



# Sex Differences in Animal Models of Traumatic Brain Injury

Journal of Experimental Neuroscience  
Volume 13: 1–10  
© The Author(s) 2019  
DOI: 10.1177/1179069519844020



Todd G Rubin<sup>1,2</sup>  and Michael L Lipton<sup>1,2,3,4</sup> 

<sup>1</sup>The Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Rose F. Kennedy Center, Bronx, NY, USA. <sup>2</sup>Gruss Magnetic Resonance Research Center, Albert Einstein College of Medicine, Bronx, NY, USA. <sup>3</sup>Department of Radiology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx NY, USA. <sup>4</sup>Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine, Bronx, NY, USA.

**ABSTRACT:** Traumatic brain injury (TBI) is highly prevalent and there is currently no adequate treatment. Understanding the underlying mechanisms governing TBI and recovery remains an elusive goal. The heterogeneous nature of injury and individual's response to injury have made understanding risk and susceptibility to TBI of great importance. Epidemiologic studies have provided evidence of sex-dependent differences following TBI. However, preclinical models of injury have largely focused on adult male animals. Here, we review 50 studies that have investigated TBI in both sexes using animal models. Results from these studies are highly variable and model dependent, but largely show females to have a protective advantage in behavioral outcomes and pathology following TBI. Further research of both sexes using newer models that better recapitulate mild and repetitive TBI is needed to characterize the nature of sex-dependent injury and recovery, and ultimately identifies targets for enhanced recovery.

**KEYWORDS:** Cognition, neuropathology, neurotrauma, neuroimaging, murine

**RECEIVED:** March 14, 2019. **ACCEPTED:** March 25, 2019.

**TYPE:** Traumatic Brain Injury and Chronic Traumatic Encephalopathy - Review

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Supported by the National Institutes of Health, National Institute of Neurologic Disorders and Stroke (R01 NS082432 and F31 NS105450-03).

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**CORRESPONDING AUTHOR:** Michael L Lipton, Gruss Magnetic Resonance Research Center, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, Bronx, NY 10641, USA. Email: Michael.lipton@einstein.yu.edu

## Introduction

Traumatic brain injury (TBI) is a major public health burden. The number of TBIs per year is not known due to the many that go undiagnosed, but it has increased steadily over the past decade and is estimated that anywhere from 1.4 to 42 million occur every year.<sup>1–4</sup>

Most head trauma, up to 80% of all cases, fall into the mild subgroup of traumatic brain injury (mTBI), also known as concussion.<sup>5,6</sup> Concussion occurs from a direct blow to or rapid acceleration-deceleration of the head, with or without loss of consciousness, that causes rapid onset of altered neurological function. Typical symptoms include headache, nausea, sensitivity to light, and impaired concentration and memory.<sup>7</sup> Although concussion produces symptoms that are understood to arise from brain pathology, clinical imaging abnormalities such as hemorrhage or skull fracture are not typically present.<sup>8,9</sup> The diverse mechanisms by which concussion occurs and its varied clinical symptoms implicate heterogeneity of the underlying neuropathology.<sup>10</sup> Although most patients recover from concussion in a matter of hours to weeks, some remain symptomatic for months or even years.<sup>11–13</sup>

Understanding why some patients recover relatively quickly whereas others remain symptomatic is of the utmost importance. Preclinical studies over the past 30 years have yielded valuable information about various aspects of injury and recovery, such as the elaboration of a prolonged innate neuroinflammatory process and acute changes in phosphorylated tau deposition following concussion.<sup>14–16</sup> Currently, the only clinical treatment for concussion is rest and supportive care. Because

strict rest may actually confer worse and prolonged symptoms, active recovery methods have started to be implemented.<sup>17–19</sup> In the search for direct biological interventions to treat concussion, animal models are critical for defining viable therapeutic targets for ameliorating symptoms and enhancing recovery. Given the heterogeneity with which concussion manifests in humans, it is important for animal models to address potential sources of inter-individual variation.

One area that has received increased attention as a possible modifier of outcome after concussion is biological sex. Multiple studies have shown that females are at greater risk than men for poor outcomes following concussion.<sup>20–23</sup> Despite the fact that men are at greater risk for concussion due to greater participation in high-risk activities, women tend to report more symptoms and more persistent sequelae following concussion.<sup>22–25</sup> These findings have been debated due to the subjective self-reporting involved to collect these measures, with some attributing the differences to societal pressure causing men to underreport symptoms.<sup>26</sup> Various mechanisms have been postulated as to why men and women have different outcomes following concussion and subconcussive injury, such as force of injury, number of injuries, skull and brain shape, neck strength, and hormonal influences.<sup>27,28</sup> Further investigation, including in animal models, will be necessary to isolate individual aspects of injury and recovery to develop new modes and methods for treatment.

With a growing body of clinical evidence that sex may be an important factor conferring risk from TBI, defining the underlying mechanisms using animal models remains an important



Creative Commons CC BY: This article is distributed under the terms of the Creative Commons Attribution 4.0 License

(<http://www.creativecommons.org/licenses/by/4.0/>) which permits any use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

goal. This review seeks to summarize the sex-dependent findings from animal models of TBI to better understand what role sex differences play, and where opportunity may lie for future research to identify actionable targets for intervention.

## Methods

This review used a structured search of PubMed to include all relevant articles through 2018. Search terms included “Sex Characteristics” or “Sex Factors”; “Disease Models. Animal”; and “Brain Injuries,” “Concussion,” “TBI,” and “mTBI.” Searches were limited to articles in English. Papers were screened to retain original research articles reporting sex effects related to TBI in animal models. Studies of stroke or other non-TBI injury models such as direct lesion, as well as reviews, editorials, letters and case reports, were excluded. References of included papers were reviewed to identify additional papers. A total of 50 articles were identified and are reviewed here (Table 1).

## Sex-Dependent Changes to Cognition and Behavior Following TBI

In studying the effects of TBI, 3 areas are most commonly assessed for behavioral changes: sensorimotor function, cognitive function, and anxiety/depression-like behavior. Most studies have reported that TBI is associated with reductions in motor performance across multiple tests. Studies that have examined sex have largely reported females to be more resilient than their male counterparts. O'Connor et al investigated the effects of different anesthetics on motor performance after TBI in male and female adult Sprague Dawley (SD) rats. They reported baseline sex differences in rotarod performance, with females outperforming males.<sup>29</sup> This finding was extended to post-injury performance. Both sexes were impaired following TBI in comparison with pre-injury, but females performed better than males, and their performance recovered at a faster rate. Other studies have also shown that females perform better than males on rotarod and other sensorimotor evaluations such as beam balance and wire grid foot fault.<sup>30–33</sup> However, in a model of juvenile TBI, no sex differences were reported for sensorimotor tasks.<sup>34–36</sup> In one of the few studies that reported on a repetitive mTBI model, adolescent female rats were more impaired than males in the beam balance task.<sup>37</sup> However, a subsequent study from the same group showed no sex difference in the beam balance using the same repetitive model.<sup>36,37</sup> It is possible that these prepubescent animals showed no differences, or even the opposite association, if sex hormones play a role in producing the behavioral effect. Another study of adolescent C57/Bl6 mice showed progesterone improved performance of males on a wire grip test, but led to worse performance in females, suggesting a ceiling effect.<sup>38</sup>

O'Connor probed spatial memory using the Barnes Maze, and females performed better post injury than males when isoflurane was used as the anesthetic during injury, but no difference between the sexes was detected when halothane was used.

