TRAUMATIC STRESS RESPONSES IN RATS REVEAL FUNDAMENTAL SEX DIFFERENCES THAT MIRROR PTSD IN MEN AND WOMEN

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ABSTRACT

TRAUMATIC STRESS RESPONSES IN RATS REVEAL FUNDAMENTAL SEX DIFFERENCES THAT MIRROR PTSD IN MEN AND WOMEN

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Post-traumatic stress disorder (PTSD) develops after exposure to trauma and is associated with dysfunction in the normal stress response. Women are twice as likely as men to develop PTSD and tend to experience different symptoms and comorbidities than men, but the neurobiological basis for these pervasive sex differences is poorly understood due to the overwhelming male bias in the preclinical research. My dissertation work tested the novel hypothesis that the neurobiological mechanisms underlying the traumatic stress response in male and female rats are fundamentally different and may be related to normal sex differences in circulating levels of adult gonadal hormones. These experiments are the first to compare adult male and female rats across two rodent models of PTSD, single prolonged stress and predator exposure. I report a highly sex-specific traumatic stress response that recapitulates fundamental differences of PTSD in men and women. Surprisingly, these sex differences were largely independent of adult circulating gonadal hormones, housing conditions, and types of stress. Two standard measures, the acoustic startle response and dexamethasone suppression test to measure the negative feedback control of the stress hormone response, suggest that female rats, unlike male rats, are resilient to the effects of traumatic stress. However, other measures like sucrose preference and social interaction make it clear that females are *not* resilient, but simply respond differently to trauma than males. Dramatic sex differences in how trauma affects cFos activation and glucocorticoid receptor expression in the brain lend further support to the idea that the trauma response of males and females is fundamentally different, and likely determined prior to adulthood. Factors that mediate differences in how individuals adjust after trauma are attractive targets for the prevention and treatment of PTSD, and identifying such factors of resilience depends on understanding the various ways the traumatic stress response manifests in different individuals. I propose that sex differences offer a promising inroad for addressing this issue.

For all those who survived the unthinkable, and especially for those who didn’t.

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KEY TO ABBREVIATIONS

ACC	anterior cingulate cortex
ACTH	adrenal corticotropic hormone
APA	American Psychiatric Association
AR	androgen receptor
ASR	acoustic startle response
BDNF	brain-derived neurotrophic factor
BLA	basolateral amygdala
CBG	corticosterone binding globulin
CORT	corticosterone
CRH	corticotropin-releasing hormone
DESNOS	disorders of extreme stress, not otherwise specified
DEX	dexamethasone
DSM-III/IV/5	Diagnostic and Statistical Manual of Mental Disorders-third, fourth, or fifth edition
DST	dexamethasone suppression test
fMRI	functional magnetic resonance imaging
GDX	gonadectomy/gonadectomized
GR	glucocorticoid receptor
HPA	hypothalamic-pituitary-adrenal
ICD-10	International Statistical Classification of Diseases and Related Health Problems-10th ed.
IHC	immunohistochemistry
IL	infralimbic region of the medial prefrontal cortex
mCPP	meta-Chlorophenylpiperazine
MDD	major depressive disorder
mPFC	medial prefrontal cortex
PET	positron emission tomography
PredX	predator exposure

PrL	prelimbic region of the medial prefrontal cortex
PTSD	post-traumatic stress disorder
PVN	paraventricular nucleus of the hypothalamus
SNS	sympathetic nervous system
SPS	single prolonged stress
T	testosterone

CHAPTER 1: INTRODUCTION

Post-traumatic stress disorder in humans

Post-traumatic stress disorder (PTSD) is a disorder that can develop after exposure to a traumatic experience and is associated with dysfunction in the normal stress response. As many as 10% of civilians and 30% of military veterans in the United States develop PTSD after trauma (1–4), and affected individuals can remain chronically symptomatic for decades with debilitating symptoms that interfere with daily functioning (5). Clinical manifestations of PTSD include a wide range of symptoms that often overlap or are co-morbid with other psychiatric conditions. At least one other co-morbid psychiatric condition occurs in 88% of people with PTSD, with major depressive disorder (MDD) diagnosed in half of all PTSD patients (1), indicating significant heterogeneity in PTSD. A history of experiencing trauma is not sufficient alone to diagnose PTSD, as over 75% of people in the United States experience a traumatic event in their lifetime and do *not* develop PTSD (6), begging the question *why do some people who experience trauma develop PTSD while others do not?* To begin to answer this question, we must first understand what makes an experience traumatic.

Traumatic stress is a unique form of stress. In 1936, Hans Selye introduced the first report of a biological stress syndrome, in which he described an “alarm reaction” and suggested that the symptoms are “independent of the nature of the damaging agent” (7). While several early hypotheses regarded stress as a non-specific response that was essentially the same for all stressors, much evidence exists today to indicate the contrary—various types of stressors impart distinct “neurochemical signatures” and activational patterns in the nervous system (8, 9). General classifications of stressor type are based on four broad categories that include physical stressors, psychological stressors, social stressors, and cardiovascular/metabolic stressors in acute or chronic duration (8). But traumatic stressors can fall into any of these categories, and a stressor that is traumatic in nature has its own unique signature, so a more clinically relevant classification scheme may be one that first identifies a stressor as traumatic or not.

The key to a stressor qualifying as traumatic is that it poses a real or perceived threat to life, physical integrity, or social affiliation (of oneself or one’s kin) and is uncontrollable in some aspect (10).

Uncontrollable stressors are those to which an individual is unable to act upon or to which the available stress responses (e.g. sympathetic fight-or-flight, neuroendocrine responses, etc.) are not applicable. Controllability is formally defined as “the probability that a given response will prevent or terminate” the stressor (11). In response to *controllable* threats, most animals exhibit physiological and behavioral changes that function as adaptive, life-preserving responses (12–14). Exposure to mild, controllable stressors early in life can be beneficial to the developing nervous system to (e.g. the “stress-inoculation theory”) (15). All animals experience mild, predictable stressors from birth and, in healthy environments, learn repeatedly which behaviors will terminate or aid in adaptation to cold, hunger, loneliness, fatigue, and discomfort (16). These associative learning experiences can impart healthy and successful coping with other stressors throughout life.

On the other hand, little can be learned from an uncontrollable stressor, and maladaptive behaviors may result from the activation of sympathetic and neuroendocrine stress response systems when the individual is unable or unequipped to act, or when the responses elicited do not result in consistent outcomes. Stressors that are more recently-established on the evolutionary time scale may be more likely to elicit PTSD (e.g. a “neuroevolutionary time-depth principle”) (17), evidenced by the fact that adversities such as fires are less likely to result in PTSD than adversities for which the human genome has had much less time to evolve such as motor vehicle accidents (1) or modern surgical procedures and treatments, after which PTSD rates range from 14-59% (18). After a train accident, for example, where a stress response was elicited but there was no opportunity to make any behavioral action, the brain subsequently searches for ways in which the individual was responsible for their own survival (or for their own harm), and maladaptive thought and behavior patterns can develop. A person might decide that they survived the train crash because they were sitting at the back of the train, so in the future they only sit at the backs of trains. Or they might decide that the reason they got in the accident in the first place was because they took an earlier train than they normally take, so in the future they will never ride trains in the morning. These types of avoidant behaviors are a key diagnostic criterion for PTSD and primarily occur after experiencing an uncontrollable stressor. Experimental studies indicate that uncontrollable stress is necessary for the onset of PTSD-like symptoms and leads to physiological and behavioral consequences

different from those associated with controllable stress (11, 16, 19, 20). Those consequences further diverge if the stress includes a psychosocial aspect.

While physical trauma can have psychological manifestations and psychological trauma can have physical manifestations (21), physical trauma nonetheless differs from psychosocial trauma in that physical trauma is caused by objective "changes of the world outside the brain" rather than being dependent on subjective perception, beliefs, assumptions, expectations, and previous experience within the brain, as happens in psychosocial conflict, which is the predominant form of stress in humans and many other social mammals (16). While the ramifications of a gunshot wound, for example, may be objectively similar at the bullet entry point between two victims, their perceptions of the psychosocial aspect of the shooting may be completely different—informing unique neurocircuitry effects—depending on their individual worldviews and experiences. This makes the traumatic stress response, especially when any psychosocial aspect is involved, extremely heterogenous (22). Considering the various ways the traumatic stress response has manifested in different individuals throughout history—and not limited to the ever-changing diagnostic definitions created by professional and political organizations—is a crucial step to fully understand how to prevent, diagnose, and treat PTSD.

