeit it at a much lower frequency, to GABA as depolarizing. This disconnection suggests two independent mechanisms are regulating the threshold for depolarization and the amount of calcium influx [119].

What do these results mean for sex differences in the hippocampus? All these studies were conducted on neuronal cultures generated from males and females and then treated with exogenous estradiol. Given that males and females have equivalent levels of estradiol it can be speculated that any mechanisms discovered are relevant to both, but can it be empirically determined? Depriving one sex or the other of its estradiol would provide insight into whether estradiol is required as a normal trophic factor during development or is simply present but not biologically relevant.

4.2.2. Estradiol depresses excitatory glutamate in developing hippocampal neurons

The hippocampus is particularly sensitive to hypoxia/ischemia-induced injury due to excitotoxicity. Over two decades of research has provided evidence implicating the amino acid glutamate in this injury [138]. NMDA receptor activation produces, in particular, calcium and sodium influx into neurons. Excessive activation produces energy failure that yields a break down of ionic gradients and disproportionate intracellular calcium. This deregulation of calcium homeostasis ultimately cascades into cell death. In addition to NMDA receptors, activation of any of the three ionotropic glutamate receptors can also be neurotoxic [19,74,112].

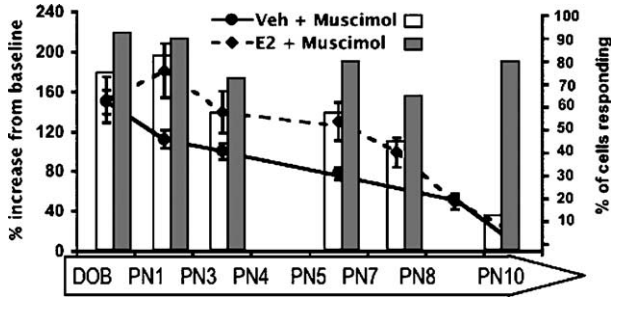

Fig. 5. A composite diagram of the developmental profile of the effects of estradiol treatment on muscimol-induced intracellular calcium levels. Percent change from baseline in peak amplitude of the calcium transients is shown on the left axis and depicted by a line graph. The percent of cells responding to $GABA_A$ receptor activation are illustrated by the bar graph; the levels are shown on the right axis (based on [119]). Note that while estradiol is acting to keep more of the older neurons responsive to $GABA_A$ receptor activation, the magnitude of the response declines dramatically.

A different story can be told about the developing brain in part due to the relative immaturity of the glutamatergic system during this time period. Damage to the hippocampus via glutamate excitotoxicity is only apparent when rats are at least one week old [105,107]. While GABA-induced neurotoxicity appears to be the predominant source of hippocampal damage before this period in life (see discussion above), we have recently shown damage from AMPA/Kainate glutamate receptor activation of the newborn. Treatment of rat pups with kainic acid on the first two days of life resulted in selective injury to the dentate gyrus exclusively in female animals [51]. The reduction in hippocampal volume and neuron number as well as the increase in pyknotic cell density associated with this injury model is prevented by estradiol pretreatment [51,52]. In an in vitro model of kainate-induced injury, estradiol pretreatment reduces the number of glutamate-induced apoptotic cells and reduces the amplitude of the calcium transient induced by kainic acid and lowers the number of neurons that respond to kainic acid with a detectable increase in intracellular calcium (Hilton and McCarthy, unpublished observation). These results parallel those reported for models of glutamate-induced excitotoxicity in older animals [114,58,160]. However, in the immature system, we found that the predominant source of kainate-induced calcium influx is via voltage gated calcium channels, secondary to depolarization of the membrane following activation of AMPA/kainate receptors. Notably, GABA-induced excitotoxicity also involves calcium influx via voltage gated calcium channels, suggesting this may be a particularly important route for damage to the developing hippocampus.