This suggests a sex-dependent effect of injury as well as anesthetic. In a subsequent study by the same group, ovariectomized (OVX) SD rats performed similarly to males on rotarod and Barnes maze after injury, but regained their pre-TBI performance advantage over males when treated with progesterone 30 minutes after injury.<sup>39</sup> Females also were shown to be less impaired in the novel context mismatch task, a test of working memory, after repetitive mTBI.<sup>37</sup> Other groups have also shown a similar sex-dependent difference in spatial memory using the Morris Water Maze (MWM), where females performed better than males following a controlled cortical impact (CCI).<sup>33</sup> However, in a previous study from the same group, MWM performance of males and females was similar, but only males improved their performance when housed in an enriched environment.<sup>40</sup> Still, others have shown the exact opposite association of sex with MWM performance after TBI, with males showing shorter latencies to find the platform.<sup>41</sup> Of note, this last study was the only one reviewed to use a model that accelerated the animal into a fixed object to produce TBI, rather than an object accelerated into the animal's head. In summary, cognitive deficits have largely been shown to be more pronounced in male animals than females, but vary across TBI models.

A few groups have examined sex-dependent effects of TBI on depression- and anxiety-like phenotypes using the open field test and the elevated plus maze (EPM). Mychasiuk et al found that both male and female rats exhibit reduced locomotion in the open field following TBI produced using the modified weight drop model, but male locomotion was depressed more than that of females.<sup>42</sup> They also showed females to have more exploration of the open arms of the EPM than males, indicating greater anxiety in the males, or loss of inhibition in the females. In a subsequent study by the same group, adolescent rats subjected to repetitive mTBI showed sex differences in the forced swim task, where females showed a greater depressive phenotype than males.<sup>37</sup> Other groups have also found greater activity of female mice in the open field following CCI,<sup>43</sup> whereas others have found no difference between males and females.<sup>44,45</sup> The few studies that have reported sex dependence of depression- and anxiety-like behaviors following TBI seem to show a protective effect of female sex, but may this may depend on the TBI model, including number of impacts. The paucity and diversity of studies to date precludes any definitive conclusions.

Finally, one group reported on social behavior in juvenile rats following TBI by using a social play fighting test. Following induction of TBI by modified weight drop, females showed reduced levels of play compared with males, and there were sex-dependent changes in the social interaction of sham animals with TBI animals. Sham females were less likely to interact with TBI females than sham males with their male TBI counterparts.<sup>35</sup> In a follow-up study, the same group investigated attention deficit hyperactivity disorder-like behavior,

**Table 1.** All papers examining sex differences after TBI.

YEAR	AUTHORS (FIRST, LAST)	TITLE	ANIMAL	BOTH SEXES	MODEL	REFERENCE
1993	(Claire Emerson, Robert Vink)	Estrogen improves biochemical and neurologic outcome following traumatic brain injury in male rats, but not in females	Adult SD Rat	Yes	FPI	55
1993	(Robin Roof, Donald Stein)	Gender influences outcome of brain injury: progesterone plays a protective role	Adult SD Rat	Yes	Trad CCI	59
1996	(Robin Roof, Donald Stein)	Progesterone rapidly decreases brain edema: treatment delayed up to 24 hours is still effective	Adult SD Rat	Yes	Trad CCI	60
2000	(Robin Roof, Donald Stein)	Estrogen-related gender difference in survival rate and cortical blood flow after impact-acceleration head injury in rats	Adult SD Rat	Yes	Modified weight drop	53
2001	(Takuji Igarashi, Linda Noble)	Regional vulnerability after traumatic brain injury: gender differences in mice that overexpress human copper, zinc superoxide dismutase	CD-1 Mice	Yes	Trad CCI	46
2002	(Amy Wagner, C. Edward Dixon)	Intervention with environmental enrichment after experimental brain trauma enhances cognitive recovery in male but not female rats	Adult SD Rat	Yes	Trad CCI	40
2003	(Nancy Kupina, Edward Hall)	Cytoskeletal protein degradation and neurodegeneration evolves differently in males and females following experimental head injury	CF-1 Mice	Yes	Weight drop	54
2003	(Christine O'Connor, Robert Vink)	Interaction between anesthesia, gender, and functional outcome task following diffuse traumatic brain injury in rats	Adult SD Rat	Yes	Weight drop	29
2003	(Takamoto Suzuki, W. Dalton Dietrich)	The importance of gender on the beneficial effects of posttraumatic hypothermia	Adult SD Rat	Yes	FPI	47
2005	(X Chen, AK Wagner)	Gender and environmental effects on regional brain-derived neurotrophic factor expression after experimental traumatic brain injury	Adult SD Rat	Yes	Trad CCI	81
2005	(Edward Hall, Kirstina Pavel)	Lack of a gender difference in post-traumatic neurodegeneration in the mouse controlled cortical impact injury model	CF-1 Mice	Yes*	Focal CCI	56
2005	(Nigel Jones, Sean Murphy)	The neuroprotective effect of progesterone after traumatic brain injury in male mice is independent of both the inflammatory response and growth factor expression	Adult C57	Yes	Aseptic cryogenic cerebral injury	48
2005	(Christine O'Connor, Robert Vink)	Both estrogen and progesterone attenuate edema formation following diffuse traumatic brain injury in rats	Adult SD Rat	Yes**	Weight drop	62
2005	(Changsheng Qu, Michael Chopp)	Effect of atorvastatin on spatial memory, neuronal survival, and vascular density in female rats after traumatic brain injury	Adult SD Rat	Only females	Trad CCI	49

(Continued)

Table 1. (Continued)