The effects of traumatic stress have been observed throughout recorded history. From depictions in the *Epic of Gilgamesh* (300 B.C.) to Homer's *Iliad* (800 B.C.) to much of Shakespeare (23, 24), historical descriptions of the effects of war, sexual assault, physical and emotional abuse, natural disasters, and accidents indicate that PTSD as we know it is not a modern phenomenon, as some have suggested (25). That life-threatening events can leave a lasting impression upon the body and psyche has been unwavering in these remarkably parallel historical descriptions of trauma, but the field of psychiatry has not been so consistent in acknowledging that external factors like trauma can impact an individual so profoundly and has been peppered with assertions that PTSD merely represents a vulnerability of the individual that was present with or without trauma (26, 27).

The earliest clinical study on the effects of trauma was published in 1866 when sleep disturbances, nightmares, chronic pain, depression, memory loss, and avoidance of railway travel manifested in railway employees and passengers who had experienced train crashes and whiplash from

the violent rocking of the train cars (28). Termed *railway spine*, these symptoms closely mirror current diagnostic criteria for PTSD (22), and when Dr. Erichsen first studied railway spine, he wrote, “Do not for a moment suppose that these injuries are peculiar to and are solely occasioned by accidents that may occur on railways” (28). However, while Erichsen thought the psychological effects of trauma must be a result of physical injury to the spine, surgeon Herbert Page claimed that both physical and psychological manifestations of trauma could arise without any detectable lesions to the central nervous system. Page compared railway spine to hysteria—characterized by almost identical symptoms—suggesting that both conditions arise from the same psychological processes and that “the change [after trauma] may be a chemical one” (29). Significant hypotheses regarding the effects of psychological trauma stemmed from studies of hysteria in the nineteenth century, in which it was suggested that childhood sexual abuse could cause symptoms of hysteria later in life in both men and women (30), a supposition we know to be true today. Other responses to trauma were associated with war, including American Civil War and World War I soldiers who had what was then described as “soldiers’ heart” and “shell shock,” respectively—the symptoms of which mirror those of currently defined PTSD (31, 32). One landmark study found similar symptoms in World War II concentration camp survivors (“Concentration Camp Syndrome”) that persisted 15 years after the war had ended (33). The term “rape trauma syndrome” was coined in 1974 to describe similar effects in some rape survivors (34). After Vietnam, nearly 25% of veterans returned from combat with psychological problems (“Post-Vietnam Syndrome”) that resembled those of soldiers in previous wars, leading to the conceptualization of PTSD as a combat disorder (22). The first official recognition of PTSD, which was issued by the American Psychiatric Association (APA) in the 1980 edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-III*), was fueled by advocates calling specifically for the treatment of the psychological problems in returning Vietnam veterans. Nonetheless, in addition to military combat, the *DSM-III* explicitly included rape, physical assault, and motor vehicle accidents as stressors that could lead to PTSD. This recognition was monumental in advancing research on the effects of different types of traumatic stress in various populations, and it is now widely accepted that both civilians and veterans who experience any type of trauma are at risk for PTSD. The other major medical manual, the World Health Organization’s *International Statistical Classification of Diseases and Related Health Problems* (*ICD*) first included PTSD in the 1992 *ICD-10*, which is still in its current edition.

When the current edition of the *DSM* (*DSM-5*) was issued in 2013, PTSD was no longer listed as an anxiety disorder but instead considered part of the newly recognized “trauma and stressor-related disorders,” reflecting the unique nature of disorders caused by trauma/stress that is not captured in a simple anxiety phenotype (also included were reactive attachment disorder, acute stress disorder, adjustment disorders, and disinhibited social engagement disorder). The *DSM-5* diagnostic criteria for PTSD require exposure to “actual or threatened death, serious injury, or sexual violation.” The exposure must result from one or more of the following scenarios, in which the individual: (1) directly experiences the traumatic event, (2) witnesses the traumatic event in person, (3) learns that the traumatic event occurred to a close family member or friend, and (4) experiences first-hand repeated or extreme exposure to aversive details of the traumatic event (e.g. emergency responders, police officers, corrections officers, etc.). This *DSM* revision aligns closely with the *ICD-10* classification of PTSD. Notably, the requisite diagnostic criterion that an individual had responded to the trauma with “fear, helplessness, or horror” was removed in the *DSM-5*, citing that it had not been proven that those subjective responses could predict the onset of PTSD (35). This position was coupled with an impetus to focus research and treatment on the measurable objective effects of trauma, as the subjective psychosocial factors proved highly variable and difficult to control or study, as learned from early studies of PTSD.

The initial understanding of PTSD was limited almost exclusively to psychological symptoms based on self-report, which propagated skepticism about the validity of PTSD as a disorder. But as progress in biological research confirmed that the disorder was caused by an external agent (i.e. a traumatic event), and biological readouts of the effects of trauma were identified (36), the medical community and public began to accept PTSD as a valid disorder worth studying and treating. It is now widely accepted that a single traumatic event is *necessary* to induce the onset of PTSD. Nonetheless, the traumatic event alone is not sufficient to lead to the development of PTSD, as the same traumatic event triggers PTSD in some individuals but not in others (37). While the characteristics of the traumatic event itself are invariably important in the subsequent development of PTSD, the subjective perception of trauma and individual susceptibilities still cannot be ruled out as contributing risk factors for the development of PTSD.

Risk factors for PTSD. Epidemiological studies indicate that 1-in-14 civilians and 1-in-3 veterans develop PTSD, but some people are more susceptible than others (1). Women are twice as likely as men to develop PTSD, with 10% of women and 5% of men meeting the diagnostic criteria for PTSD (see section: *Sex differences in PTSD*) and a history of multiple traumatic experiences increases the risk for PTSD (38). Additionally, some types of stressors are more likely to lead to PTSD than others, and the resources available to an individual in the aftermath of trauma have a large impact on whether PTSD develops (39).

Some hypothesize that "stress exposure only affects individuals with a susceptible genetic background" (40), but even the most liberal estimates find that only about 30% of the variance in responses to trauma can be attributed to heredity (41, 42). Genetic backgrounds with certain polymorphisms in genes encoding the serotonin transporter (43, 44), brain-derived neurotrophic factor (BDNF) (45, 46), the pituitary adenylate cyclase-activating polypeptide receptor in females (47, 48), 5α-reductase in males (49), and others (50, 51), confer greater risk for developing PTSD after trauma, but the effects of genetics alone on PTSD is limited and likely strongly dependent on gene by environment interactions (52) including epigenetic modifications.

Experience-dependent epigenetic changes, such as changes in gene methylation that correspond to gene expression (53), occur with PTSD and increase with each traumatic event experienced (54). Specific types of genes particularly vulnerable to trauma-induced epigenetic changes include the glucocorticoid receptor (GR) gene, various immune function genes, and sensory perception genes (54–56). Differential epigenetic states in the genes that regulate the epigenetic process itself may represent pre-existing risk factors for how an individual responds to trauma (57). Some epigenetic changes remain even after PTSD symptoms have remitted (58). Increased PTSD risks exist for people whose mother had PTSD, which are unique from the risks for people whose father had PTSD (59, 60). While trauma-related epigenetic effects influence parenting behavior (61), these epigenetic imprints themselves are also highly heritable and passed down through generations (62, 63).

Other physiological risk factors for PTSD include low cortisol level at the time of trauma (64, 65) and small hippocampal volume (66). While evidence indicates these are pre-trauma risk factors for PTSD, traumatic stress can also impact cortisol levels and hippocampal volume via changes to hypothalamic-

pituitary-adrenal (HPA) axis negative feedback of cortisol and inflammatory responses that decrease neurogenesis in the hippocampus (67). It is likely that individuals with cortisol and hippocampi on the low end of the average are simply more affected by the trauma-induced changes to those systems, representing both a risk factor and consequence of trauma (68).

Nonetheless, to say that *only* those with a certain genetic background or physiological predisposition are susceptible to the negative effects of stress likely underestimates the damage that some severe or repeated stressors can cause. Trauma repeated in occurrence or high in severity may simply overwhelm even the least vulnerable nervous system. PTSD becomes more likely as individuals experience more cumulative traumatic events, and experiencing four or more traumatic events is associated with significantly greater impairment of functioning than experiencing any fewer number of traumatic events (69, 70). This dose-response of trauma may impart damage by increasing allostatic load and overwhelming the systems that adapt to environmental challenge and maintain homeostasis (71). Additionally, some types of traumatic events may constitute a larger "dose" of trauma and are themselves associated with increased risk for PTSD.

Interpersonal trauma (e.g. physical and sexual assault, emotional abuse, domestic violence) is unique from other trauma and is the class of events most likely to result in PTSD (72–74). In contrast, non-interpersonal trauma like natural disasters, accidents, and witnessing trauma all have low conditional risks for PTSD (4-9%) (1). Sexual trauma is the most common cause of PTSD in both men and women and accounts for the largest proportion of people with PTSD (1, 70). Moreover, sexual trauma perpetrated by someone known to the victim is more likely to result in PTSD than sexual trauma perpetrated by a stranger (75), a phenomenon described by *betrayal trauma theory*.

