Experimental Neurology 317 (2019) 168–179

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Review Article

Sex differences in pediatric traumatic brain injury

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ARTICLE INFO

Keywords:
Sex
Gender
Neuroinflammation
Excitotoxicity
Cerebral metabolism
Mitochondria

ABSTRACT

The response of the developing brain to traumatic injury is different from the response of the mature, adult brain. There are critical developmental trajectories in the young brain, whereby injury can lead to long term functional abnormalities. Emerging preclinical and clinical literature supports the presence of significant sex differences in both the response to and the recovery from pediatric traumatic brain injury (TBI). These sex differences are seen at all pediatric ages, including neonates/infants, pre-pubertal children, and adolescents. As importantly, the response to neuroprotective therapies or treatments can differ between male and females subjects. These sex differences can result from several biologic origins, and may manifest differently during the various phases of brain and body development. Recognizing and understanding these potential sex differences is crucial, and should be considered in both preclinical and clinical studies of pediatric TBI.

1. Introduction

Pediatric traumatic brain injury (TBI) is a significant public health problem, with marked emotional, societal and economic impacts (Taylor et al., 2017; Brener et al., 2004). It is a leading cause of long-term disability in children (Keenan and Bratton, 2006), with TBI related deficits persisting for years and even into adulthood (Anderson et al., 2006; Yeates et al., 2004). Injury to the developing brain can alter developmental trajectories, leading to psychosocial, cognitive, emotional and behavioral abnormalities. Importantly, emerging literature suggests that both the response to injury and the path to recovery may be different between male and female infants, children and adolescents. The goals of this review are to summarize the literature on sex differences in pediatric TBI, describe the types and origins of sex differences in brain injury, and to highlight the convergence of sex differences with developmental brain injury pathobiology.

2. Sex differences in pediatric TBI

2.1. Differences between girls and boys after TBI: clinical studies

Boys are approximately 2 times more likely than girls to sustain a TBI, with boys 0–4 years of age having the highest incidence rates of all pediatric patients (Coronado et al., 2011; Berney et al., 1994). There are also sex-based differences in functional outcomes after pediatric TBI. Morrison et al. used the National Pediatric Trauma Registry to evaluate 16,586 pediatric cases of nonpenetrating TBI (Morrison et al., 2004). They hypothesized that sex hormones may influence outcomes, so analysis was conducted on age-based subgroups: prepubertal (0–7 years), indeterminate pubertal (8–12 years), and probable pubertal (13–19 years). Overall, there was no difference in mortality rates between boys and girls. However, girls had a longer ICU length-of-stay (LOS) than boys and a trend towards worse outcomes, when controlling for injury severity using the Injury Severity Score. In age subgroups, adolescent girls (13–19 years) had longer hospital and ICU LOS, and greater rehabilitation needs. Potential reasons for sex-based differences in outcomes were not assessed in this retrospective cohort study. Another large study used the National Trauma Data Bank to study 20,280 pediatric TBI patients (Ley et al., 2013). Similarly, in the prepubescent group (0–12 years) they found no overall differences in mortality rates between boys and girls. However, in contrast to the prior study, adolescent girls (13–18 years) had a reduced mortality rate compared to adolescent boys. The authors hypothesized that this difference could relate to endogenous hormone production.

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https://doi.org/10.1016/j.expneurol.2019.02.016
Received 4 January 2019; Received in revised form 21 February 2019; Accepted 28 February 2019
Available online 02 March 2019
0014-4886/

The literature on longer term functional outcomes in girls versus boys after TBI is relatively limited. One group of investigators found girls had better performance on the California Verbal Learning Test compared to boys, in a relatively small group of 60 pediatric TBI cases (50% male) (Donders and Hoffman, 2002). The same investigators, using a slightly larger cohort ($n = 70$, 50% male) and including a control group, reported boys performed worse than girls on the Wide Range Assessment of Memory and Learning (Donders and Woodward, 2003). Interestingly, a recent study evaluated sex differences in psychosocial functioning in adulthood following pediatric TBI (Scott et al., 2015). Females were significantly more likely than males to report internalizing problems, such as depression and anxiety, while males were significantly more likely to report externalizing problems, such as substance abuse or criminal behavior. These findings were consistent across all severities of TBI. This study suggests that lifelong psychosocial outcomes following TBI in childhood could be influenced by sex and/or gender, and recognition of this is important for targeting interventions.

Many of the studies previously cited focused on moderate to severe pediatric TBI. But the number of children that experience mild TBI and sports-related concussion is much greater, and recent studies suggest there are sex based differences in pediatric concussion. Childhood and adolescent concussion rates and reporting have increased over the last 10 years, with a study of high school athletes showing a 2.3-fold increase from 2005 to 2015 (Schallmo et al., 2017). Notably, in the most recent years studied (2014–15), girls' soccer had the highest rates of adolescent sports-related concussion versus any other sport in boys or girls. Similarly, other studies found that concussions occur in girls' sports at rates higher than those in boys' sports (Lincoln et al., 2011; Rosenthal et al., 2014; Marar et al., 2012; Covassin and Elbin, 2011; Dick, 2009). Study of high school athletes from a school system in Fairfax County, Virginia found that in sports played by both boys and girls, such as soccer, basketball, and baseball/softball, concussion risk was higher in girls (Lincoln et al., 2011). The reasons for greater rates of concussion in girls' sports are likely multifactorial, and could include biomechanical mechanisms, such as sex differences in head-neck mass, neck strength, neck girth, and ball-to-head ratio (Covassin and Elbin, 2011). In addition to higher rates of concussion, there is also higher reporting of concussive symptoms in female athletes compared to males (Covassin and Elbin, 2011; Ono et al., 2016). A study of middle and high school athletes (ages 10–18) in Atlanta found that aggregate concussive symptoms were higher in females, both at baseline (prior to injury) and immediately after injury, while the trajectory of recovery was similar between males and females (Ono et al., 2016). Female athletes also have a longer period of concussive symptoms following injury (Covassin and Elbin, 2011). A recent systematic review of concussion in student athletes synthesized the findings from 101 articles and 13 abstracts with a focus on sex differences and concluded that although there are mixed reports, females generally take longer to recover after concussion (Iverson et al., 2017). However, the authors highlight that a number of factors such as injury biomechanics, injury rates, and higher rates of symptom reporting in females, may account for these differences. Taken together, the growing body of literature on sex differences in sports-related concussion in adolescent athletes suggests that gender differences should be considered when diagnosing, monitoring, and treating concussed athletes. In addition, understanding the baseline level of symptom frequency is important for assessing recovery of female athletes after concussion.