YEAR	AUTHORS (FIRST, LAST)	TITLE	ANIMAL	BOTH SEXES	MODEL	REFERENCE
2005	(Amy Wagner, C. Edward Dixon)	Gender and environmental enrichment impact dopamine transporter expression after experimental traumatic brain injury	SD rat	Yes	Trad CCI	82
2006	(Christine O'Connor, Robert Vink)	The temporal profile of edema formation differs between male and female rats following diffuse traumatic brain injury	Adult SD Rat	Yes	Weight drop	61
2007	(Annadora J Bruce-Keller, Stephen Scheff)	Gender and estrogen manipulation do not affect traumatic brain injury in mice	Adult C57	Yes	Trad CCI	78
2007	(William Armstead, Monica Vavilala)	Adrenomedullin reduces gender-dependent loss of hypotensive cerebrovasodilation after newborn brain injury through activation of ATP-dependent K channels	Piglet	Yes	FPI	67
2007	(D Meffre, R Guennoun)	Steroid profiling in brain and plasma of male and pseudopregnant female rats after traumatic brain injury: analysis by gas chromatography/mass spectrometry	Adult SD Rat	Yes***	Trad CCI	84
2007	(Christine O'Connor, Robert Vink)	Effects of progesterone on neurologic and morphologic outcome following diffuse traumatic brain injury in rats	Adult SD Rat	Yes**	Weight drop	39
2007	(Amy Wagner, C. Edward Dixon)	Gender associations with chronic methylphenidate treatment and behavioral performance following experimental traumatic brain injury	Adult SD Rat	Yes	Trad CCI	33
2007	(Ye Xiong, Michael Chopp)	Role of gender in outcome after traumatic brain injury and therapeutic effect of erythropoietin in mice	Adult C57	Yes	Trad CCI	32
2009	(Helen Bramlett, Robert Keane)	Sex differences in XIAP cleavage after traumatic brain injury in the rat	Adult SD Rat	Yes	FPI	58
2009	(Symeon Missios, Ann-Christine Duhaime)	Scaled cortical impact in immature swine: effect of age and gender on lesion volume	Pigs, 3 ages	Yes	Trad CCI	50
2010	(William Armstead, Monica Vavilala)	Adrenomedullin prevents sex-dependent impairment of autoregulation during hypotension after piglet brain injury through inhibition of ERK MAPK upregulation	Piglet	Yes	FPI	68
2010	(William Armstead, Monica Vavilala)	Impaired cerebral blood flow autoregulation during posttraumatic arterial hypotension after fluid percussion brain injury is prevented by phenylephrine in female but exacerbated in male piglets by extracellular signal-related kinase mitogen-activated protein kinase upregulation	Piglet	Yes	FPI	73
2010	(William Armstead, Monica Vavilala)	SNP improves cerebral hemodynamics during normotension but fails to prevent sex dependent impaired cerebral autoregulation during hypotension after brain injury	Piglet	Yes	FPI	69

Table 1. (Continued)

YEAR	AUTHORS (FIRST, LAST)	TITLE	ANIMAL	BOTH SEXES	MODEL	REFERENCE
2011	(William Armstead, Monica Vavilala)	Phenylephrine infusion prevents impairment of ATP- and calcium-sensitive potassium channel-mediated cerebrovasodilation after brain injury in female, but aggravates impairment in male, piglets through modulation of ERK MAPK upregulation	Piglet	Yes	FPI	70
2011	(Kristin Russell, Beth Levant)	Sensorimotor behavioral tests for use in a juvenile rat model of traumatic brain injury: Assessment of sex differences	Immature LE Rat	Yes	Trad CCI	34
2012	(William Armstead, Monica Vavilala)	TBI sex dependently upregulates ET-1 to impair autoregulation, which is aggravated by phenylephrine in males but is abrogated in females	Piglet	Yes	FPI	71
2013	(William Armstead, Monica Vavilala)	Dopamine prevents impairment of autoregulation after traumatic brain injury in the newborn pig through inhibition of Up-regulation of endothelin-1 and extracellular signal-regulated kinase mitogen-activated protein kinase	Piglet	Yes	FPI	74
2015	(Rachel Lazarus, Gregory Mueller)	Protein carbonylation after traumatic brain injury: cell specificity, regional susceptibility, and gender differences	Adult SD Rat	Yes	Trad CCI	79
2014	(Rebekah Mannix, Shenandoah Robinson)	Sex differences in the effect of progesterone after controlled cortical impact in adolescent mice: a preliminary study	Adolescent C57	Yes	Trad CCI	38
2014	(Richelle Mychasiuk, Michael Esser)	Mean girls: sex differences in the effects of mild traumatic brain injury on the social dynamics of juvenile rat play behaviour	Juvenile SD Rat	Yes	Modified weight drop	35
2015	(Mattias Gunther, Marten Rising)	COX-2 regulation and TUNEL-positive cell death differ between genders in the secondary inflammatory response following experimental penetrating focal brain injury in rats	Adult SD Rat	Yes	Trad CCI#	57
2015	(Richelle Mychasiuk, Michael Esser)	A mild traumatic brain injury (mTBI) induces secondary attention-deficit hyperactivity disorder-like symptomatology in young rats	Juvenile SD Rat	Yes	Modified weight drop	45
2015	(Courtney Robertson, Manda Saraswati)	Progesterone protects mitochondrial function in a rat model of pediatric traumatic brain injury	Immature SD Rat	Yes	Trad CCI	51
2016	(Rastafa Geddes, Iqbal Sayeed)	Progesterone treatment shows benefit in female rats in a pediatric model of controlled cortical impact injury	P28 SD Rat	Females only##	Trad CCI	30
2016	(William Armstead, Monica Vavilala)	Preferential protection of cerebral autoregulation and reduction of hippocampal necrosis with norepinephrine after traumatic brain injury in female piglets	Piglet	Yes	FPI	75

(Continued)

Table 1. (Continued)

YEAR	AUTHORS (FIRST, LAST)	TITLE	ANIMAL	BOTH SEXES	MODEL	REFERENCE
2016	(Richelle Mychasiuk, Michael Esser)	The direction of the acceleration and rotational forces associated with mild traumatic brain injury in rodents effect behavioural and molecular outcomes	Adult Rat	Yes	Modified weight drop	42
2016	(Xiupeng Xu, Jing Ji)	Sex-related differences in striatal dopaminergic system after traumatic brain injury	Adult CD-1 Mouse	Yes	Trad CCI	43
2017	(William Armstead, Monica Vavilala)	Sex and age differences in epinephrine mechanisms and outcomes after brain injury	Piglet	Yes	FPI	76
2017	(Peter Wirth, Melissa Glenn)	New method to induce mild traumatic brain injury in rodents produces differential outcomes in female and male Sprague Dawley rats	Adult SD Rat	Yes	Impact acceleration novel model	41
2017	(Kristin Free, Anthony Kline)	Comparable impediment of cognitive function in female and male rats subsequent to daily administration of haloperidol after traumatic brain injury	Adult SD Rat	Yes	Trad CCI	31
2017	(Sonia Villapol, Mark Burns)	Sexual dimorphism in the inflammatory response to traumatic brain injury	Adult C57	Yes	Trad CCI	52
2017	(David Wright, Richelle Mychasiuk)	Sex matters: repetitive mild traumatic brain injury in adolescent rats	Adolescent SD	Yes	Modified weight drop	37
2017	(Victor Curvello, William Armstead)	Sex and age differences in phenylephrine mechanisms and outcomes after piglet brain injury	Piglet	Yes	FPI	72
2018	(Amandine Jullienne, Andre Obenaus)	Male and female mice exhibit divergent responses of the cortical vasculature to traumatic brain injury	Adult C57	Yes	Trad CCI	44
2018	(Anna Taylor, Richard Sutton)	Sex differences in thermal, stress, and inflammatory responses to minocycline administration in rats with traumatic brain injury	Adult SD Rat	Yes**	Trad CCI	63
2018	(David Wright, Richelle Mychasiuk)	Telomere length and advanced diffusion MRI as biomarkers for repetitive mild traumatic brain injury in adolescent rats	Adult SD Rat	Yes	Modified weight drop	36