Trauma involving social betrayal "violates a fundamental ethic of human relationships" (76) and presents an especially high risk for the dissociative subtype of PTSD (see section: *Dissociative subtype of PTSD*), shame, and more pervasive mental and physical health difficulties than other forms of trauma (77). Because all interpersonal traumas have some degree of betrayal (i.e. betrayal by fellow humankind), betrayal trauma theory may explain why interpersonal traumas have such a high risk for PTSD. That trauma especially high in betrayal (e.g. perpetrated by a partner or family member) represents a risk even beyond general interpersonal trauma undoubtedly points to the involvement of an individual's perception

of the traumatic event in the trauma sequelae. Indeed, in addition to perceived betrayal, the traumatic stress response is influenced by perceived level of threat, perceived violations of assumptions, and perceived level of social support.

Individuals with a high perceived level of danger (those who thought their own lives or the lives of loved ones were in danger) exhibit a significantly increased risk for PTSD compared to those who did not perceive a high level of danger (70, 73, 78, 79). Additionally, there is an increased risk for PTSD when the trauma violates a strongly held worldview (e.g. “My hometown is a safe place.”), and a greater stress response is elicited when trauma is experienced in a context that previously signaled safety (11). Subjective perceptions of trauma and the subsequent psychological reactions to trauma are also heavily influenced by sociocultural norms and widespread ethnocultural variations of PTSD exist (80–82).

While PTSD disproportionally affects socioeconomically disadvantaged populations and people in conflict zones, (39, 83–86), high-income countries have a significantly greater prevalence of PTSD than low- or middle- income countries (69). The United States has the second highest worldwide prevalence of PTSD (behind Northern Ireland and before New Zealand), which may reflect that the PTSD diagnosis is a Western construct that doesn’t capture the trauma experience of other cultures (i.e. a “culture-bound disorder”) (81). Alternatively, these nations may have historical and cultural antecedents that predicate a high risk for PTSD. For example, societies of predominately Western-European heritage de-emphasize communal living, shared grieving, and other systems of social support in favor of individual responsibility, which may lead to social isolation and over-reliance on maladaptive coping mechanisms. That people in Western cultures exhibit a prodromal form of PTSD (see section: *Delayed-onset PTSD*) more than people from non-Western cultures might be a reflection of cultural differences in help-seeking behavior or post-trauma resources available (87). Part of the PTSD diagnosis involves respondents reporting distress and severe role impairment in the domain of work, and disruptions in this domain can be especially distressing for the working class in capitalist societies where productivity is considered a measure of individual value. Additionally, many of the nations with the highest PTSD prevalence are also driven by systems that oppress certain groups of people (i.e. “pathogenic societies”) who are consequently more likely to be exposed to trauma and to develop PTSD (81). Indeed, in primate social systems with established dominance hierarchies, individuals of lower social status are faced with persistent uncontrollable social

stress such that their adrenal gland weight and circulating glucocorticoid levels are significantly elevated above individuals of higher social status (88–90), and these biological consequences of social structures may influence subsequent responses to trauma. While research focuses primarily on those factors that moderate increased risks for the dysfunctional long-term effects of stress, some of these changes may actually be adaptive in coping with traumatic stress (91, 92). Whether biological mechanisms confer an adaptive advantage or an increased risk for psychiatric disorders may be largely dependent on context—what may be adaptive in one culture or life stage may lead to significant impairment in another (93). And indeed, life stage is an important mediator of the traumatic stress response (94).

The risk for developing PTSD after interpersonal trauma is even higher if the trauma was experienced in childhood (75). Early-life interpersonal trauma (age 14 or under) leads to greater emotional dysregulation and self-destructive behavior compared to early-life non-interpersonal trauma and any adult trauma (95). While exposure to mild, predictable stressors early in life can be beneficial (e.g. the “stress-inoculation theory”), chronic adversity or any type of trauma can impart difficulties later in life including mood and anxiety disorders, susceptibility to drugs of abuse, and learning and memory problems. (40). While some children fully recover after trauma (37), children exposed to trauma before age 16 have almost double the rate of any psychiatric disorder compared to those not exposed to trauma (38), and the effects of childhood abuse can last into adulthood presenting as problems with emotion regulation, interpersonal skills, sleep difficulties, anxiety, depression, and suicide (96–99). But even if a child recovers after early trauma, that experience could impart an increased risk for developing PTSD after subsequent trauma later in life. A history of childhood maltreatment is one of the most robust predictors of developing PTSD after experiencing trauma in adulthood (73, 100). Similar to the cumulative effect of trauma on increasing PTSD risk in adults, adverse childhood experiences show a dose-response in children for increasing risk of a variety of detrimental health outcomes, including PTSD (101, 102). Some evidence indicates that adult trauma only has a cumulative effect if childhood trauma is also present (103). As with adult trauma, the mechanisms by which early-life stress can have lasting effects involves genetic polymorphisms (44, 48, 50) and modification of the epigenome (55, 104, 105). Early-life stress may also alter the development of brain regions that normally go through postnatal development, such as the hippocampus and prefrontal cortex (106). While early-life trauma is often thought to act as a

kind of toxic agent that damages healthy brain development (e.g. the “glucocorticoid cascade hypothesis”) (107), these changes may instead promote an “alternative neurodevelopmental pathway” (e.g. an *organizing* effect of glucocorticoids) that is adaptive to surviving a high-stress environment (97). The idea of adaptive responses to trauma can be examined in people who do not develop PTSD after trauma.

After trauma, most people display symptoms common to PTSD patients, but these effects usually seem to resolve within a few weeks (108–110), suggesting that at least part of the susceptibility to the lasting effects of trauma may come not only from vulnerability to the initial impact of trauma but from factors during the aftermath of recovering from the trauma such as social support. Among 14 separate risk factors for PTSD, social support is the single greatest factor (100). More specifically, *lack* of social support in the form of negative responses from others after trauma significantly predicts PTSD, as opposed to positive social support, which doesn’t itself protect from PTSD *per se* but may serve more of a buffering function (111). Effective social support after trauma may be a mechanism by which an individual gains a sense of control over an uncontrollable stressor (11). Many individual coping mechanisms are maladaptive, including thought suppression, avoidance, distraction, and denial, all of which increase PTSD symptom severity and are often relied upon less in individuals with high levels of social support (112). Social support on a neighborhood or community-wide level is also important in the PTSD trajectory (39). While a variety of risk factors influence whether an individual will develop PTSD, even among those who develop PTSD, symptom severity and presentation can vary tremendously.

Symptoms and subtypes of PTSD*.* Defining features of PTSD in the *DSM-5* include 20 symptoms in 4 symptom clusters: (1) persistent re-experiencing of the traumatic event, (2) avoidance of stimuli associated with the trauma, (3) negative cognitions and mood, and, (4) increased physiological arousal (Table 1). Each diagnostic cluster consists of diverse symptoms that have unique presentations in each individual, and no individual will meet all 20 symptoms at any one time. Indeed, some of these diagnostic measures seem to oppose one another (e.g. recurrent intrusive memories of the trauma vs. amnesia of the trauma). There are 636,120 unique combinations of *DSM-5* symptoms that could lead to a PTSD diagnosis (113). To explain this symptom heterogeneity, subtypes of PTSD have been identified.

Dissociative subtype of PTSD. The dissociative subtype of PTSD includes the standard *DSM-5* criteria plus prominent depersonalization and derealization symptoms, which presents as disruptions in memory (including amnesia for all or part of the trauma), identity, body awareness, and self-perception. Depersonalization is an "out-of-body" experience during which individuals may describe observing their own body from above and is typically accompanied by a cognitive impression that "this is not happening to *me*." Derealization creates the perception that an experience itself is not real or may be a dream, and both derealization and depersonalization are associated with an attenuation of the intensity of emotional experience (84).

Over a hundred years ago, Pierre Janet coined the term dissociation as we know it and developed the original theories of two distinct types of responses to traumatic stress: hysteria, characterized by the dissociation of feelings or memories related to traumatic experiences, and psychasthenia, characterized by ruminations, phobias, and anxiety related to traumatic experiences (114). Janet hypothesized that some traumatized individuals cannot integrate the memories of painful events and the intense emotions associated with them into their narrative memory—so both the memory and the accompanying emotions remain dissociated from consciousness (115). Independently from Janet, Freud drew similar conclusions about trauma and memory when he described patients recalling memories of childhood abuse who "are recalling these infantile experiences to consciousness…suffer the most violent sensations of which they are ashamed and try to conceal, and even after having gone through them once more in such a convincing manner…they have no feeling of remembering the scenes…and assure so emphatically of their unbelief" (30).