2.2. Differences between females and males after TBI: experimental studies

Many studies have evaluated sex differences in animal models of adult TBI, however less is known about immature animals. The following section will summarize the literature on sex differences following experimental TBI during development. One of the more comprehensive, recent studies evaluated sex differences in psychosocial outcomes and neuronal morphology in the social brain network after pediatric TBI in immature mice (postnatal day, PND, 21), using the controlled cortical impact (CCI) model (Semple et al., 2017). TBI reduced dendritic arborization and complexity in the medial prefrontal cortex and hippocampus of male mice, an effect not seen in female mice. In correlation with these histologic findings, they also found that while both sexes had a reduction in same-sex sociability after TBI, male mice had a deficit in social recognition and memory. Female mice had an increase in social avoidance in sociosexual testing and spent more time immobilized in the forced swim test than males. Overall, they conclude that sex specific dendritic changes may affect sex differences in adult social behavior following early life TBI. In contrast, Russell et al. did not find a sex difference in behavioral outcomes after CCI in PND 17 rats (Russell et al., 2011). They performed a large panel of sensorimotor tests from 1 to 28 days post-TBI, including gridwalk, gait analysis, rotarod, beam walk, spontaneous forearm elevation test, and general motor activity assessment. There were no major differences between male and female rats in either baseline sensorimotor function (in sham rats) or in the sensorimotor response to TBI. They concluded that sex differences after TBI may relate primarily to post-pubertal onset of sex hormone production, but note that the prepubertal tests did not involve evaluation of social behaviors. In other studies, cerebral blood flow (CBF) autoregulation is more impaired in male than female piglets after fluid percussion TBI, and females responded with improvement in CBF after infusion of most vasoactive agents, whereas male piglets experienced no effect or detrimental effects from these agents (Armstead et al., 2010; Armstead et al., 2011; Armstead et al., 2012; Armstead et al., 2013; Armstead et al., 2016a; Armstead et al., 2016b; Curvello et al., 2017; Armstead et al., 2017a; Armstead et al., 2017b). Potential mechanisms responsible for these findings include sex differences in mitogen-activated protein kinase (MAPK), extracellular signal-related kinase (ERK), endothelin-1, and interleukin 6 (IL-6) (Kosty et al., 2012; Armstead et al., 2017a; Armstead et al., 2013; Armstead et al., 2016a; Curvello et al., 2017).

A few studies comparing sex differences following mild TBI revealed subtle, but potentially important, differences between males and females. Use of a modified weight drop model of concussion in PND 30 rats to assess social play fighting and beam walking 6–7 days after injury found that mild TBI altered play-fighting relationships, and this was dependent on the sex of the pairing and the injury status (Mychasiuk et al., 2014). Female rats receiving mild TBI were rejected and excluded from play experiences more frequently than male mild TBI or sham rats. Furthermore, injured male rats were able to learn play strategies from their sham peers, while injured female rats showed worse impairments and were more likely to respond to playful attacks by peers with avoidance. Using a similar model, female rats with mild TBI on PND 25–28 had more immediate and prolonged changes in long-term potentiation than male rats after mild TBI (White et al., 2017). This group also studied another model of mild TBI that used a modified CCI method to deliver 2 injuries per day to the helmeted, closed skull of male and female rats over a two-day period (PND 25–28) (Meconi et al., 2018). In this model, there were no major sex differences in lesion volume or most functional outcomes tested. But injured females made more errors on Barnes maze testing compared to males and there was a subset of female subjects that performed much worse than other injured and uninjured rats. Taken together, these studies suggest that important sex differences in both histologic and neurologic outcomes exist in both mild and moderate/severe TBI animal models.

In addition to sex differences in response to injury, emerging literature suggests there are sex differences in response to neuroprotective therapies after pediatric TBI. For instance, progesterone is a steroid hormone that protects the brain after TBI through many potential mechanisms, including anti-inflammatory, antioxidant, and anti-apoptotic pathways (De Nicola et al., 2009; Sayeed and Stein, 2009) (De Nicola et al., 2009; Sayeed and Stein, 2009), and over the last 20 years, multiple preclinical studies showed that progesterone has neuroprotective

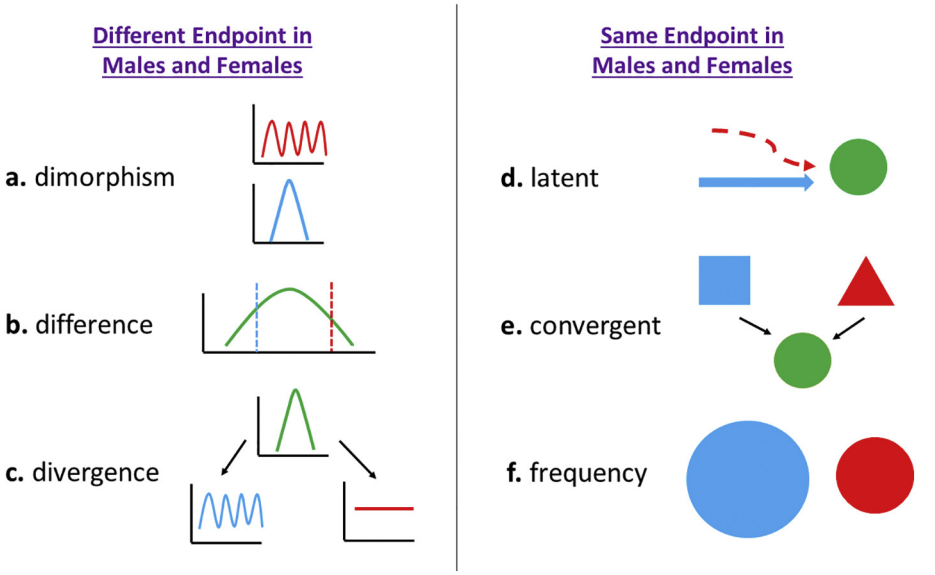

Fig. 1. There are multiple types of sex differences in the brain. The most frequent conceptualizations of sex differences in the brain involve different endpoints in males and females. *a,b)* These may be of different forms, and hence referred to as *a) sexually dimorphic* (red and blue lines representing each sex differ in form), or they may be a difference in average response along a continuum, and thus a *b) sex difference* (green line represents a continuum of possible endpoints, and sexes represented by dashed lines differ in their average value on this continuum). *b,c)* In some instances males and females are at the same point but in response to a challenge, such as stress, or over the course of the lifetime, they may diverge to different places, either along the same continuum (*b*) or taking on different forms all together (*c*). *d)* Alternatively, there are instances where males and females appear to be the same but there are underlying or latent sex differences. For instance, neural responses (represented by green circle) may appear similar in males and females but the cellular and/or molecular mechanisms by which they are achieved may be quite distinct (blue and red arrows represent unique pathways to the same endpoint). *e)* Or, one sex may be constrained by physiology inherent to reproduction, but evolved new ways of achieving the same endpoint, such as unique neural circuits to promote parenting behavior in males, referred to as convergence (each sex, represented by the square and triangle, converge on the same endpoint represented by the green circle). *f)* Lastly, a disease or other endpoint may be highly similar (circle) in the two sexes but occur at varying frequency (size of circle). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effects in adult animal models of TBI (De Nicola et al., 2009; Sayeed and Stein, 2009; Roof et al., 1994; Roof et al., 1996; Roof et al., 1997; Yao et al., 2005; O'Connor et al., 2007; Robertson et al., 2006a, 2006b; Pan et al., 2007; Chen et al., 2008). Recently, 2 studies on progesterone treatment were conducted in pediatric TBI rat models. Using the CCI model in PND 17–21 male and female rat pups, progesterone treatment resulted in sex based differences in outcomes (Robertson and Saraswati, 2015). TBI altered mitochondrial respiration at 24 h in male rats only and progesterone was able to prevent mitochondrial respiratory changes. TBI also induced loss of mitochondrial glutathione in both males and females, while progesterone treatment prevented this loss in male rats only. Interestingly, normal (naïve) male rat pups had significantly lower concentration of mitochondrial glutathione than normal female pups. Finally, progesterone treatment reduced total tissue loss only in female rats. In a study of progesterone in adolescent mice (4 week old), CCI produced deficits in vestibulomotor function using wire grip scoring (Mannix et al., 2014). Remarkably, progesterone treatment improved wire grip scores in male mice, but worsened wire grip score in female mice. There was no effect of progesterone treatment on spatial memory testing (Morris water maze) or on lesion volume in either males or females. In contrast to the sex differences seen with progesterone, the neuroprotective drug minocycline did not produce sex differences in response to treatment in a closed head injury model of CCI in PND 11 rats (Hanlon et al., 2017). Of note, in this young animal model, longer term minocycline, an antibiotic that also calms microglia, actually worsened neurodegeneration and memory deficits. Finally, the response to post-traumatic secondary insults, such as seizures, can be influenced by ovarian hormones (Hoffman et al., 2006). In adult animal studies, estrogen decreases seizure thresholds, while progesterone increases them (Hoffman et al., 2003). However, despite progesterone's ability to decrease seizure burden, cell death was worsened in progesterone treated animals that did have breakthrough seizures (Hoffman et al., 2003). These findings could be important in the management of TBI in adolescent females, where menstrual cycle changes in levels of ovarian hormones could influence the susceptibility to post-traumatic seizures, and the response of the brain to this secondary insult.