Abbreviations: FPI, fluid percussion injury; SD, Sprague Dawley; Trad CCI, traditional controlled cortical impact; TBI, traumatic brain injury.

\*Used animals of same weight, females 5-6 weeks older than Males.

\*\*Ovariectomized females.

\*\*\*Pseudopregnant females.

#Penetrating injury, very severe and not diffuse.

#Compared with males from another study from the same lab.



following experimental TBI, and found males exhibited reduced inhibitory control compared with females.<sup>45</sup>

### Sex-Dependent Changes to Pathophysiology

Quantifying the extent of tissue damage is important for assessment of TBI pathology. Depending on the severity of TBI, however, frank tissue loss, necrosis, or apoptosis may be absent. In studies of more severe injuries that produce macroscopic brain lesions due to TBI, females consistently exhibit a smaller lesion than males receiving the same TBI, across various species and TBI model mechanisms.<sup>30,31,46–52</sup> Moreover, in studies of the most severe TBI, females generally have lower mortality than males,<sup>53,54</sup> with one exception.<sup>55</sup> Limited reports on neuropathological aspects have detected no divergence of neurodegeneration and cell death by sex, using a silver stain,<sup>56</sup> while another identified greater apoptosis in males compared with females using TUNEL staining.<sup>57</sup> Structural magnetic resonance imaging (MRI) studies have also been limited and their results were mixed, such as reduced prefrontal cortex (PFC) volume after mTBI in female SD compared with males in one study and reduced curvature of the corpus callosum in males after repetitive experimental mTBI in another.<sup>36,37</sup> Lesion recovery after injury was found to diverge between the sexes, demonstrated by labeling with BrdU (bromodeoxyuridine), a marker of newly proliferated neurons. Female mice showed greater cortical, but not hippocampal neurogenesis following CCI than males, indicating regional specificity of the sex dependence.<sup>32</sup> Further investigation of pathways governing the sex dependence of cell death after TBI has found that X-linked inhibitor of apoptosis (XIAP) is upregulated in female rats after fluid percussion injury (FPI), possibly protecting females from cell death.<sup>58</sup> Another group showed that male and OVX female rats treated with progesterone after TBI showed reduced expression of caspase-3, a key component of the apoptotic pathway, and produced a reduction in dark cell change in the hippocampus, suggesting less cell death.<sup>39</sup> Overall, studies indicate females have a protective advantage over males when considering numerous pathologic features such as lesion size, cell death, and recovery/neurogenesis. However, others have shown no sex difference, or an advantage for males over females, effects that appear to be model dependent.<sup>41,44,55</sup>

Deeper investigations into the root causes of sex-dependent injury and recovery of tissue have revealed secondary physiologic changes that may prove informative. Some of the earliest studies of sex dependence investigated TBI-induced edema by measuring cerebral water content. In a series of studies, Roof et al<sup>59,60</sup> showed that adult SD rats elaborate edema after CCI, with males showing a greater increase than females. This difference was attributed to the action of progesterone, because males who received progesterone 1 hour following injury exhibited levels of edema similar to females.<sup>60</sup> Another group followed the evolution of post-injury edema attributed to

changes in blood brain barrier (BBB) permeability. Male rats elaborated greater edema than females following a weight drop TBI.<sup>61</sup> In addition, the peak of edema occurred at 5 hours post injury for males, but was delayed to 24 hours for females. Edema resolved by 5 days post injury in females, whereas it persisted in males at 5 days post injury, the last day assayed. Studies from the same group showed administration of estrogen or progesterone 30 minutes after injury reduced brain water content following TBI.<sup>62</sup> Sex divergence of post-TBI hyperthermia has also been reported. Males remained hyperthermic for 5 days post TBI, whereas females recovered normal body temperatures after 1 day.<sup>63</sup> Post-injury brain edema and hyperthermia are important prognosticators of impairment<sup>64,65</sup> that affect males to a greater extent than females.

In association with brain swelling and edema, hypoperfusion with reduction of cerebral blood flow (CBF) is a hallmark of severe TBI that confers significantly worse outcome.<sup>66</sup> One group has reported sex differences in CBF using the FPI model of TBI in piglets. They were able to show that pial arteries in the parietal cortex constricted in a sex-dependent manner following TBI, where males were more constricted than females.<sup>67</sup> In subsequent studies, a sex-dependent increase in adrenomedullin was shown to dilate the pial artery to a greater extent in females, by acting on arterial potassium channels.<sup>67,68</sup> Various pressors were then administered to assess restoration of CBF. Sodium nitroprusside was shown to increase CBF compared with vehicle-treated TBI animals of both sexes but failed to improve outcome.<sup>69</sup> Phenylephrine was shown to upregulate endothelin-1 (ET-1) in a sex-dependent manner and to act on potassium channels and regulate vasodilation.<sup>70–72</sup> ET-1 itself was upregulated in a sex-dependent manner following TBI, and phenylephrine further worsened CBF in males, but improved in females.<sup>71,73</sup> Dopamine was investigated in the same model and blocked TBI-induced upregulation of ET-1 in both males and females, protecting them from hypoperfusion.<sup>74</sup> Norepinephrine and epinephrine were shown to have differential effects, with norepinephrine augmenting recovery of CBF in females but not males, whereas epinephrine improved CBF for both infants, but failed to confer the same protection for juvenile males.<sup>75,76</sup> The choice of pressors used to manage cerebral perfusion in severe TBI may be greatly influenced by sex, a finding with important translational implications.