As many as 30% of PTSD patients report with symptoms of depersonalization and derealization, and individuals with the dissociative subtype of PTSD are more likely to have experienced early-life trauma (prior to age 14), sexual trauma, and have comorbid MDD (116–120). Additionally, dissociative PTSD is accompanied by a distinct neurobiological, physiological, and stress-hormone profile (121–125). Because individuals with dissociative PTSD show blunted, rather than increased, heart rate in response to stress (126), the *ICD-10* classification of PTSD does not include a dissociative subtype but allows for the diagnosis of PTSD in the absence of hyperarousal symptoms *if* dissociation or amnesia is present.

Peritraumatic dissociation is one of the strongest predictors of subsequent development of PTSD (73, 79). Dissociation may be a survival mechanism by which consciousness is altered in the face of overwhelming experience when actual escape is not possible. This may reflect the strong link between child abuse and dissociation where children are most often abused by caregivers or other power figures, and because children rely on those adults for survival, traumatic amnesia may not simply reduce suffering but promote survival (76). Dissociative PTSD most often follows trauma high in social or institutional betrayal (e.g. abuse that occurs with complicity from a church or educational institution) as an adaptive response when an individual must "preserve a necessary relationship in the face of mistreatment" and this most often occurs when the betrayal trauma occurred in childhood (77, 127, 128).

Child subtypes of PTSD. The *DSM-IV* PTSD diagnostic criteria were developed from adult literature and did not identify the children most adversely affected by trauma or reflect how trauma manifests in children (38, 97). The same trauma that leads to hypervigilance in adults leads to dissociation in children (127), indicating fundamental differences in how trauma is processed across development. Indeed, people with PTSD who experienced childhood trauma have a completely different (98% non-overlapping) epigenetic profile than people with PTSD whose trauma occurred only in adulthood (129). A new diagnosis called Developmental Trauma Disorder was proposed for inclusion in the *DSM-5*, citing that children who experience trauma are diagnosed with 3-8 co-morbid disorders that can all be explained by one etiological factor: trauma (130, 131). Developmental Trauma Disorder was not accepted as a new diagnosis, but the *DSM-5* included a child subtype (or "preschool subtype") specification that simply requires fewer symptoms than necessary for an adult diagnosis, but does not take into account the evidence indicating that PTSD in children is not simply a "miniature" form of adult PTSD (132, 133). Interestingly, some children who experience trauma do not present with detectable symptoms until later in life, which may be related to delayed-onset PTSD.

Delayed-onset PTSD. While it isn't a subtype *per se*, the delayed-onset specification of PTSD has been included since the first release of the *DSM-IV* based on the indication that some individuals do not meet full PTSD criteria until more than six months (sometimes even years) after the traumatic event (35). About 25% of people who develop PTSD are considered to have delayed PTSD (87). Individuals with delayed-onset PTSD usually still experience several PTSD symptoms immediately after the trauma (134,

135), but at a clinical subthreshold level that does not progress to full PTSD until some time later, often after exposure to new traumatic events or reminder triggers (136). The *ICD-10* has a separate diagnosis for "the late chronic sequelae of devastating stress, i.e. those manifest decades after the stressful experience," which is classified under "enduring personality changes, not attributable to brain damage and disease." Roger Pitman, building off of Eysenck and Kelly's 1987 hypotheses on the "incubation of neurotic disorders," proposed a model for delayed onset of PTSD as a stress-hormone facilitated "positive feedback loop in which subclinical PTSD may escalate into clinical PTSD" (36). Another possibility is that what was speculated to be "subclinical" PTSD was really an unrecognized phenotype of PTSD, such as the internalizing subtype of PTSD.

Internalizing and externalizing subtypes of PTSD. While not yet a formal diagnostic distinction, subgroups of individuals with either dominant internalizing symptoms or dominant externalizing symptoms have been revealed as distinct from "simple PTSD" by their presentation of prominent personality alterations (117, 137). People with simple PTSD have low comorbidity with other disorders and normal personality functioning (138). The internalizing phenotype is characterized by low-positive emotion, avoidant personality, and high comorbid depression and anxiety, and the externalizing phenotype is characterized by low levels of constraint, aggression, antisocial or narcissistic personality, and comorbid substance abuse (139, 140). The idea that trauma can lead to pronounced personality alterations in some individuals was addressed in a previously proposed phenotype: *complex PTSD*. Internalizing and externalizing phenotypes of PTSD have been found to be an analogous construct to complex PTSD and may represent subtypes of complex PTSD (138).

Complex PTSD. The complex PTSD construct was proposed by Judith Herman in 1992 for inclusion in the *DSM-IV* under "disorders of extreme stress, not otherwise specified (DESNOS)". Herman argued that the *DSM-III* PTSD criteria did not capture the effects of prolonged, repeated trauma such as those observed in survivors of captivity (prisons, concentration camps, sex trafficking) and domestic abuse (intimate partner violence, child abuse) who exhibit pronounced changes in personality and identity and diffuse symptoms in multiple domains (somatic, cognitive, affective, behavioral, relational) (141). DESNOS was ultimately not included in the manual because most people who met the criteria for DESNOS also met the criteria for PTSD, so it was thought of as just a more severe form of PTSD (142).

Calls for a complex PTSD diagnosis continued during the preparation of *DSM-5*, as some thought the practice of diagnosing PTSD along with several comorbidities was less clinically useful than a single complex PTSD diagnosis (103, 143), and the complex PTSD symptoms of dissociation, affect dysregulation, and somatization were not captured with the standard PTSD diagnosis. Nonetheless, complex PTSD was again not included in the *DSM-5*, but the new PTSD criteria moved closer toward accounting for complex PTSD symptoms (e.g. the addition of the "negative alterations in cognitions and mood" symptom category and the dissociative subtype) (35). What still remains to be recognized by any official diagnosis is the well-documented somatic symptomatology related to trauma: headache, gastrointestinal disturbance, autoimmune disorder, non-epileptic seizure, and chronic pain (141). The link between trauma and the various symptoms and phenotypes described here can only be fully elucidated by research on the pathophysiology of the traumatic stress response.

Table 1. PTSD diagnostic criteria in *DSM-5*. Items highlighted in red are new additions to the *DSM-5*. Adapted from Friedman, 2013**.**

Criterion	Symptom category	# symptoms required	Specific symptoms
A	Exposure to a traumatic event	N/A	1. Directly experiencing the event(s) 2. Witnessing the event(s) 3. Learning that the event(s) occurred to a close relative or close friend 4. Experiencing repeated or extreme exposure to aversive details of the event(s)
B	Intrusion symptoms	1	1. Intrusive distressing memories of the event(s) 2. Recurrent distressing trauma-related dreams 3. Dissociative reactions (e.g. flashbacks) 4. Intense psychological distress when exposed to traumatic reminders 5. Marked physiological reactions to traumatic reminders
C	Avoidance symptoms	1	1. Persistent avoidance of thoughts and memories 2. Persistent avoidance of external reminders
D	Negative alterations in cognitions and mood	2	1. Dissociative amnesia of the event(s) 2. Persistent negative expectations **3. Persistent distorted blame of self or others about the event(s)** **4. Persistent negative emotional state** 5. Diminished interest or participation in significant activities 6. Feeling of detachment or estrangement from others 7. Persistent inability to experience positive emotions
E	Alterations in arousal and reactivity	2	1. Irritable behavior or angry outbursts **2. Reckless or self-destructive behavior** 3. Hypervigilance 4. Exaggerated startle response 5. Problems with concentration 6. Sleep disturbance
F	Duration of symptoms >1 month		
G	Symptoms cause significant distress or functional impairment		
H	Symptoms not due to alcohol, drugs, or medication		**1. Specify if: dissociative subtype (full PTSD + derealization or depersonalization** **2. Specify if: preschool subtype (1 B and 2 E, but only 1 C or D symptoms needed)** 3. Specify if: with delayed expression of symptoms

Pathophysiology. While identifying PTSD subtypes has been important in recognizing symptom heterogeneity and providing more accurate diagnoses and treatment options for patients, PTSD cannot fully be prevented, diagnosed, or treated without a clear fundamental understanding of the underlying neurobiology of the traumatic stress response. Studying PTSD pathophysiology in humans is mostly limited to brain functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) scans and biomarkers in blood, urine, and saliva. The effects of PTSD are most reliably measured during "challenge" tests (e.g. traumatic script reminders, glucocorticoid injection), as baseline measures have not consistently detected PTSD (144). A postmortem PTSD brain collection is currently being built by the Harvard Brain Bank and Cohen Veterans Bioscience, but to date, no studies from this initiative have been published. Like other types of stress, responses to traumatic stress involve neuroendocrine activation of the HPA axis, neuronally mediated activation of the sympathetic nervous system (SNS), and limbic system responses (110), but the neurobiological underpinnings of these trauma-induced alterations are very different from those seen in non-traumatic stress responses (145). These systems likely interact with one another in synergistic and inhibitory ways to modulate the traumatic stress response.