In summary there are differences in male and female response after TBI in the developing brain, including physiological response, neuropathology, and behavioral outcomes. These differences are seen in animal models of concussion/mild TBI, as well as in models of moderate/severe injury. Notably, several pediatric animal models of TBI detect sex differences in the response to therapies after injury. However, despite the growing number of preclinical studies evaluating sex differences, it is too early to identify a consistent pattern conferring benefit for one sex. In both preclinical and clinical pediatric TBI studies, this is likely to depend on many factors, such as age at injury, mechanisms of injury, and timing of post-injury outcome being measured. In conclusion, both clinical and experimental data indicate that there are sex differences following pediatric TBI and highlight the necessity of separately evaluating males and females in future studies. Importantly, future studies should also include the appropriate sex- and age-matched control groups, in both animal and human studies. Recognizing sex differences in the context of brain injury and understanding potential mechanisms have important implications for the development of translational therapies and treatments in pediatric TBI.

3. Types of sex differences

To say there is a sex difference in a particular behavioral outcome, diagnostic or anatomical measure seems like a straightforward unambiguous descriptor meaning males and females are different. But, there are in reality many ways that males and females can differ and appropriately categorizing them is useful, if not essential, to understanding the biological origins of sex differences. Even if the categorization is not perfect, it provides a conceptual context for interpretation and evaluation of the relative importance or impact of a particular sex difference. When neuroscientists discuss sex differences there are two frequently made mistakes. First is the use of the word “gender” in discussion of research on animals. Gender is a human construct that combines self-perception and others' perceptions of one's sex. We can't ask animals what sex they think they are, nor what sex they think others are, and so they do not have gender. The second common mistake is the over use of the term “sexually dimorphic” to describe any and all sex differences. Dimorphic is a compound word consisting of di = two, and morph = form, so two forms. If something is sexually dimorphic this means it has two different forms in males and females, which does happen but not very often (Fig. 1a). However, many people use the term sexually dimorphic for what is really a sex difference, often a very small one. The descriptor “sex difference” is the appropriate moniker to

apply to any continuous variable that on average differs between males and females (Fig. 1b). In many instances there is overlap in the response or endpoint in males and females, but the mean is significantly different.

A difference in the average frequency of a disease between men and women, boys and girls is also a sex difference, meaning the condition or state is the same but occurs more or less frequently in one sex versus the other (Fig. 1f). Alzheimer's disease is a good example. It is the same disease in men and women, presenting with the same symptoms and trajectory, but more likely to occur in women. Conversely, stuttering is more common in boys than girls, and so on. This has been referred to as a population sex difference (Miller et al., 2017).

There are additional complex and still poorly understood sex differences in which the responses of males and females differ according to circumstance. The best known example is the work of Tracy Shors and colleagues demonstrating that learning improves in males following stress but deteriorates in females (Shors, 2006). This behavioral shift is paralleled by changes in synaptic profile of hippocampal pyramidal neurons, with the number of synapses increasing in stressed males compared to same-sex controls and decreasing in stressed females. Importantly, in the unstressed control group there are no sex differences in this particular learning paradigm or in synaptic density. Thus, there is a sex difference induced by a divergence in responses that occurs only in the context of stress (Fig. 1c).

In contrast to sex differences involving divergence away from a common baseline is the existence of convergence, also referred to as compensation and first articulated by Geert De Vries (De Vries, 2004). Here the two sexes begin at different places but then converge on the same endpoint under certain circumstances (Fig. 1e). This may involve the lack of a specific physiology in one sex. For instance, males of most rodent species do not behave in a nurturing manner towards young, including their own offspring, whereas females that have given birth universally do so. The physiology of pregnancy and parturition is critical to inducing maternal behavior and obviously males are deprived of this experience. However, in a monogamous species of voles males have evolved a vasopressinergic circuitry that promotes paternal behavior, in other words they have compensated for the lack of exposure to the hormones of pregnancy by generating a new independent regulatory circuit. A related but distinct principle has been articulated by Catherine Woolley who has discovered latent sex differences. A latent sex difference is one that is not apparent when measuring a particular endpoint, say synaptic plasticity of hippocampal pyramidal neurons, which is the same in males and females, but the cellular mechanisms mediating that plasticity are distinct, occurring via different signal transduction pathways or different receptors all together (Huang and Woolley, 2012) (Fig. 1d). Latent sex differences may be far more pervasive than we realize simply because there has so far been little motivation to look for them. It is generally assumed that a particular physiological or functional outcome is achieved via the same mechanisms in males and females, yet this may not always be the case. In one particularly interesting example for this special issue, the cellular mechanisms leading to cell death following ischemic stroke differ in males versus females, being dominated by caspase activation in females but caspase independent pathways in males (Liu et al., 2011). This work highlights the limitations of preclinical research conducted on only one sex when it comes time for translation to clinical treatment. For these reasons, conceptualizing the various ways in which the sexes can differ is essential when attempting to unravel the origins of a particular adult response profile.

4. Origins of sex differences

4.1. Sex chromosome complement influences brain sex differences

Considering the substantial and complex role that sex and gender play in shaping our lives, ranging from societal expectations to biological realities of reproductive capacity and differential vulnerability to various diseases, it is easy to overlook the fact that it is all determined by the random winner of a race; the first sperm to successfully penetrate the egg that will eventually become a human being. Did it contain an X or a Y chromosome?

Historically and still persistently so today, the most profound and well understood attribute of the sex chromosomes is in determining the development of male or female gonads, testes (XY) and ovaries (XX), respectively. Female development, the formation of ovaries, is considered the default developmental pathway that is only eluded in males by expression of the testes-determining gene, *Sry*, located on the Y chromosome. In fact, models of XX animals which have been genetically engineered to include *Sry* on an autosome will develop male gonads, and likewise models of XY animals with *Sry* deleted will develop female gonads. The gonads are responsible for producing and putting into circulation the sex hormones that during the perinatal period organize the sexually specific neuroarchitecture required for dimorphic sex behavior and parental care, and post-pubertally activate these sexually differentiated regions. Although it has experimentally been difficult to parse out the role of the sex chromosomes outside of sex hormones, with the advent of novel mouse models (four core genotypes and chromosome Y consomic mice) the intrinsic influence that sex chromosomes have on non-gonadal tissues is gaining in appreciation (Table 1) (De Vries et al., 2002; Negrin et al., 2001).