Many groups have reported on the neuroinflammatory response of the brain after TBI, which is recognized as a crucial component of injury, repair, and recovery. However, relatively few studies have reported sex divergence of TBI-related neuroinflammation.<sup>77</sup> Widespread elevation of the neuroinflammatory markers IL-6, TNF $\alpha$ , and MCP-1 and activated microglia were reported in both sexes following CCI.<sup>78</sup> When OVX female mice were resupplemented with exogenous estrogen, reduction of proinflammatory IL-6 and MCP-1, an elevation in anti-inflammatory IL-4 was shown in comparison with

males, but it made no difference in TBI lesion size.<sup>78</sup> Taylor et al<sup>63</sup> also profiled TNF $\alpha$ , IL-6, and IL-1 $\beta$  after TBI, and found female rats expressed more cortical IL-6 than males, but males expressed more cortical TNF $\alpha$  and IL-1 $\beta$ . A study by Villapol et al<sup>52</sup> did more detailed profiling of microglial morphology and activation state showing male mice exhibited more activated microglia 1 day following CCI than females, as well as greater peripheral macrophage infiltration at 1 and 3 days post CCI. In addition, more astrogliosis was seen by glial fibrillary acidic protein (GFAP) immunofluorescence in males compared with females at 1 and 7 days post CCI in the same study. Jullienne et al,<sup>44</sup> however, demonstrated greater GFAP expression in female mice at 1 day post CCI but no difference between the sexes at 7 days post injury.

Differences seen in neuroimmune cell populations following injury (above) may have resulted from, and contributed to, differential cytokine expression following injury. Females expressed more IL-1 $\beta$  at 4 hours post injury than males, whereas males expressed more anti-inflammatory TG-F $\beta$  than females at 1 day post injury.<sup>52</sup> In addition to cytokines, proinflammatory enzymes COX-2 and iNOS play important roles in neuroinflammation and oxidative stress in the brain. Gunther et al<sup>57</sup> found that TBI induces a greater increase of COX-2 in male rats, and a greater increase in iNOS in females. Although females produced more iNOS, they did not report a sex-dependent difference in oxidative stress by measuring 3-nitrotyrosine. However, Lazarus et al<sup>79</sup> measured protein carbonylation, a quantifiable consequence of oxidative stress, after CCI and found region-specific increases in males that were significantly greater than females. Although the neuroinflammatory response following experimental TBI is highly dependent on injury model, sex appears to play a crucial role in nature and evolution of the post-TBI neuroinflammatory response.

Few studies have explored sex differences in TBI pathogenesis related to individual genes and pathways associated with brain injury and recovery. Brain-derived neurotrophic factor (BDNF) is an important trophic factor that helps maintain existing neuronal function, and supports new neuron growth, among many other functions.<sup>80</sup> Chen et al<sup>81</sup> investigated BDNF expression in adult rats following CCI and found spatial and temporal sex-based differences in BDNF protein levels. Males and females showed significantly different increases in BDNF within frontal cortex ipsilateral to the impact site and in the contralateral hippocampus, respectively, while both males and females showed reductions in BDNF in the ipsilateral hippocampus.

Wagner et al<sup>82</sup> examined the sex-dependent changes to the dopamine system following TBI and showed the dopamine transporter (DAT) expression to be reduced in cortical-striatal circuits in rats of both sexes, but relatively more so in males.

Hormonal status can have profound effects on behavior and pathology.<sup>83</sup> To test the effects of TBI on hormone levels in the brain, Meffre et al<sup>84</sup> performed CCI to produce diffuse TBI in

adult rats and measured endogenous hormones by gas chromatography/mass spectrometry. They found increases in progesterone and its metabolites following TBI that varied by sex, location, and time following CCI. The authors suggest that progesterone is reduced into its metabolites locally within the brain following injury, and that it may contribute to differences in recovery between males and females.

## Conclusion

It is still largely unknown what features underlie variation of risk and susceptibility to long-term adverse outcome among concussion patients as well as those who experience repetitive head impacts (RHI). However, epidemiologic and animal studies have given us clues regarding specific pathologies and molecular pathways to explore. The heterogeneous nature of injury complicates the situation, but there remains optimism toward finding better treatment strategies.<sup>85</sup>

At the moment, there is clinical and preclinical evidence that sex may play a role in susceptibility to worse outcomes following brain trauma. Although it is not yet known what aspect of biological sex confers this increased risk, the knowledge of its existence provides a path for investigation into its molecular underpinnings. However, most animal studies have studied males only.<sup>86,87</sup> In the relatively few studies that have investigated sex in relation to experimental TBI, sex-specific hormones have mainly been the focus. Progesterone has been most commonly associated with beneficial effects, but translation to human studies has not been shown beneficial in any clinical trial.<sup>85</sup> With the National Institutes of Health (NIH) acknowledging and mandating research address sex as a biological variable, hopefully, the discovery of new mechanisms to target will be expedited.

Although post-concussion syndrome (PCS) can be assessed quite readily in the clinic, it is still very much unclear why some people recover rapidly, but others do not. One aspect that has been consistently shown to exacerbate outcome is repeated injury.<sup>19,88–90</sup> However, only 2 studies that have examined sex differences in experimental TBI employed more than a single impact.<sup>36,37</sup> Moreover, a recent systematic review of experimental RHI found all published studies exclusively used male animals.<sup>91</sup> Studies of human subjects show that PCS is more likely to persist after multiple concussions, and mounting evidence suggests RHI may contribute to long-term disease and dysfunction, even in the absence of recognized concussion.<sup>92–94</sup> In light of these clinical realities, there seems to be some degree of disconnect from current preclinical research approaches.<sup>3,92,95</sup> Uncovering mechanisms underlying RHI pathology and dysfunction is an imperative goal because of the tremendous “window of opportunity” that exists to treat the disease, or possibly prevent it altogether. Identifying risk factors on the population and molecular level should provide legitimate targets to intervene and steer at-risk individuals toward recovery from head trauma.



To progress toward these goals, we suggest the following:


- Inclusion of both sexes in all preclinical TBI research to determine sex dependence of injury and recovery.
- Increased use of newer and more translatable models for mild TBI that do not require craniotomy and direct brain impact.
- Increased focus on multiple impact models to reveal how behavioral and pathophysiological sequelae differ from isolated concussion.
- Increased use of advanced imaging techniques to facilitate reverse translation of the human condition into animal models for more robust and relevant findings.

### Author Contributions

TGR performed all searches and all research related to the manuscript, as well as wrote the main manuscript text and table. MLL reviewed and edited the manuscript.