Sympathetic nervous system. Within seconds of stress exposure, catecholamines (e.g. epinephrine/adrenaline and norepinephrine/noradrenaline) are released from the adrenal medulla through sympathetic neuron activity (146). In PTSD, the SNS response is exaggerated, exhibited by elevated resting heart rate and blood pressure, exaggerated acoustic startle responses, and increased skin conductance, which do not seem to be risk factors for PTSD but indicators of PTSD (110, 112). Catecholamines enhance memory storage for events associated with emotional arousal (147) and norepinephrine is specifically associated with the hyperarousal and re-experiencing symptom clusters of PTSD (148). Flashbacks and panic attacks can be elicited by acute yohimbine injection in PTSD patients but not healthy controls, indicating an increased noradrenergic sensitivity in PTSD; however, a subset of PTSD patients do not experience yohimbine-induced reactivity but rather exhibit meta-Chlorophenylpiperazine (mCPP)-induced flashbacks and panic, indicating enhanced serotonergic sensitivity (149). This evidence suggests that flashbacks and hyperarousal are not specific to SNS hyperactivity and distinct biological subtypes of PTSD exist. These SNS responses both influence and are influenced by HPA responses to stress (110).

Hypothalamic-pituitary-adrenal axis. Within minutes after stress exposure, glucocorticoids (e.g. cortisol) are released from the adrenal cortex via activation of the HPA axis. In response to stress, the paraventricular nucleus of the hypothalamus (PVN) releases corticotropin-releasing hormone (CRH), stimulating the release of adrenal corticotropic hormone (ACTH) from the pituitary, which then stimulates the release of adrenal glucocorticoids. Cortisol in humans is the major adrenal HPA stress response hormone, and its release is regulated by a negative feedback system modulated by GR in the PVN and pituitary immediately after a cortisol increase, and again hours later, in distinct processes of *fast feedback* and *delayed feedback* (150). GR in the hippocampus is a primary indirect mediator of HPA negative feedback (151). This system is specifically disrupted in PTSD, characterized by decreased levels of ACTH and cortisol due to exaggerated or sensitized negative feedback control over cortisol release, resulting in persistent low levels of cortisol (152–154), the opposite of what is seen in MDD (155, 156) and the dissociative subtype of PTSD (125). These differences may reflect that some patients with PTSD have an increased number of GR and patients with MDD have a decreased number of GR on blood lymphocytes (157). However, MDD co-occurs in 48% of PTSD patients (1), which may explain some inconsistencies in the literature regarding the HPA axis and PTSD. Nonetheless, low cortisol is associated with the avoidance symptom cluster in PTSD (158). Since cortisol potentiates memory consolidation, low cortisol in response to a traumatic event could contribute to aberrant memory formation in PTSD (159). Alternatively, low cortisol in response to trauma may permit catecholamines release to remain unchecked, leading to PTSD symptoms (160), as glucocorticoids, in addition to their function in the HPA axis, suppress stress-induced increases in catecholamines (161). Indeed, high-dose cortisol administration in the hours before or after trauma exposure can attenuate or prevent the development of PTSD symptoms (162, 163). Together, these results suggest that both adrenal glucocorticoid and catecholamine response at the time of trauma exposure may influence the subsequent trauma sequelae. While these biomarker tests have provided insight to the biological underpinnings of PTSD, structural and functional neuroimaging studies of the brain have provided a broader lens of the effects of trauma and PTSD on psychological functioning.

Limbic system. Limbic system structures including the amygdala, hippocampus, medial prefrontal cortex (mPFC), and anterior cingulate cortex (ACC) are involved in the emotional and memory-related

processes of PTSD and show functional and structural changes with PTSD (9, 164–166). The amygdala detects threats in the environment, the ACC and mPFC are activated during emotional states and decision making, and the hippocampus is involved in explicit memory processes and fear memory (144).

The widely-reported smaller hippocampal volume in PTSD patients (see section: *Risk factors for PTSD*) was initially thought to be caused by damage from excess glucocorticoids, but as evidence of chronic low cortisol in PTSD patients mounted, it became clear that this was not the case and lower hippocampal volume is a pre-trauma risk factor for PTSD (66, 145). Results of hippocampal functional tests have been inconsistent, with some showing decreased hippocampal activation and gray matter volume in PTSD and some showing increased hippocampal activation and gray matter volume, possibly depending on differences in tasks and analyses used (9, 165, 166).

PTSD is traditionally associated with decreased activity in the prefrontal cortex and increased activity in the amygdala (167–169). While overactive amygdala activity is also observed in people with social anxiety disorder, phobic disorder, and fear conditioning of healthy subjects, underactive mPFC activity is unique to PTSD (169). The reduction of mPFC activity and gray matter in PTSD patients is coupled with reduced ACC activity and gray matter, and some hypotheses posit that this underactivation of frontal circuits results in amygdala hyperactivity, inhibiting fear extinction (166, 170–172), but others show no evidence that amygdala hyperactivity is due to a failure of frontal lobe inhibition (173), while still others show no amygdala hyperactivity at all (9). The pattern of reduced frontal cortex and enhanced amygdala activity has been associated with an inability of people with PTSD to use contextual cues to assess safety, resulting in feelings of endangerment in safe contexts, and “low fear” in danger contexts (174). The prefrontal cortex detects whether a stressor is under a person’s control, and if it is, it inhibits brainstem stress responses; thus, the *presence of control* blocks the “default” behavioral sequelae of uncontrollable stress (19). This evidence argues that “from an evolutionary perspective, it may be sensible that activation of ‘lower’ centers by strongly aversive events came first, and that as species developed the ability to cope with such events by behavioral means, inhibition from ‘higher’ centers under conditions of behavioral coping then developed;” so, if trauma reduces activation of the mPFC, then a loss in the “perception of control” may follow (19).

Recently, however, people with dissociative PTSD have been observed with increased prefrontal cortex activity and decreased amygdala activity (123, 124), the opposite of the presumed typical PTSD pattern. What this means for the perception of control is yet to be examined, but this natural dichotomy will be a useful tool to examine the role of stressor controllability and mPFC activity in PTSD and certainly strengthens the argument that distinct subtypes of PTSD exist.

While fMRI scans can aid in the diagnosis of PTSD (175), discrepancies in the literature as to what a "PTSD brain" looks like preclude robust imaging diagnostics, and similar challenges exist for physiological biomarker tests. One contributor to these inconsistencies is related to the fact that sex differences in PTSD, while well-documented in epidemiological reports, have received little attention in PTSD physiological research. To the extent that sex differences have been reported, women with PTSD almost always show very different responses than men with PTSD.

Sex differences in PTSD. Sex differences pervade nearly every aspect of the traumatic stress response, from PTSD prevalence to epigenetic changes at the molecular level (176, 177). One of the most commonly reported epidemiological findings is that PTSD is twice as prevalent in women than it is in men, even though women are less likely to experience a traumatic event (1, 3, 70, 108, 178, 179). This increased prevalence in PTSD in women is a reflection of a higher overall conditional risk of developing PTSD after any kind of trauma in women compared to men, a sex difference that is partially mediated by women's higher risk for revictimization (i.e. cumulative trauma effect) (1, 180) and for developing PTSD after physical attack or threat with a weapon (1). Interestingly, the latter two traumas confer the lowest risk for developing PTSD among men, which is perhaps a reflection that women experience physical violence as more threatening than men do (70). Women have been reported to perceive any type of traumatic event as more distressing than do men (70), and perception of danger is a significant risk factor for PTSD (78). Indeed, women respond to negative emotional stimuli with greater amygdala activation than men and men respond to positive emotional stimuli with greater amygdala activation than women (181). Additionally, women face a more negative social environment (e.g. victim blaming) than men following trauma (111). A meta-analysis confirmed that decreased hippocampal volume is correlated with PTSD but revealed that this effect is independent of gender, suggesting that the higher prevalence of PTSD in

women is not attributed to decreased hippocampal volume (182), further implicating the amygdala as an important neurobiological substrate for women's increased response to traumatic stress. While women's increased risk for PTSD certainly calls for more research on the effects of trauma in women, what is perhaps more intriguing is that, of the women who do develop PTSD, their phenotype is characteristically and biologically different from men with PTSD.