The sex differences driven by chromosome complement primarily stem from three main differences implicit to XX and XY cells: 1)

Table 1
Models to study the contribution of sex chromosomes and sex hormones in observed sex differences

Effects of Chromosomes	Effects of Hormones
Four core genotypes: Model in which testes determining region *Sry* is moved to an autosome, resulting in four genotypes XX^{-} (ovaries), XX^{Sry} (testes), XY^{-} (ovaries), and XY^{Sry} (testes). ***Broad Conclusions***: If sex differences persist in gonad-matched, chromosome-divergent mice, effects are hormone driven and chromosome independent. If differences persist between chromosome-matched, gonad-divergent mice, effects are chromosome driven. If XY^{Sry} uniquely differs from all groups, there is a chromosome-hormone interaction.	***Organizational Effects of Steroid Hormones***: Treat females with male hormone or males with hormone antagonists in the first days of life. ***Broad conclusions***: If sex differences persist, effects may be chromosome driven, and activation effects of hormones should be assessed. If sex differences are abolished, effects are hormone-mediated.
Consomic Y Strains: Mouse and rat strains in which all chromosomes remain equal except for strain-divergent Y chromosomes. ***Broad Conclusions***: If a sex difference observed in one strain but not the other is transferrable or reversible by exchanging only the Y chromosome, the effect is Y driven. Further experiments are required to determine if there is a chromosome-hormone interaction.	For a more complete review of methods to study hormonal and chromosomal contributions to sex differences see references.[ab]

[a] McCarthy et al., 2012.
[b] Arnold and Chen, 2009.

expression of Y specific genes, 2) random X-inactivation in females, and 3) gene dosage for those genes that escape X-inactivation. *Sry* has additional roles beyond gonadal development and spermatogenesis. For example, it is expressed in dopaminergic neurons where it promotes the transcription of enzymes involved in dopamine synthesis and was found to be necessary for normal motor function (Czech et al., 2014; Czech et al., 2012; Dewing et al., 2006). In addition to the divergent role of genes like *Sry*, there is evidence that genes on the Y chromosome also serve a convergent role ensuring that male and female physiology remains overall similar. For example, symptoms associated with Turner Syndrome, ascribed to females with XO genotype, do not occur with greater frequency in males, suggesting that the Y chromosome compensates for some of the genes expressed in females with a second X chromosome (Fisher et al., 1990; Hughes and Page, 2015; Watanabe et al., 1993). X-inactivation, driven by female specific expression of the lncRNA *Xist*, prevents most double gene dosage in females by conversion of one X chromosome, randomly chosen, to heterochromatin. This establishes features unique to XX organisms, including body-wide mosaicism in the expression of maternal or paternal X chromosome, a feature that can promote resilience to X-linked disorders (Arnold, 2017). Additionally, only XX organisms express the paternally imprinted X chromosome. This can result in differential gene expression when a gene is normally inactivated in the paternal versus maternally acquired X chromosome. A number of genes have also been discovered that escape X-inactivation thereby doubling the gene dose in females, which can be protective against loss-of-function X-linked gene mutations (Dunford et al., 2017). Lastly, X-inactivation requires additional demand of the cellular epigenetic machinery, a work-load that is absent in XY organisms (Silkaitis and Lemos, 2014; Wijchers and Festenstein, 2011). The role that these unique features of XX and XY complement play in the response to disease and injury have not been thoroughly examined.

4.2. Steroid-mediated sex differences

Steroid hormones are a major source of sex differences in the brain. Considerable research in a variety of animal models has uncovered an abundance of sex differences modulated by developmental exposure to steroid hormones. Among these are volumetric differences due to differential cell death and survival, neurogenesis, dendritic morphology and synaptic patterning, and changes in astrocyte and microglial morphology and activation (reviewed in (Lenz et al., 2012, McCarthy, 2016, McCarthy et al., 2015). In the classic view of brain sexual differentiation, endogenous gonadal hormones act on the brain during a restricted critical period that begins in utero to organize the developing neuroarchitecture differently in males and females. In rodents, this process occurs during the perinatal period when sex differences in levels of gonadal hormones are large. Masculinization of the brain is initiated by exposure to high levels of intracerebral estrogens synthesized locally through the aromatization of testosterone generated by the male fetal testis (McEwen et al., 1977; Naftolin et al., 1975). In contrast, the female fetal ovary remains quiescent, thus the female rodent brain develops in the relative absence of steroid hormones (Weisz and Ward, 1980). Additionally, both male and female brains are protected from the influence of maternal estradiol in the fetal circulation by the steroid binding protein α-fetoprotein (Bakker et al., 2004; McEwen et al., 1975). In humans, studies on brain sexual differentiation are constrained by practical and ethical limitations, however extensive evidence from non-human primates suggest the critical period of sexual differentiation is largely prenatal and that testosterone acts directly on the brain to drive masculinization (reviewed in (Wallen, 2005)). In addition to the well-known perinatal period of steroid-dependent brain development, converging lines of evidence indicate that steroid hormones further refine the nervous system during adolescence when levels of gonadal steroid hormones increase with the onset of puberty (reviewed in (Schulz and Sisk, 2016)), For example, in the male rat hippocampus, androgen receptor activation by testosterone during puberty leads to long-term depression in the CA1 region, indicating that pubertal hormones may organize certain types of learning and memory (Hebbard et al., 2003),

Although our understanding is incomplete, the impact of steroid hormones on brain sex differences is robust, complex, and varies markedly by brain region. Knowing how these processes differ between the sexes can help us understand fundamental mechanisms underlying gender differences in pediatric TBI with respect to vulnerability to injury.

4.3. Neuroinflammation and neuronal excitation contribute to brain sex differences

Many of the pathways classically associated with inflammation are active in sexual differentiation of the brain. Although in adulthood many immune-based disorders, such as systemic lupus erythematosus, multiple sclerosis, and even major depression, are female biased in their prevalence, during early development it is the male brain that shows consistent evidence of increased inflammation. Much of the research done in the field of sexual differentiation has focused on the preoptic area (POA), a highly differentiated region of the telencephalon that contributes to male sexual behavior and maternal behavior. During the critical period of sexual differentiation, a higher concentration of estradiol in the male POA triggers production of the inflammatory mediator, prostaglandin E_2 (PGE_2) (Amateau and McCarthy, 2002). In the POA, PGE_2 induces cAMP production, leading to protein kinase A activation, phosphorylation of AMPA receptors, and stabilization of the receptors at dendritic spines resulting in an enduringly higher density of spines in the male POA (Amateau and McCarthy, 2002).