### ORCID iDs

Todd G Rubin  <https://orcid.org/0000-0002-2821-5877>

Michael L Lipton  <https://orcid.org/0000-0002-4702-2114>

### REFERENCES

1. Ma VY, Chan L, Carruthers KJ. Incidence, prevalence, costs, and impact on disability of common conditions requiring rehabilitation in the United States: stroke, spinal cord injury, traumatic brain injury, multiple sclerosis, osteoarthritis, rheumatoid arthritis, limb loss, and back pain. *Arch Phys Med Rehabil*. 2014;95:986–995.e1.
2. Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil*. 2006;21:375–378.
3. Gardner RC, Yaffe K. Epidemiology of mild traumatic brain injury and neurodegenerative disease. *Mol Cell Neurosci*. 2015;66:75–80.
4. Taylor CA, Bell JM, Breiding MJ, Xu L. Traumatic brain injury-related emergency department visits, hospitalizations, and deaths—United States, 2007 and 2013. *MMWR Surveill Summ*. 2017;66:1–16.
5. Centers for Disease Control Prevention (CDC) National Center for Injury Prevention and Control. *Report to congress on mild traumatic brain injury in the United States: steps to prevent a serious public health problem*. Atlanta, GA: Centers for Disease Control and Prevention; 2003.
6. Gardner AJ, Zafonte R. Neuroepidemiology of traumatic brain injury. *Handb Clin Neurol*. 2016;138:207–223.
7. Kushner D. Mild traumatic brain injury: toward understanding manifestations and treatment. *Arch Intern Med*. 1998;158:1617–1624.
8. Halstead ME, Walter KD, Moffatt K; Council on Sports Medicine and Fitness. Clinical report—sport-related concussion in children and adolescents. *Pediatrics*. 2010;126:597–615.
9. Shenton ME, Hamoda HM, Schneiderman JS, et al. A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. *Brain Imaging Behav*. 2012;6:137–192.
10. Rosenbaum SB, Lipton ML. Embracing chaos: the scope and importance of clinical and pathological heterogeneity in mTBI. *Brain Imaging Behav*. 2012;6:255–282.
11. McCrea M, Guskiewicz KM, Marshall SW, et al. Acute effects and recovery time following concussion in collegiate football players: the NCAA concussion study. *JAMA*. 2003;290:2556–2563.
12. Karr JE, Areshenkoff CN, Garcia-Barrera MA. The neuropsychological outcomes of concussion: a systematic review of meta-analyses on the cognitive sequelae of mild traumatic brain injury. *Neuropsychology*. 2014;28:321–336.
13. Alexander MP. Mild traumatic brain injury: pathophysiology, natural history, and clinical management. *Neurology*. 1995;45:1253–1260.
14. Helmy A, De Simoni MG, Guilfoyle MR, Carpenter KL, Hutchinson PJ. Cytokines and innate inflammation in the pathogenesis of human traumatic brain injury. *Prog Neurobiol*. 2011;95:352–372.
15. Mouzon BC, Bachmeier C, Ferro A, et al. Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model. *Ann Neurol*. 2014;75:241–254.
16. Tsitsopoulos PP, Marklund N. Amyloid-beta peptides and tau protein as biomarkers in cerebrospinal and interstitial fluid following traumatic brain injury: a review of experimental and clinical studies. *Front Neurol*. 2013;4:79.
17. Thomas DG, Apps JN, Hoffmann RG, McCrea M, Hammeke T. Benefits of strict rest after acute concussion: a randomized controlled trial. *Pediatrics*. 2015;135:213–223.
18. McCrory P, Meeuwisse W, Dvorak J, et al. Consensus statement on concussion in sport—the 5(th) international conference on concussion in sport held in Berlin, October 2016. *Br J Sports Med*. 2017;51:838–847.
19. Asken BM, Hack DC, McCrea MA. The modern landscape of sport-related concussion research: key achievements and future directions. *Handb Clin Neurol*. 2018;158:269–278.
20. Preiss-Farzanegan SJ, Chapman B, Wong TM, Wu J, Bazarian JJ. The relationship between gender and postconcussion symptoms after sport-related mild traumatic brain injury. *PM R*. 2009;1:245–253.
21. Broshek DK, Kaushik T, Freeman JR, Erlanger D, Webbe F, Barth JT. Sex differences in outcome following sports-related concussion. *J Neurosurg*. 2005;102:856–863.
22. Bazarian JJ, Blyth B, Mookerjee S, He H, McDermott MP. Sex differences in outcome after mild traumatic brain injury. *J Neurotrauma*. 2010;27:527–539.
23. Berz K, Divine J, Foss KB, Heyl R, Ford KR, Myer GD. Sex-specific differences in the severity of symptoms and recovery rate following sports-related concussion in young athletes. *Phys Sportsmed*. 2013;41:58–63.
24. Carroll LJ, Cassidy JD, Peloso PM, et al. Prognosis for mild traumatic brain injury: results of the WHO collaborating centre task force on mild traumatic brain injury. *J Rehabil Med*. 2004;36:84–105.
25. Cassidy JD, Carroll LJ, Peloso PM, et al. Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO collaborating centre task force on mild traumatic brain injury. *J Rehabil Med*. 2004;36:28–60.
26. Laker SR. Epidemiology of concussion and mild traumatic brain injury. *PM R*. 2011;3:S354–S358.
27. Caccese JB, Buckley TA, Tierney RT, et al. Head and neck size and neck strength predict linear and rotational acceleration during purposeful soccer heading. *Sports Biomech*. 2017;17:1–15.
28. Catenaccio E, Mu W, Kaplan A, et al. Characterization of neck strength in healthy young adults. *PM R*. 2017;9:884–891.
29. O'Connor CA, Cernak I, Vink R. Interaction between anesthesia, gender, and functional outcome task following diffuse traumatic brain injury in rats. *J Neurotrauma*. 2003;20:533–541.
30. Geddes RI, Peterson BL, Stein DG, Sayeed I. Progesterone treatment shows benefit in female rats in a pediatric model of controlled cortical impact injury. *PLoS ONE*. 2016;11:e0146419.
31. Free KE, Greene AM, Bondi CO, Lajud N, de la Tremblaye PB, Kline AE. Comparable impediment of cognitive function in female and male rats subsequent to daily administration of haloperidol after traumatic brain injury. *Exp Neurol*. 2017;296:62–68.
32. Xiong Y, Mahmood A, Lu D, et al. Role of gender in outcome after traumatic brain injury and therapeutic effect of erythropoietin in mice. *Brain Res*. 2007;1185:301–312.
33. Wagner AK, Kline AE, Ren D, et al. Gender associations with chronic methylphenidate treatment and behavioral performance following experimental traumatic brain injury. *Behav Brain Res*. 2007;181:200–209.
34. Russell KL, Kutcho KM, Fowler SC, Berman NE, Levant B. Sensorimotor behavioral tests for use in a juvenile rat model of traumatic brain injury: assessment of sex differences. *J Neurosci Methods*. 2011;199:214–222.
35. Mychasiuk R, Hehar H, Farran A, Esser MJ. Mean girls: sex differences in the effects of mild traumatic brain injury on the social dynamics of juvenile rat play behaviour. *Behav Brain Res*. 2014;259:284–291.
36. Wright DK, O'Brien TJ, Mychasiuk R, Shultz SR. Telomere length and advanced diffusion MRI as biomarkers for repetitive mild traumatic brain injury in adolescent rats. *Neuroimage Clin*. 2018;18:315–324.
37. Wright DK, O'Brien TJ, Shultz SR, Mychasiuk R. Sex matters: repetitive mild traumatic brain injury in adolescent rats. *Ann Clin Transl Neurol*. 2017;4:640–654.
38. Mannix R, Berglass J, Berkner J, et al. Sex differences in the effect of progesterone after controlled cortical impact in adolescent mice: a preliminary study. *J Neurosurg*. 2014;121:1337–1341.
39. O'Connor CA, Cernak I, Johnson F, Vink R. Effects of progesterone on neurologic and morphologic outcome following diffuse traumatic brain injury in rats. *Exp Neurol*. 2007;205:145–153.
40. Wagner AK, Kline AE, Sokoloski J, Zafonte RD, Capulong E, Dixon CE. Intervention with environmental enrichment after experimental brain trauma enhances cognitive recovery in male but not female rats. *Neurosci Lett*. 2002;334:165–168.
41. Wirth P, Yu W, Kimball AL, Liao J, Berkner P, Glenn MJ. New method to induce mild traumatic brain injury in rodents produces differential outcomes in female and male Sprague Dawley rats. *J Neurosci Methods*. 2017;290:133–144.
42. Mychasiuk R, Hehar H, Candy S, Ma I, Esser MJ. The direction of the acceleration and rotational forces associated with mild traumatic brain injury in rodents