Since its first inception in 1980, the *DSM* PTSD diagnosis has reflected the traumatic stress sequelae in primarily men, with the assumption that women with PTSD would show the same responses. Much evidence exists to counter this assumption. Indeed, while exaggerated HPA negative feedback is considered a hallmark symptom of PTSD (145), many women with PTSD do not show this response (125, 183–185). Similarly, an enhanced startle response to an acoustic stimulus is also a presumed core symptom of PTSD, but women with PTSD have been found to show a diminished startle (186). Additionally, the symptoms of PTSD may be more severe (116) and persist up to four times longer in women than in men (187).

Sex differences in the traumatic stress response also interact with PTSD subtypes. Men tend to exhibit the externalizing PTSD phenotype and women tend to exhibit the internalizing PTSD phenotype (188). While both men and women can exhibit any of the PTSD phenotypes, what factors contribute to the observed sex bias in not yet known. Furthermore, the dissociative subtype of PTSD was initially found in a male sample to be associated with increased severity of flashbacks and exaggerated startle (118), but when female samples were examined by the same group, the dissociative subtype was not associated with increased flashbacks or startle (120). Additionally, women are more likely to exhibit dissociative symptoms, with 30% of women with PTSD fitting the dissociative subtype of PTSD compared to 15% of men with PTSD, but this could be due to the increased exposure of female sexual trauma in this sample, which has been specifically linked to dissociation (116, 120). In contrast, a larger worldwide survey found *males* were at higher risk for dissociative symptoms (119). Additionally, women but not men with dissociative PTSD have a higher rate of comorbid personality disorders compared to same-sex groups without dissociative PTSD (120), further indicating that regardless of differences in prevalence, women seem to respond to traumatic stress with a different phenotype than men. This is reflected in

studies indicating that, in general, men and women with PTSD exhibit different physiological responses to stressful stimuli (189–192).

Women with PTSD show greater psychophysiological (skin conductance and electromyography) responses to recollection of personal trauma than men with PTSD, but only men with PTSD show greater amygdala activation to personal traumatic reminders (193). However, another study showed that women with PTSD did have increased amygdala activation in response to fearful faces compared to happy faces (194). This may reflect sex differences in how stressful stimuli are processed, with male amygdalae more reactive to personal trauma reminders and female amygdalae more reactive to general threats, highlighting further nuances in the sex-specific amygdala activation mentioned earlier in this section. Current diagnostic criteria and clinical practices have not been revised to reflect these sex differences in the trauma response, largely due to a lack of basic research on the sex differences in the traumatic stress response. The vast extant literature on sex differences in the brain and behavior of rodents provides an invaluable guidepost for approaching this gap in the traumatic stress literature.

Sex differences in rodent brain and behavior

Gonadal hormones, sex chromosomes, and the development of sex differences. The organizational-activational hypothesis of sexual differentiation posits that some effects of gonadal hormones that occur early in development permanently and irreversibly influence brain structure and circuitry (organizational effects), while other effects of gonadal hormones change as circulating steroid hormone levels fluctuate throughout life (activational effects) (195). In rats, males undergo two testosterone (T) surges, one during prenatal day 17-18 and another 1-3 hours after birth that, via both androgen receptors (AR) and estrogen receptors (e.g. the "aromatization hypothesis"), are necessary for the permanent masculinization of the male rat (196). While feminization was once thought to be a "default" state that resulted from the absence of testicular secretions (197), feminization is likely an active process that is independent of masculinization (198). Further evidence indicates that sex differences resulting from gonadal secretions are downstream from the primary driver: the sex chromosomes.

Sex differences in the mammalian brain and behavior all stem from the "inherent sexual inequality of the sex chromosomes," which lead to a host of sexually-biased effects including sex-specific uses of epigenetic modifications (199), downstream effects of gonadal differentiation and gonadal hormone secretion, sex differences in autosomal gene expression, and cell-autonomous use of the sex chromosome complement (199, 200). The postnatal environment is also a source of sex-specific information, especially in humans where males and females are socially treated differently (200). Together, these sex differences may create or reduce sex differences in physiology and behavior, and the well-documented sex differences in the HPA axis are especially relevant to PTSD.

Sex differences in the rat HPA axis. Compared to males, female rats have a higher concentration of total plasma corticosterone (CORT), which is analogous to human cortisol (201, 202), and possibly to compensate for this sex difference, females also have higher CORT binding globulin (CBG) that brings their free CORT levels down to a level similar to that of males (203, 204). However, elevated CBG doesn't account for the sex differences in CORT secretion in which females release more CORT in response to ACTH stimulation (196), which may be a result of hypertrophied adrenal glands in

females due to low T (202). In rodents, activational T inhibits the HPA response to stress (202, 205–207) while activational estradiol enhances the HPA response to stress (206, 208). This gonadal hormone-dependent aspect of the HPA response is regulated by AR in males and by GR in females (203, 206, 209–212). It is likely that both AR, which is expressed in the brain of female rodents and humans (213, 214), and ER may have a role in both the male and female stress response (196, 215, 216).

Higher CORT in females may also be due to diminished negative feedback of the HPA axis in females (203, 210). Indeed, female rats are less sensitive to the negative feedback effects of glucocorticoids (204, 215, 216). Sex differences in rat CORT binding capacity and GR binding site affinity in the hippocampus and hypothalamus are regulated by ovarian hormones and may contribute to sex differences in HPA negative feedback or may be a compensatory mechanism for differences in binding capacity to *prevent* sex differences in downstream effects (217). The fast feedback inhibition of the HPA axis is sensitive to the *rate* at which plasma glucocorticoids increases, such that inhibition occurs when the glucocorticoid rate of increase rises above a certain threshold—and that threshold is higher in female rats than in male rats (150). This sex difference could be a mechanism to compensate for females' faster rate CORT increase (203). These sex differences are only partially reversed by ovariectomy, indicating that ovarian hormones play some role in modulating the HPA axis, but organizational effects are also likely involved (204). Hormonal fluctuations in the female reproductive cycle have historically been thought to be a primary source of activational sex differences.

Rat estrous cycle. While the influence of cycling estrogens have been presumed to play a large role in stress-related sex differences, the overall increased HPA activity in females is apparent in populations of normally cycling females at random stages of the estrous cycle, which is analogous to the human menstrual cycle (211, 218). Additionally, most neurobiological measures that show sex differences are not driven by changes in the female hormone cycle (219), including footshock-induced CORT release (220), behavioral responses to predator exposure (221), and the higher ACTH and CORT response to restraint stress in females compared to males (222). However, even though females have a greater stress-induced activation of the HPA axis compared to males, females exhibit reduced fear-related behavior and estradiol shows a protective effect in fear conditioning (206, 223, 224) possibly via estradiol-

mediated increases mPFC dendritic spine development and axonal branching in response to stress in females (225). This indicates that while the female reproductive cycle may not play as large of a role in activational sex differences as previously thought, ovarian hormones such as estradiol clearly do mediate some aspects of the stress response. The relationship between gonadal hormones and the stress response is well-documented, but the neurobiological mechanisms underlying these sex differences needs further basic research, particularly with respect to traumatic stress.

Merging the fields of PTSD and sex differences research with animal models

Studying sex differences in rodents. There exists some discord among neuroendocrinologists about what constitutes "true" sex differences, what they mean, and how to study them. Over the past ten years, researchers have shifted from suggesting that only those differences initiated by sex chromosome-encoded genes "count" as sex differences, while differences that can be attributed to gonadal hormonal modulation do not (226) to later differentiating types of sex differences as sexual dimorphisms, sex differences, and sex convergences/divergences (227) to more recently, simply calling for the use of "sex as a biological variable," and acknowledging that contributions of sex chromosomes *and* gonadal hormones likely shape a spectrum of differences between sexes (228) while warning that not all sex differences are "direct, context-independent and persistent" (229). Regardless of the semantics of defining sex differences, designing a study to detect sex differences can be done in several ways.

Strategies to parse out which sex differences are related to gonadal sex and which are related to chromosomal sex include using the "four core genotypes" that result from mating a rat with the *Sry* gene knocked out (XY^- female) with a rat in which the *Sry* gene is moved to an autosome (XY^-*Sry* male), using a "classic two-step endocrine experiment" in which the gonads are removed via gonadectomy and specific hormones replaced (227, 230), and using rodents with a spontaneous *Testicular Feminization Mutation* which renders ARs nonfunctional (231). While a handful of studies have used both sexes in basic PTSD research, to date, no formal sex differences studies have been applied to animal models of PTSD.