The increase in PGE_2 in the POA stems from an estradiol-mediated increase in the expression of cyclooxygenase-2 (COX-2), an enzyme that converts arachidonic acid to prostaglandin, which in turn increases microglia activation (Amateau and McCarthy, 2004a). Males have more of these innate immune cells in their POA, and the morphology of the microglia suggests that they are in a more reactive state (Lenz et al., 2013). When the reactivity of microglia is inhibited by treatment with minocycline females can no longer be masculinized by exposure to estradiol, pointing to the necessity of microglia activity in normal masculinization (Lenz et al., 2013). Even beyond the POA, in the period just following the testosterone surge in males, many brain regions including the parietal cortex, amygdala, and hippocampus display higher microglia counts in males (Schwarz et al., 2012a). Moreover, both the male POA and hippocampus have more mast cells (Lenz et al., 2018). Induction of mast cell degranulation is sufficient to masculinize the female POA, an effect achieved by histamine promoting microglia to produce more prostaglandins. This predilection towards inflammatory pathways in the male brain appears to be necessary for normal masculinization, but also may contribute to male vulnerability for neurodevelopmental disorders like autism and early onset schizophrenia (see for review, (McCarthy, 2019), as well as more severe outcomes in pediatric TBI.

Along with genetic predispotion, one of the most well defined risk factors for autism spectrum disorders (ASD) and schizophrenia is maternal infection during pregnancy. ASD is 4–5 times more common in males than females, and males present with earlier onset and less treatment responsive schizophrenia (Goldstein et al., 2013; Supekar et al., 2017; Yau et al., 2013). Animal models of maternal immune activation (MIA) have recapitulated the effects of maternal infection on adverse neurodevelopmental outcomes and behavioral deficits in offspring. Although these studies have not focused on sex differences, it is hypothesized that the heightened inflammatory milieu present in the male brain during this sensitive period of development exacerbates the effects of MIA to result in reduced resilience in males. Indeed, there is evidence that the maternal environment may already be skewed against the male conceptus even in the absence of maternal infection. Evidence

for this resides in the association of antibodies against fetal brain antigens in mothers of ASD patients, a male biased disorder, and association of maternal antibodies to male specific antigens and male birth order with decreased birthweight and increased rates of homosexuality (Braunschweig et al., 2008; Croen et al., 2008; Bogaert et al., 2018).

Numerous studies also provide compelling evidence of sex differences in neuronal excitability during development and suggest that the developing male brain is in a higher state of excitation. For instance, the neurotransmitter γ-Aminobutyric acid (GABA) induces depolarizing, or excitatory, responses early in development. As the brain matures, action of GABA shifts from excitatory to inhibitory, or from depolarizing to hyperpolarizing, as the result of changing intracellular chloride concentrations that are determined by the relative expression of two cation chloride cotransporters, $NKCC_1$ and $NKCC_2$, in the cellular membrane (Rivera et al., 1999). The timing of this developmental switch varies among brain regions, but a fundamental sex difference exists whereby the excitatory effects of GABA are stronger and endure longer in the developing male brain. This was first demonstrated in the rat substantia nigra pars reticulata, where $GABA_A$ mediated signaling becomes inhibitory in females around PND 10 and in males around PND 17 (Galanopoulou, 2005; Galanopoulou, 2006; Galanopoulou et al., 2003; Kyrozis et al., 2006). Similarly, the CA1 region of the developing hippocampus in male rats undergoes a longer period of GABA-directed excitatory function than in females (Galanopoulou, 2008; Nunez and McCarthy, 2007).

In summary, while sexual differentiation was once thought to hinge almost entirely upon gonadal hormones (i.e., the brain develops as male in the presence of these hormones, and as female in their absence), it is now clear that this process is more nuanced, multifactorial, and varies by brain region. Understanding how sex-chromosome complement, inflammation, and excitation contribute to sex-specific brain development are emerging areas of priority. Converging evidence, however, suggests the cellular mechanisms mediating masculinization of the brain frequently involve heightened neuroinflammation and neuronal excitability and this may increase the vulnerability of males to neuropsychiatric and neurological disorders with origins in development, and may exacerbate the impact of brain injury. Elucidating the mechanisms by which sex-specific brain development arises will increase our capacity to more reliably prevent early life brain injuries and has important implications for the diagnosis and treatment of TBI.

5. Convergence of sex differences and TBI

5.1. Normal developmental trajectories

An inherent challenge to the understanding and treatment of brain injury during development is the existence of critical and sensitive periods. Critical periods are those during which a set of developmental events must occur or forever be precluded. One of the best examples is the coinciding events of eye opening and innervation of the lateral geniculate in altricial mammals. Light is the essential external stimulus and coordinated neuronal activity is the essential internal biological response. The result is a functioning visual system. If light is withheld during the critical period, the animal will be permanently blind. Going one step further, if an animal is raised during the critical period in an environment consisting of only vertical lines, no horizontal lines, it will be enduringly blind to horizontal lines. In this way the nervous system fine tunes its responses during the critical period, and those responses are then permanently embedded in the brain. Sensitive periods are a variant of a critical period as it is a time during which the brain is responsive to particular stimuli, be they naturally occurring, such as light in the above example, or exogenously imposed such as lead exposure or thyroid deficiency, both of which severely and permanently impact the developing brain but may go entirely unnoticed in the mature brain.

The establishment of sex differences in the brain is a unique example of a combination of a critical and sensitive period involving both endogenous and potential exogenous or extraneous stimuli. As reviewed above, sexual differentiation of the brain begins with sex determination by the Sry gene of the Y chromosome, which codes for the formation of testis from the bipotential gonad (Goodfellow and Lovell-Badge, 1993). If there is no Sry gene, ovaries will develop. Everyone is aware that puberty is a time of marked divergence for males and females in terms of the hormonal milieu. But many are not as aware that as early as fetal life there is a similar marked divergence created when the fetal testis becomes steroidogenic and synthesizes sufficiently high levels of androgens that the steroid enters the bloodstream and gains access to the fetal brain. In rodents this process begins during the last days of an approximately 3-week gestation and extends into the first hours after birth (Weisz and Ward, 1980). In humans fetal steroidogenesis starts early in the 2nd trimester and is waning by birth but followed by a later active period postnatally that is sometimes referred to as “mini-puberty” (Kuiri-Hanninen et al., 2014). The opening of the critical period for sexual differentiation of the brain is operationally defined by the onset of testicular androgen production. Artificially elevating androgens prior to the naturally occurring period of steroidogenesis is largely without effect as the essential biological processes are not yet in play. The closing of the critical period is another matter. In males, the critical period closes when the surge of androgen production ends. Once the system is fully exposed to testosterone the programming process is initiated and cannot be undone, meaning that within one day of birth masculinization is largely complete and the critical period closed. Females, however, remain sensitive to the programming potential of testosterone well into the postnatal period, up to a week (Fig. 2). If females are injected with either testosterone or estradiol, the masculinization process is initiated, reflecting a sensitive period as opposed to a critical period. Moreover, if the downstream signaling pathways by which steroids exert their effect are extraneously activated, this too can impact female brain development, pushing it towards a male-like phenotype for that particular endpoint (McCarthy et al., 2018). This lucky accident of timing in rodents provides an excellent tool for studying the mechanisms of steroid mediated programming, by essentially using the female as a platform for exogenously induced masculinization. In non-human primates, however, the end of the critical and the sensitive period are both prenatal (Wallen and Baum, 2002), confounding our ability to replicate mechanistic findings from rodents and instead forcing us to consider other routes for translational insight. To the best of our current knowledge, the critical and sensitive periods for sexual differentiation in humans are also prenatal (Breedlove, 1994). However, since we cannot experiment on humans we do not know if there is a postnatal sensitive period in addition to the prenatal critical period.