- effect behavioural and molecular outcomes. *J Neurosci Methods*. 2016;257:168–178.
43. Xu X, Cao S, Chao H, Liu Y, Ji J. Sex-related differences in striatal dopaminergic system after traumatic brain injury. *Brain Res Bull*. 2016;124:214–221.
  44. Jullienne A, Salehi A, Affeldt B, et al. Male and female mice exhibit divergent responses of the cortical vasculature to traumatic brain injury. *J Neurotrauma*. 2018;35:1646–1658.
  45. Mychasiuk R, Hehar H, Esser MJ. A mild traumatic brain injury (mTBI) induces secondary attention-deficit hyperactivity disorder-like symptomology in young rats. *Behav Brain Res*. 2015;286:285–292.
  46. Igarashi T, Huang TT, Noble LJ. Regional vulnerability after traumatic brain injury: gender differences in mice that overexpress human copper, zinc superoxide dismutase. *Exp Neurol*. 2001;172:332–341.
  47. Suzuki T, Bramlett HM, Dietrich WD. The importance of gender on the beneficial effects of posttraumatic hypothermia. *Exp Neurol*. 2003;184:1017–1026.
  48. Jones NC, Constantine D, Prior MJ, Morris PG, Marsden CA, Murphy S. The neuroprotective effect of progesterone after traumatic brain injury in male mice is independent of both the inflammatory response and growth factor expression. *Eur J Neurosci*. 2005;21:1547–1554.
  49. Qu C, Lu D, Goussev A, Schallert T, Mahmood A, Chopp M. Effect of atorvastatin on spatial memory, neuronal survival, and vascular density in female rats after traumatic brain injury. *J Neurosurg*. 2005;103:695–701.
  50. Missios S, Harris BT, Dodge CP, et al. Scaled cortical impact in immature swine: effect of age and gender on lesion volume. *J Neurotrauma*. 2009;26:1943–1951.
  51. Robertson CL, Saraswati M. Progesterone protects mitochondrial function in a rat model of pediatric traumatic brain injury. *J Bioenerg Biomembr*. 2015;47:43–51.
  52. Villapol S, Loane DJ, Burns MP. Sexual dimorphism in the inflammatory response to traumatic brain injury. *Glia*. 2017;65:1423–1438.
  53. Roof RL, Hall ED. Estrogen-related gender difference in survival rate and cortical blood flow after impact-acceleration head injury in rats. *J Neurotrauma*. 2000;17:1155–1169.
  54. Kupina NC, Detloff MR, Bobrowski WF, Snyder BJ, Hall ED. Cytoskeletal protein degradation and neurodegeneration evolves differently in males and females following experimental head injury. *Exp Neurol*. 2003;180:55–73.
  55. Emerson CS, Headrick JP, Vink R. Estrogen improves biochemical and neurologic outcome following traumatic brain injury in male rats, but not in females. *Brain Research*. 1993;608:95–100.
  56. Hall ED, Gibson TR, Pavel KM. Lack of a gender difference in post-traumatic neurodegeneration in the mouse controlled cortical impact injury model. *J Neurotrauma*. 2005;22:669–679.
  57. Gunther M, Plantman S, Davidsson J, Angeria M, Mathiesen T, Risling M. COX-2 regulation and TUNEL-positive cell death differ between genders in the secondary inflammatory response following experimental penetrating focal brain injury in rats. *Acta Neurochir (Wien)*. 2015;157:649–659.
  58. Bramlett HM, Furones-Alonso O, Lotocki G, Rodriguez-Paez A, Sanchez-Molano J, Keane RW. Sex differences in XIAP cleavage after traumatic brain injury in the rat. *Neurosci Lett*. 2009;461:49–53.
  59. Roof RL, Duvdevani R, Stein DG. Gender influences outcome of brain injury: progesterone plays a protective role. *Brain Res*. 1993;607:333–336.
  60. Roof RL, Duvdevani R, Heyburn JW, Stein DG. Progesterone rapidly decreases brain edema: treatment delayed up to 24 hours is still effective. *Exp Neurol*. 1996;138:246–251.
  61. O'Connor CA, Cernak I, Vink R. The temporal profile of edema formation differs between male and female rats following diffuse traumatic brain injury. *Acta Neurochir Suppl*. 2006;96:121–124.
  62. O'Connor CA, Cernak I, Vink R. Both estrogen and progesterone attenuate edema formation following diffuse traumatic brain injury in rats. *Brain Res*. 2005;1062:171–174.
  63. Taylor AN, Tio DL, Paydar A, Sutton RL. Sex differences in thermal, stress, and inflammatory responses to minocycline administration in rats with traumatic brain injury. *J Neurotrauma*. 2018;35:630–638.
  64. Feickert HJ, Drommer S, Heyer R. Severe head injury in children: impact of risk factors on outcome. *J Trauma*. 1999;47:33–38.
  65. Marmarou A. Traumatic brain edema: an overview. *Acta Neurochir Suppl (Wien)*. 1994;60:421–424.
  66. Coates BM, Vavilala MS, Mack CD, et al. Influence of definition and location of hypotension on outcome following severe pediatric traumatic brain injury. *Crit Care Med*. 2005;33:2645–2650.
  67. Armstead WM, Vavilala MS. Adrenomedullin reduces gender-dependent loss of hypotensive cerebrovasodilation after newborn brain injury through activation of ATP-dependent K channels. *J Cereb Blood Flow Metab*. 2007;27:1702–1709.
  68. Armstead WM, Kiessling JW, Bdeir K, Kofke WA, Vavilala MS. Adrenomedullin prevents sex-dependent impairment of autoregulation during hypotension after piglet brain injury through inhibition of ERK MAPK upregulation. *J Neurotrauma*. 2010;27:391–402.
  69. Armstead WM, Kiessling JW, Kofke WA, Vavilala MS. SNP improves cerebral hemodynamics during normotension but fails to prevent sex dependent impaired cerebral autoregulation during hypotension after brain injury. *Brain Res*. 2010;1330:142–150.