Animal models of PTSD. Because trauma exposure is generally an unpredictable event, most clinical PTSD studies are retrospective; thus, it is not known whether many of the functional, structural, and chemical brain abnormalities associated with PTSD are a consequence of the disorder or constitute a pre-existing condition that predisposes a person to PTSD. For this reason, animal models of PTSD are invaluable in examining the pathophysiology of PTSD. Moreover, because responses to life threatening challenges are highly conserved across mammalian species, PTSD may need not necessarily be "modeled" in animals, as PTSD-like responses are not unique to humans—other animals that survive life-

threatening experiences also show lasting biological effects on behavior, reproduction, and other domains of life (12). Still, it is important to develop criteria to ensure we are studying what we intend to study. In 1993, Rachel Yehuda and Seymour Antleman defined five criteria for evaluating the relevance of animal stress paradigms to PTSD (232):

(1) **Very brief exposure to the stressor should be able to induce the biological and behavioral symptoms of PTSD**. In humans, the same magnitude of PTSD symptoms can result from traumatic experiences that range in duration from seconds (e.g. traffic accidents) to months (e.g. captivity), so the extent to which a stressor is traumatizing should predict the development of PTSD rather than the duration of the event (i.e. acute or chronic).

(2) **The stressor should produce PTSD symptoms in a dose-dependent manner, in which the measured endpoints of an animal model respond differentially to different levels of the same stressor.** In humans, PTSD only occurs after experiencing a threshold dose of stress and there is evidence for a relationship between stressor intensity and severity of PTSD symptoms.

(3) **The stressor should induce PTSD symptoms that persist or increase in severity over time.** Any biological changes that occur immediately following stressor exposure and return to baseline shortly thereafter can be considered a normal acute stress response. In humans, the onset of PTSD could occur immediately following trauma exposure or could be delayed for months or years, but the symptoms can persist for many years; thus, the time since stressor exposure is correlated with development of PTSD symptoms.

(4) **The stressor should be capable of inducing both increased and decreased environmental responsiveness to stimuli that recall the stressor.** In humans, both increased (i.e. intrusive re-experiencing, hyperarousal) and decreased (i.e. avoidance, numbing) responsiveness to stimuli can exist concurrently but usually alternate between dominant re-experiencing and dominant avoidance.

(5) **Individual responses to a particular stressor should vary based on previous experience or genetics.** A traumatic experience can induce PTSD in some people, while other people experiencing the exact same event do not develop PTSD or develop unique individual phenotypes, suggesting that factors other than the trauma contribute to the induction of PTSD.

In addition to these criteria, Siegmund and Wotjak suggested that a PTSD animal model should exhibit both the *associative* (re-experiencing, avoidance of and exaggerated response to trauma reminders) and *non-associative* (hyperarousal, irritability, hypervigilance, increased startle, emotional numbing, social withdrawal) components after trauma exposure (233). Animal models used in PTSD research include predator exposure, footshock, single prolonged stress, and fear conditioning. Because the fear circuit is conserved across most vertebrate species, fear conditioning in animal models has been suggested as clinically applicable way to explore mechanisms of the fear circuit in PTSD (167), but because fear conditioning involves repeated exposure to an unconditioned stimulus in order to produce the fear response, this may not be an appropriate model for PTSD unless a single exposure to the unconditioned stimulus can induce PTSD symptoms in a manner defined by the previously described criteria. Fear conditioning is likely more useful in examining the phenomenon of "triggers" that induce PTSD symptoms rather than being used as a PTSD model itself.

Single prolonged stress. Single prolonged stress (SPS) is one of the most replicated and well-validated models for PTSD, and experimental evidence supports the face, construct, and predictive validity of the model (234). The effects of SPS recapitulate the PTSD symptoms in humans, including increased anxiety behavior (235–238), enhanced arousal (239, 240), and increased negative feedback of the HPA axis (241, 242), with the caveat that these responses can only be assumed to relevant to males. In humans, at least one month post-trauma exposure is required for a PTSD diagnosis (*DSM-5*). Similarly, in rodents, a minimum of 7 days post-SPS (equivalent to one month in humans) is required to produce the full PTSD-like response in regard to HPA axis dysregulation (242) and fear extinction deficits (243). Selective serotonin reuptake inhibitors and atypical antipsychotics are the standard

pharmaceutical treatment for PTSD (159, 244, 245), and these same drugs reduce the SPS-induced enhanced fear memory and anxiety behavior in rats (246–248).

The SPS model meets all five criteria proposed by Yehuda and Antleman. The SPS paradigm employs psychological (restraint), physiological (forced swim), and chemical/endocrinological (ether) stress, which are often all simultaneously part of the human traumatic experience. The combination of the three SPS stressors is required to produce the full PTSD phenotype, as any combination of two of the three stressors does not produce all of the effects observed with the full SPS paradigm (243). Using three different stressors, as with SPS, can also reduce the risk for habituation that sometimes occurs in other PTSD models that use repeated footshock or predator exposures (234).

Predator exposure. The predator exposure (PredX) model is another valid animal model for PTSD (249). A single, short (10-60 min) exposure to a predator or predator scent in a closed (i.e. inescapable) environment results in lasting changes in HPA axis activity, startle response, anxiety behaviors, social interaction, conditioned fear responses, prefrontal cortex activation, and hippocampal cell morphology (250–255). These responses to predator stress exposures can be prevented with early-intervention selective serotonin reuptake inhibitor treatment (256), but as with SPS, can only be assumed to be relevant to males.

Measuring responses to traumatic stress. While PTSD shares many symptoms with other psychiatric disorders, increases in acoustic startle response (ASR) and HPA negative feedback are two that are unique to PTSD and readily quantifiable in both clinical and preclinical research settings.

Dexamethasone suppression test. One of the most robust measures used in the SPS paradigm is the dexamethasone suppression test (DST) (235, 239, 240), which surprisingly has not been tested in any PredX paradigm. The DST is a tool used in clinical and experimental studies to detect disruption of the HPA axis (257). Dexamethasone (DEX) is a pituitary GR agonist (258) that diminishes any subsequent CORT response via negative feedback. In humans, high-dose DEX administration at night normally results in very low baseline CORT levels the next day; thus, shifting down the post-stress CORT levels (259). But under conditions of acute stress such as a CRH injection (260), patients with MDD show no CORT suppression with DEX (DEX non-suppression) (257, 261–263), whereas patients with PTSD typically show exaggerated suppression of CORT with DEX (155, 156, 160). While a high dose of DEX

(1.0mg in humans) is more sensitive in detecting DEX non-suppression, a low dose of DEX (0.5mg in humans) is more sensitive in detecting exaggerated DEX suppression (156). Interestingly, as many as 10% of healthy individuals are reported to be DEX non-suppressors (257), and women are more likely to be DEX non-suppressors than men (261). Women with PTSD do not show any different response to the DST compared to women without PTSD (183). Post-menopausal women are more likely to have DEX non-suppression but age does not appear to affect males (264).

Acoustic startle response. The startle response is a brainstem reflex to sudden stimuli manifesting as a whole-body or an eyeblink reflex, and the exaggeration of this response is a hallmark symptom of PTSD that indicates trauma-induced increased arousal and fear (265–268). In humans, eyeblink reflex is the most common method for measuring startle response, and in rodents, whole-body movement is the most common method for measuring startle response. However, women with PTSD are less likely to show enhanced ASR, and may in fact show a *diminished* startle (186). The ASR is a reliable measure for enhanced arousal in both the SPS and PredX models, and the sex differences in both the ASR and DST warrant more investigation as a potential mechanism by which to measure sex differences in the traumatic stress response.

Overview of chapters

While much attention has been given to how sex differences in gonadal hormones may influence sex differences in the prevalence of depression (269), the same consideration has not been given to the influence of gonadal hormones on the traumatic stress response. Indeed, gonadal hormones modulate negative feedback of the HPA axis (264, 270, 271), and to the extent that gonadal hormones have been studied relevant to PTSD, the focus is limited almost entirely on the female menstrual cycle with no direct sex comparisons to men (272–275). The few studies that have examined the role of T in PTSD are limited only to men, with no comparisons to women (276). While some evidence indicates that T in healthy males correlates with increased fear reactivity (276), treating healthy women with T reduces fear and startle (277, 278), indicating sex differences in how T mediates stress responses. The following experiments will begin to address this gap in the understanding of the traumatic stress response.

Chapter 2 begins by characterizing sex differences in response to SPS in gonadally intact, adult male and female Sprague Dawley rats with the DST, ASR, and relevant measures in the brain (cFos activation and GR expression). The potential sex-specific effects of social support are tested, and whether measures of depression (sucrose preference and social interaction) capture the traumatic stress phenotype in females is examined. Finally, whether SPS induces conditioned anxiety behavior in male and females is tested.

Chapter 3 characterizes sex differences in a PredX model of PTSD to determine whether the sex differences found in SPS are generalizable to another type of traumatic stress. The DST, ASR, and GR expression in the PVN are examined.