At the end of the critical period of sexual differentiation of the brain, there is a period called the juvenile hiatus during which there are no circulating gonadal steroids, thus equalizing males and females in terms of hormonal profile. In rodents this is from the first few postnatal days until puberty onset starting around 35 days old. In humans this is from 6 months to a year after birth until the onset of adrenarche and the beginning of gonadal steroid production at around 8 to 12 years old respectively. But the brain is clearly still a remarkably immature organ during that time and is designed to respond to the environment and program itself in accordance with what it encounters, i.e. learn. The programming that occurs during development is particularly important as it is anticipatory of future events and circumstances. This often means that one developmental path is followed at the exclusion of another. It also usually means the decision point is restricted to a critical period during which the relevant environmental stimulus is more salient and the biological process mediating fate determination are in sync.

In the case of the visual system as reviewed above, the environmental signal is external light. In the case of sexual differentiation the signal is internal, being produced by the gonads. But there are many

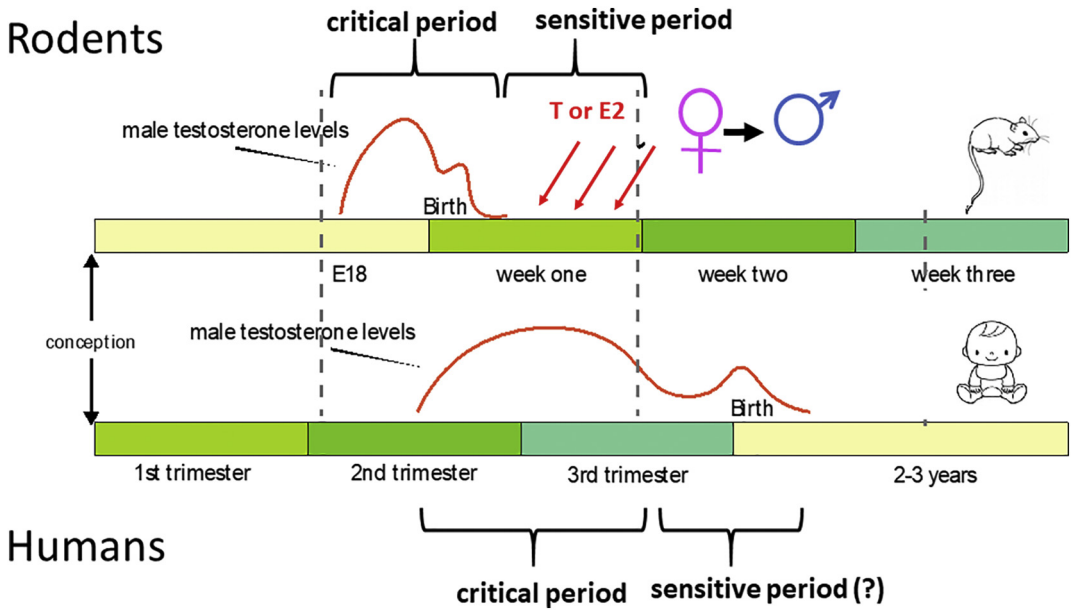

Fig. 2. Critical and sensitive periods in the establishment of sex differences in the brain. In rodents there is a critical period in males that begins in utero with the onset of testicular androgen production which initiates the process of masculinization of the brain. Females, however, remain sensitive to the impact of exogenous hormone treatment into the first postnatal week and can be essentially sex reversed if exposed during that time. Thus, the timing of the critical period in males and sensitive period in females are overlapping but not identical. In humans convergent evidence indicates there is a critical period for masculinization that begins and ends in utero but we remain unclear about the potential for a postnatal sensitive period in females as well as the functional significance of the "mini-puberty" that occurs in the first few months of life.

other critical and sensitive periods of brain development. The amount and type of nutrition experienced prenatally programs future metabolomics. Similarly, the amount and type of stress before and shortly after birth impacts adult behavioral and physiological phenotypes associated with anxiety, emotionality and activation of the hypothalamic-pituitary-adrenal axis (Bale and Epperson, 2015). In both these instances, males and females may differ in sensitivity or other aspects of critical period programming. Moreover, the developmental stage of a particular brain region may be different for males and females at any given time. There are regions of the male brain that mature faster than those of females, and vice versa. This is best illustrated by longitudinal MRI studies in children measuring cortical volume as well as white and gray matter to provide a maturation index, revealing markedly different trajectories across regions and between the sexes (Lenroot et al., 2007).

There are also critical periods that are intrinsically programmed. The cerebellum is one of the latest brain regions to develop, with birth being a clear demarcation for its maturation across species (Hibi and Shimizu, 2012). A narrowly constrained period in cerebellar development was identified in the rodent during the 2nd postnatal week when there is a peak in the production of estradiol that is essential to full maturation of the Purkinje cells, the principle neurons of the cerebellum (Hoffman et al., 2016). The control of estradiol synthesis is local to the cerebellum and is from an unexpected source, via stimulation of the steroidogenic enzyme, aromatase, by PGE_2 (Dean et al., 2012). The action of estradiol is further limited by a peak in expression of the alpha form of the estrogen receptor, ERα, during the 2nd postnatal week which then drops to undetectable by the 3rd week, essentially rendering the cerebellum blind to estradiol until adulthood when it is again exposed by estradiol coming from the gonads. Importantly, while in this case PGE_2 is a normal endogenous regulator of brain development, it can also be upregulated by either infection or inflammation or down regulated by drugs meant to combat both. Indeed, treatment with either the bacterial mimetic, LPS, or cyclooxygenase inhibitors deleteriously impacts Purkinje cell development, but only if exposure occurs during this narrow sensitive period (Hoffman et al., 2016).

An additional intrinsically programed sensitive period is adolescence, a time when the brain undergoes substantially more pruning and network refining than either immediately before or forever after (Blakemore, 2012). A time of heightened risk taking, this is also a developmental stage during which TBI is more likely to occur, including due to the increased intensity of athletic activities. Use of drugs and alcohol further add to the volatile confluence of variables leading to increased chances of TBI which can have unique enduring consequences if occurring during this dynamic stage of brain development. The combination of factors at play demand a multidisciplinary approach to fully appreciate the adolescent brain and how it might be impacted by TBI (see for review, see Ronald E Dahl, https/doi/org.https://doi.org/10.1196/annals.1308.001).

Mechanistically the uniqueness of the adolescent brain also appears to be multifactorial, involving a combination of an intrinsic developmental trajectory and the onset of gonadal hormone secretion occurring with puberty (Piekarski et al., 2017; Sisk and Zehr, 2005). Given that the timing of puberty can vary on average between boys and girls by as much as 5 years, with girls earlier, there is a clear distinction in the intersection between brain maturation and hormone exposure. The result can be substantial differences in the overall rate of maturation of brain regions in boys and girls (Lenroot and Giedd, 2006), and in animal models we can detect microlevel differences such as the number of particular cells of a given phenotype in the prefrontal cortex (Willing and Juraska, 2015; Koss et al., 2015).

These examples highlight the potential for many more undiscovered critical and sensitive periods and any study of developmental brain injury should consider the possibility of hidden variables that either enhance or constrain the impact of an insult. Moreover, the timing of critical period in males and females may be different such that a TBI at the same age in a boy versus a girl may be happening on a markedly different baseline of maturation, thereby impacting both the severity and consequences of the injury and possibly one day in the future informing us as to the best therapeutic intervention.