In both our models of perinatal brain damage, one induced by GABA and one by glutamate, our goal was to elucidate sex differences in susceptibility to damage and the potential role that estradiol might play in this sex difference. In the case of GABA-induced damage, we found only a moderate sex difference [118,120]. Moreover, estradiol pretreatment exacerbated the GABA-induced damage regardless of the sex of the neonate [121]. When damage is induced by glutamate receptor activation via kainic acid administration, there is a sex difference but it is highly specific to the dentate gyrus of females. Estradiol pretreatment exerts a protective effect in females but has little effect in males [51]. Taken

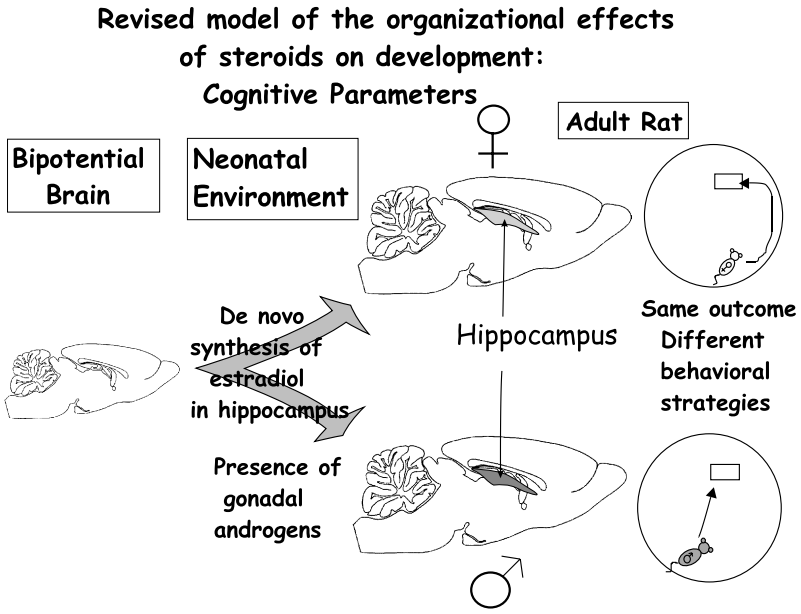

Fig. 6. Revision of Fig. 1 schematic for organization of telencephalic sex differences. This model takes into account the potential contribution of de novo steroid synthesis in these brain sites, in both sexes. In the rat, estrogens may be acting to maintain similarities between the sexes whereas androgens may be responsible for the induction of the small differences that characterize the hippocampus.

together, these findings suggest that either estradiol is not the endogenous agent protecting the male hippocampus from kainic acid-induced injury or alternatively, that estradiol is only synthesized in discrete regions of the developing female hippocampus. Mostly, these findings illustrate how much we still have to learn regarding the effects of steroids, both endogenous and exogenous, on the developing hippocampus.

5. Conclusion

The classic view of sex differences in the brain begins with differential development of the gonads. Testicularly derived testosterone and its neuronally aromatized end product, estradiol, impact on the development of the brain and consequently behavior, through endocrine actions in adulthood (see Fig. 1). Though this represents the model for sexual differentiation of the reproductively relevant neural substrate and related function, it has also become the template by which we think about and study sex differences overall. Through decades of work, the clarity and simplicity of the model have emerged. Thus dissimilarities between the sexes, in behavior or morphology and biochemistry of the brain, even those unrelated to reproduction, are assumed to follow the same rules laid out for sexual differentiation of the reproductively important hypothalamus.

The purpose of this review is to suggest that a new set of rules must be created for a complete understanding of sex differences in non-reproduction related neural structures, particularly those in the telencephalon (see Fig. 6). Sex differences in cognitive endpoints have been overly simplified and must be re-examined in a new light, without relying on the well-established dogma of sexual differentiation. Related to this but not discussed here is the emerging evidence for a contribution of genetic sex to brain differentiation, particularly in regard to non-reproductively relevant endpoints [4]. Understanding how the sexes are the same is just as important as how they differ, but the latter receives far less attention and little value as a genuine scientific finding. The imperativeness of equalizing the focus between sex-sameness and sex-differences is self evident in the use of biological arguments to justify social inequities, which regrettably are as prevalent in the year 2005 as they were in the previous century.

Acknowledgments

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