  70. Armstead WM, Kiessling JW, Riley J, Kofke WA, Vavilala MS. Phenylephrine infusion prevents impairment of ATP- and calcium-sensitive potassium channel-mediated cerebrovasodilation after brain injury in female, but aggravates impairment in male, piglets through modulation of ERK MAPK upregulation. *J Neurotrauma*. 2011;28:105–111.
  71. Armstead WM, Riley J, Vavilala MS. TBI sex dependently upregulates ET-1 to impair autoregulation, which is aggravated by phenylephrine in males but is abrogated in females. *J Neurotrauma*. 2012;29:1483–1490.
  72. Curvello V, Hekierski H, Riley J, Vavilala M, Armstead WM. Sex and age differences in phenylephrine mechanisms and outcomes after piglet brain injury. *Pediatr Res*. 2017;82:108–113.
  73. Armstead WM, Kiessling JW, Kofke WA, Vavilala MS. Impaired cerebral blood flow autoregulation during posttraumatic arterial hypotension after fluid percussion brain injury is prevented by phenylephrine in female but exacerbated in male piglets by extracellular signal-related kinase mitogen-activated protein kinase upregulation. *Crit Care Med*. 2010;38:1868–1874.
  74. Armstead WM, Riley J, Vavilala MS. Dopamine prevents impairment of autoregulation after traumatic brain injury in the newborn pig through inhibition of up-regulation of endothelin-1 and extracellular signal-regulated kinase mitogen-activated protein kinase. *Pediatr Crit Care Med*. 2013;14:11.
  75. Armstead WM, Riley J, Vavilala MS. Preferential protection of cerebral autoregulation and reduction of hippocampal necrosis with norepinephrine after traumatic brain injury in female piglets. *Pediatr Crit Care Med*. 2016;17:e130–e137.
  76. Armstead WM, Riley J, Vavilala MS. Sex and age differences in epinephrine mechanisms and outcomes after brain injury. *J Neurotrauma*. 2017;34:1666–1675.
  77. Kumar A, Loane DJ. Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. *Brain Behav Immun*. 2012;26:1191–1201.
  78. Bruce-Keller AJ, Dimayuga FO, Reed JL, et al. Gender and estrogen manipulation do not affect traumatic brain injury in mice. *J Neurotrauma*. 2007;24:203–215.
  79. Lazarus RC, Buonora JE, Jacobowitz DM, Mueller GP. Protein carbonylation after traumatic brain injury: cell specificity, regional susceptibility, and gender differences. *Free Radic Biol Med*. 2015;78:89–100.
  80. Gray JD, Milner TA, McEwen BS. Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors. *Neuroscience*. 2013;239:214–227.
  81. Chen X, Li Y, Kline AE, Dixon CE, Zafonte RD, Wagner AK. Gender and environmental effects on regional brain-derived neurotrophic factor expression after experimental traumatic brain injury. *Neuroscience*. 2005;135:11–17.
  82. Wagner AK, Chen X, Kline AE, Li Y, Zafonte RD, Dixon CE. Gender and environmental enrichment impact dopamine transporter expression after experimental traumatic brain injury. *Exp Neurol*. 2005;195:475–483.
  83. McEwen BS, Milner TA. Understanding the broad influence of sex hormones and sex differences in the brain. *J Neurosci Res*. 2017;95:24–39.
  84. Meffre D, Pianos A, Liere P, et al. Steroid profiling in brain and plasma of male and pseudopregnant female rats after traumatic brain injury: analysis by gas chromatography/mass spectrometry. *Endocrinology*. 2007;148:2505–2517.
  85. Stein DG, Geddes RI, Sribnick EA. Recent developments in clinical trials for the treatment of traumatic brain injury. *Handb Clin Neurol*. 2015;127:433–451.
  86. Babikian T, Prins ML, Cai Y, et al. Molecular and physiological responses to juvenile traumatic brain injury: focus on growth and metabolism. *Dev Neurosci*. 2010;32:431–441.
  87. Prins ML, Hales A, Reger M, Giza CC, Hovda DA. Repeat traumatic brain injury in the juvenile rat is associated with increased axonal injury and cognitive impairments. *Dev Neurosci*. 2010;32:510–518.
  88. Broglio SP, McCreary M, McAllister T, et al. A national study on the effects of concussion in collegiate athletes and US military service academy members: the NCAA-DoD concussion assessment, research and education (CARE) consortium structure and methods. *Sports Med*. 2017;47:1437–1451.
  89. Guskiewicz KM, McCreary M, Marshall SW, et al. Cumulative effects associated with recurrent concussion in collegiate football players: the NCAA Concussion Study. *JAMA*. 2003;290:2549–2555.
  90. Guskiewicz KM, Marshall SW, Bailes J, et al. Recurrent concussion and risk of depression in retired professional football players. *Med Sci Sports Exerc*. 2007;39:903–909.
  91. Hoogenboom WS, Branch CA, Lipton ML. Animal models of closed-skull, repetitive mild traumatic brain injury [published online ahead of print February 26, 2019]. *Pharmacol Ther*. doi:10.1016/j.pharmthera.2019.02.016.
  92. Gavett BE, Stern RA, McKee AC. Chronic traumatic encephalopathy: a potential late effect of sport-related concussive and subconcussive head trauma. *Clin Sports Med*. 2011;30:179.xi.
  93. Huber BR, Alosco ML, Stein TD, McKee AC. Potential long-term consequences of concussive and subconcussive injury. *Phys Med Rehabil Clin N Am*. 2016;27:503–511.
  94. Bahrami N, Sharma D, Rosenthal S, et al. Subconcussive head impact exposure and white matter tract changes over a single season of youth football. *Radiology*. 2016;919–926.
  95. Bailes JE, Petraglia AL, Omalu BI, Nauman E, Talavage T. Role of subconcussion in repetitive mild traumatic brain injury. *J Neurosurg*. 2013;119:1235–1245.