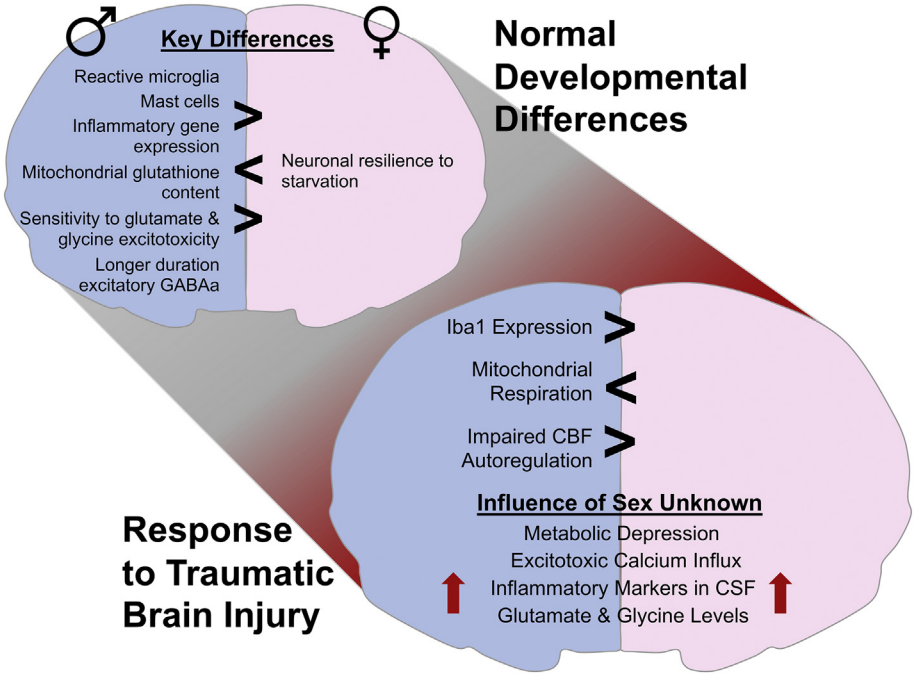

Fig. 3. Innate sex differences in the developing brain can tip the balance in pathobiology of pediatric TBI. Key differences in mechanisms of neurodevelopment may put males closer to a pathobiologic threshold. During early life, males show a greater propensity towards inflammatory pathways, sensitivity to excitotoxicity, reduced mitochondrial glutathione content and a prolonged period of excitatory GABAa activity. These differences may explain observed increases in Iba1 expression, reduced mitochondrial respiration, and impaired CBF autoregulation seen in response to pediatric TBI. However, for many known pathobiologic pathways active in the pediatric TBI response, sex has not been examined as a factor.

5.2. *Pathobiology of pediatric TBI and sex differences*

There are a multitude of pathobiologic pathways involved in secondary injury, as well as repair and recovery, after TBI. In both animal and human studies, a growing literature provides evidence that many of these occur in the developing brain after injury. As described throughout this review, many of these pathways demonstrate important sex differences, which was recently summarized in an extensive review on sex-related responses after TBI in adults (Spani et al., 2018). Here we will discuss three of the primary pathobiologic pathways found in the developing brain after TBI, including neuroinflammation, altered cerebral metabolism and mitochondrial dysfunction, and excitotoxicity (Fig. 3). In addition, we will focus on evidence for sex differences in these pathways in the young brain.

TBI is well known to initiate an inflammatory response, which begins early after injury and can persist for months to years (McKee and Lukens, 2016; Kumar and Loane, 2012; Simon et al., 2017). The inflammatory response consists of resident immune cells (microglia, perivascular cells) and infiltrating cells (macrophages, neutrophils), and is mediated by many cytokines, chemokines and other inflammatory molecules. The developing brain may have a particularly unique inflammatory response after TBI, given the normal role that microglia play in brain development and homeostasis, their location in white matter tracts in early development, and the relative pro-inflammatory phenotype of microglia in the young brain (Pierre et al., 2017; Bhalala et al., 2015). There is relatively strong evidence for neuroinflammation after pediatric TBI in both animal and human studies (Cederberg and Siesjo, 2010; Simon et al., 2017; Merkel et al., 2017; Moretti et al., 2016; Zhang et al., 2018; Hanlon et al., 2017; Schober et al., 2016; Newell et al., 2015), including a series of studies from Pittsburgh delineating the profile of inflammatory markers in the cerebrospinal fluid of infants and children after TBI (Newell et al., 2015; Buttram et al., 2007; Walko 3rd et al., 2014; Wallisch et al., 2017).

As described in this review, there are several reasons to believe that males and females may have a different severity and/or pattern of neuroinflammation after developmental TBI. The developing male brain has high levels of inflammatory mediators and reactive microglia during critical periods of sexual differentiation (Amateau and McCarthy, 2004b; Lenz et al., 2013). In addition, steroid hormones mediate changes in microglial morphology and activation (Lenz et al., 2013), and many brain regions in males show higher microglial counts after the testosterone surge (Schwarz et al., 2012b). Males also have greater numbers of other inflammatory cells, such as mast cells (Lenz et al., 2018). Overall, the propensity towards greater inflammatory pathways in the normal, developing male brain would suggest that there could be a greater degree of neuroinflammation following TBI in young males. In fact, one preclinical study of repetitive mild TBI in adolescent rats showed that male rats had increased Iba-1 positive cells in the ventromedial hypothalamus, which was not seen in female rats (Yamakawa et al., 2017). This highlights the necessity for separately studying male and female subjects, and considering the age-at-injury effect on neuroinflammation in pediatric TBI research.

In addition to inflammation, TBI also induces marked changes in cerebral metabolism and mitochondrial function (Robertson et al., 2009; Robertson et al., 2006a, 2006b; Fiskum, 2000; Yonutas et al., 2016; Yokobori et al., 2014). In the young brain, this can be particularly devastating, as the injury occurs during periods of relatively high metabolic needs due to rapid brain growth and development (reviewed in (McKenna et al., 2015). In addition, the metabolic machinery of the brain, including glucose and monocarboxylic acid transporters, enzymes for glycolysis and TCA cycle metabolism, and mitochondrial electron chain complexes all have maturational profiles during brain development (McKenna et al., 2015). Uniquely, the developing brain also has the ability to utilize alternative fuels, such as lactate and ketone bodies, for energy, which could be particularly important following brain injury (Prins, 2017; Deng-Bryant et al., 2011; Prins, 2011). Multiple preclinical and clinical studies show direct and indirect evidence for altered cerebral metabolism after pediatric TBI (Babikian et al., 2006; Aaen et al., 2010; Scafidi et al., 2009; Robertson et al., 2009). Importantly, the time course of altered metabolism may be age-dependent following TBI (Prins, 2017). After fluid percussion injury in rats, the post-traumatic period of metabolic depression lasted 10 days in adult rats, but was only 7 days in PND 35 rats, and was shortened to 3 days in PND 17 rats (Yoshino et al., 1991; Thomas et al., 2000; Prins and Hovda, 2009). It is not known if there are similar age-based differences in post-traumatic metabolic depression in humans. An additional metabolic pathway with developmental regulation is the complex biochemistry of the essential amino acid, tryptophan (O'Mahony et al., 2015; Schwarcz and Stone, 2017). In pathologic conditions, such as

following TBI, tryptophan metabolism can shift from producing protective and homeostatic products, such as serotonin, melatonin, and kynurenic acid, to producing excitotoxic products, such as quinolinic acid (reviewed in (Palego et al., 2016)). This can be especially true in the setting of robust neuroinflammation, and evidence for this metabolic shift is seen in animal and human TBI studies (Sinz et al., 1998; Meier et al., 2016; Chung et al., 2009), including pediatric TBI (Zhang et al., 2018; Bell et al., 1999; Berger et al., 2004). Furthermore, there is emerging literature that sex hormones can influence the tryptophan pathway (Barth et al., 2015), which could be an important consideration in adolescent victims of TBI.

Sex based differences in cerebral metabolism were originally attributed to the influence of circulating sex hormones. However, more recently there is evidence for innate sex differences in neuronal metabolism, which may be more pronounced after injury (reviewed in (Manole et al., 2011). For example, neurons from female rodents had a greater capacity to tolerate starvation than those from male rodents (Du et al., 2009). During a 72 h period of complete nutrient deprivation, female neurons in vitro were able to maintain cellular respiration longer, with improved survival, relative to male neurons (Du et al., 2009). This appeared to be related to increased capacity of female neurons to utilize fat sources (lipids, fatty acids) for cellular metabolism. Sex differences in the metabolic response to injury in the developing, pre-pubertal brain have also been seen. In a preclinical study of pediatric TBI, using CCI in PND 17–21 rats, trauma resulted in depressed mitochondrial respiration in male rats only, while uninjured (control) male rats had lower mitochondrial glutathione content than uninjured female rats (Robertson and Saraswati, 2015). As reviewed in section 1b, studies of CBF autoregulation in piglets by Armstead et al. show more impairment in males after TBI. Although these studies did not directly investigate cerebral metabolism, proposed mechanisms responsible for these sex differences relate closely to and can influence metabolic pathways. Direct measurements of sex differences in brain metabolism are more limited in humans. Indirect measurements using functional magnetic resonance imaging (fMRI) in adolescents (Caldwell et al., 2005) and resting EEG in 8–12 year old children (Barry et al., 2004) would suggest that there are age- and brain region-specific differences in brain metabolism in normal brain.

A third important pathobiologic pathway after TBI is excitotoxicity. Traumatic injury to the brain causes very early excitotoxicity, resulting in high levels of intracellular calcium. NMDA-type glutamate receptors are responsible for most of the pathologic influx of calcium after TBI, with other receptors (AMPA, kainite) also contributing. Importantly, all of these glutamate receptors are developmentally regulated, with generally greater expression in early postnatal development (Monyer et al., 1994; Anderson et al., 1999; McDonald et al., 1992). As a result of this developmental profile, as well as developmental shifts in NMDA receptor subunit expression and sensitivity, it is generally believed that the young brain has increased sensitivity to excitotoxic insults (Johnston et al., 2001; McDonald et al., 1988; McDonald and Johnston, 1990). In addition to glutamate receptors, the GABA receptor has a unique developmental profile, with excitatory, depolarizing responses in early development (Rivera et al., 1999; Ben-Ari et al., 1997). Once calcium has gained entry into the cell, mitochondria are responsible for intracellular calcium homeostasis, and mitochondria from the developing brain may have differing capacities for buffering calcium than those from mature, adult brains. Using isolated brain mitochondria, we demonstrated that brain mitochondria from PND 16–18 rats had a lower maximal calcium uptake capacity than those from adult rats, in the presence of ATP (Robertson et al., 2004). However, in the absence of ATP, as may occur after TBI, immature brain mitochondria had greater maximal calcium uptake. Other groups found age-dependent changes in basal calcium uptake of mitochondria (Sharma et al., 2000). In the pediatric TBI literature, studies demonstrate evidence for significant excitotoxicity. In a study of lateral fluid percussion TBI in PND 17, PND 28 and adult rats, investigators showed an acute and sustained accumulation of calcium after TBI at all ages, with the time course and location of calcium accumulation being age-dependent (Osteen et al., 2001). In clinical studies, children with severe TBI have elevated glutamate and glycine in the cerebrospinal fluid, which correlates with poor outcomes (Ruppel et al., 2001). Pediatric patients also have increases in glutamate/glutamine content in the injured brain, as measured by proton magnetic resonance spectroscopy (Ashwal et al., 2004).

Over the last two decades, there has been growing evidence for sex differences in excitatory neurotransmission in the developing brain. In a very informative study, Du et al. compared the response of male and female neurons to cytotoxic agents and treatments, and showed a sex difference in response (Du et al., 2004). Male cortical neurons were more sensitive to glutamate/glycine exposure than female cortical neurons, using cell viability as a bioassay. This same trend was seen in isolated hippocampal neurons. Furthermore, male neurons were more sensitive than female neurons to exposure to NMDA, kainite and AMPA. Sex differences in excitatory signaling have also been seen in developing rodents. A series of studies by McCarthy et al., demonstrate that the developing male brain has more significant and longer lasting excitatory effects of GABA (reviewed above). Collectively, experimental studies suggest that children may experience a sex dependent difference in the excitatory response to TBI, with the youngest male patients potentially being the most vulnerable.

6. Conclusions and future directions

In summary, the response to TBI in the developing brain is unique from that of the adult, and there are significant sex differences in this response. These sex differences can arise from a variety of biologic origins, including differences due to chromosome complement, the influence of circulating steroid hormones, and cellular mechanisms responsible for masculinization of the brain. Preclinical and clinical research supports the presence of sex differences in both the response to and the recovery from pediatric TBI. In addition, emerging literature reveals sex differences in the response to neuroprotective therapies after injury.

Although many studies demonstrated worsened neuropathology and functional outcomes after TBI in males, it would be a gross over simplification to conclude that males always fare worse. Importantly, one must consider the age at injury as there is an intersection of the endogenous developmental trajectory and the biological and cultural influences of sex, relationships that are still being elucidated. Important age sub-categories to be considered are the early development period (neonate, infant), when there is the potential for critical and sensitive periods of brain development, the juvenile hiatus period (toddler to prepuberty), when there are no circulating gonadal steroids, and adolescence (pubertal and post-pubertal), when there are major sex differences in circulating gonadal steroids as well as differences in the timing of puberty and the societal responses to that developmental milestone. Studies of pediatric TBI should take into account these development sex differences when designing preclinical protocols and clinical trials. Specifically, there appears to be a paucity of literature in the infant and toddler ages, and this may be one of the most important periods for understanding sex differences in order to provide precision medicine level therapies. Another important consideration when designing future studies is the concept of latent sex differences. It is possible that the measured, long-term outcome after pediatric TBI (i.e., injury volume, functional outcome) could be the same between male and female subjects in a given study, but the mechanisms by which that outcome was achieved could be completely different, involving different pathobiologic cascades or recovery pathways. This is especially important for the development and study of neuroprotective therapies, where both the response to injury and the response to the therapy could be influenced by sex.

In conclusion, it is imperative that future studies of pediatric TBI consider sex as a biological variable. This is important across all age

sub-categories of the pediatric population, and should be encouraged and supported in preclinical and clinical studies equally. Importantly, failure to consider sex could result in ineffective translation of preclinical therapies for acute interventions, and lack of effective, targeted clinical approaches for long-term recovery and rehabilitation following pediatric TBI.

Acknowledgements

This work was supported by the National Institutes of HealthNS092747 (CLR), P01HD085928 (MMM), and F32HD097816 (ELR).

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