Chapter 4 examines the role of gonadal hormones in mediating the traumatic stress response using a classic-two step endocrine experiment in which adult male and female rats are gonadectomized and later exposed to SPS. A subset of rats receive T replacement to determine the role of activational T in the traumatic stress response.

Chapter 5 presents concluding remarks on the interpretation of the data presented and future directions.

CHAPTER 2: CHARACTERIZING SEX DIFFERENCES IN RESPONSE TO SPS

Introduction

The neurobiological basis of marked sex differences in PTSD is not understood (178). While women are twice as likely as men to develop PTSD following a traumatic experience and tend to experience different symptoms and comorbidities than men (1, 139, 140, 279), current understanding of PTSD is largely based on studies of males. Recent reviews emphasize the need to examine the neurobiology behind sex differences in PTSD (280), but this appeal has been largely unfulfilled. While the animal literature is replete with reports showing various forms of stress affect males and females differently, with often opposing outcomes on behavior, physiology and the brain (281–283), focus has been largely on acute or chronic stress as models of anxiety and depression, with negligible attention given to traumatic stress and how it might also affect males and females differently. One of the most well-validated and commonly used rodent models of PTSD is SPS. To date, over 140 published SPS studies have been published, but only six have used females (223, 284–287), with only one directly comparing males and females (288). It is clear that the sex-specific effects of traumatic stress merit further investigation.

Measures of hyper-responsiveness to stressful stimuli, including enhanced ASR and exaggerated negative feedback control of the HPA axis, are presumed core attributes of PTSD and readily observed in men with PTSD (159) and trauma-exposed male rodents (239, 256). However, whether these same readouts reflect the effects of trauma in females is unclear (289). Indeed, more than half of women with PTSD do *not* show the male-typical increase in negative feedback of the HPA axis (183, 290). Similarly, women with PTSD are less likely to show enhanced ASR, and may in fact show a *diminished* startle (186). Current diagnostic criteria and clinical practices have not been revised to reflect these sex differences in the trauma response, largely due to a lack of basic research on females.

To address this issue, we examined the response of male and female rats to the same traumatic stress, SPS. We began by directly comparing intact males to intact, normally cycling females, since sex differences in fear-conditioning are present regardless of estrogen levels in women with PTSD (190). We

now report robust sex differences in the traumatic stress response at every level of analysis, from behavior to the stress hormone response to cellular measures in the brain.

Methods and Materials

Experiment 1: Sex differences in the SPS model. Refer to Fig 1 for experimental timeline.

Animals. 8wk old adult Sprague-Dawley male and female rats were housed in same-sex pairs on the day of arrival and handled 3min daily for one week before any testing or stress exposure. Rats were purchased from Charles River and housed with 12h reversed light-dark cycle, ad lib food and water. Cage bedding was changed weekly and no testing was conducted on days of cage changes. All behavior tests were conducted in the dark-phase > 2h after beginning of dark phase. Female rats were freely-cycling and assigned to treatment groups without regard to estrous cycle stage. All animal procedures and care met or exceeded the NIH guidelines, were approved by Michigan State University Institutional Animal Care and Use Committee.

Acoustic startle response. ASR was tested as previously described (239). Rats were placed in a Plexiglas tube attached to an accelerometer inside a dark, soundproof chamber (SR-Lab, San Diego Instruments) and allowed to acclimate for 5 min (68 dB background noise) before delivery of a startle stimulus (50 ms burst of 110 dB white noise every 30 sec for 15 min). The chamber and Plexiglas tube were cleaned with 70% ethanol between each test. Peak whole-body startle response was recorded every 1 ms for 100 msec, beginning with each startle stimulus. The average peak value for each rat was normalized to body weight. Baseline measures were taken on the day before stress exposure, with rats randomly selected and counterbalanced by group and cagemates tested simultaneously in two separate chambers. Rats were then assigned to control or stress groups so that each group had equal average ASR (291). ASR testing was repeated 11 days after stress exposure in the same order as baseline ASR.

SPS paradigm. All rats were singly housed immediately before exposure to SPS or control conditions to eliminate any sex-specific effects of social support on the stress response. SPS was performed as previously described (241). SPS consists of a psychological stressor (2 hr tube-restraint stress), a physiological stressor (20 min group forced swim in 24°C water, n=6 same sex rats per 75-liter tub with 28cm water depth), and a chemical stressor (brief exposure to diethyl ether until immobile and

lacking toe-pinch response). Rats were given a 15min rest period in their home cages after the forced swim before ether exposure. Ether was used as a chemical/endocrinological stressor to recapitulate an important phenotypic aspect of PTSD and not as an anesthetic. Control rats were also similarly removed from the vivarium for 2.75h. Rats were left undisturbed for one week after SPS, a requisite delay for the long-lasting PTSD responses to develop (239, 243).

Dexamethasone suppression test. To assess the strength of negative feedback control of the HPA axis, the DST was done two wks post-SPS, as previously described (239). DEX (Sigma-Aldrich) was dissolved with ethanol and diluted to 5% in sterile saline. Low-dose DEX (0.05 mg/kg, i.p.) or vehicle was administered 2h prior to 30min tube-restraint. Tail-nick blood samples were collected at 0 and 30min of restraint. Blood samples were collected in the rats' dark phase, matching the time of day across experimental groups. Plasma CORT levels were determined using an enzyme immunoassay kit. Rats were sacrificed 30min after the restraint ended by pentobarbital (i.p.) overdose 30min after the end of restraint (1h after restraint onset) then intracardially perfused with saline and 4% buffered paraformaldehyde, with brains harvested for IHC staining.

cFos and GR immunohistochemistry. Only rats that received vehicle injections were used to map specific neuronal populations activated by restraint stress and expressing GR. Brains were sectioned and labeled for cFos or GR, as previously described (292). A rabbit IgG polyclonal cFos antiserum (1:10,000; Santa Cruz Biotech, cat# sc-52) and an immunohistochemistry (IHC) approach was used to visualize cFos expression with biotinylated goat-anti-rabbit antibody (1:500; Vector Labs; cat# BA-1000) and avidin-biotin complex Vectastain Elite ABC kit (Vector Labs, cat# PK-6200). GR IHC used the same basic protocol, with a GR primary antiserum (1:2500; rabbit polyclonal IgG; Santa Cruz Biotech, cat# M-20) on alternate sections from the same brains. Specificity of cFos staining was confirmed by observing a loss of nuclear staining in the suprachiasmatic nucleus when the cFos antiserum was preadsorbed with the immunizing peptide. Specificity of GR staining was confirmed by observing a loss of nuclear staining when the GR antiserum was preadsorbed with the immunizing peptide and observing the expected regional staining (e.g. in the dentate gyrus and CA1 but not CA3).

Using a stereotaxic rat brain atlas (Paxinos and Watson 6th ed., 2007) regions of interest were defined as follows: the mPFC included the prelimbic (PrL) and infralimbic (IL) areas before the corpus

callosum crossed midline (5.16 - 2.52 mm bregma), the dorsal hippocampus included the CA1, CA2, and dentate gyrus regions before the lateral ventricle appeared next to the optic tract (-1.72 – 3.80 mm bregma), and the amygdala included the central, basal, and medial amygdalar nuclei in the same tissue sections as the hippocampal regions of interest. These distinct landmarks allowed for the collection of comparable sections from each animal. Four serial sections (thickness 30 µm) from a 1 in 6 series were analyzed for each region of interest in each animal. A Zeiss Axioplan II microscope equipped with a motorized stage and an MBF CX9000 digital video camera was used to quantify immuno-labeled cells within each region of interest. Stereo Investigator software (SI v. 10.55, MBF Biosciences, Williston, VT) was used to guide the microscope with 1µm precision to trace the perimeter of each region of interest in serial sections at low magnification (2.5x). After tracing regions of interest, an optical fractionator probe (SI v. 10.55) inserted a counting frame at a random location within the outlined region, and the number of labelled cells within the counting frame were identified by the observer at 20x magnification and recorded by the software. Criteria for identifying a labelled cell included distinct black nuclear staining within the plane of focus of the counting frame. Counting frame dimensions were 130 µm x 130 µm with a height of 10 µm, which allows for the inclusion of ~3 cells per counting frame, and the coefficient of error (Gundersen m=1) for each hemisphere was at or below 0.10. A 2µm no-counting guard volume was employed at the top and bottom of the 3D counting probe to avoid edge effects that arise from distortion of objects passing through the leading edge of the knife during sectioning. The systematic and random counting frame placement proceeded at fixed intervals for a total of 25-30 frames to generate an unbiased estimate of labelled cells within each region of interest. Sampling parameters were optimized to minimize systematic error from oversampling while still allowing for reproducible estimates of labelled-cell density, as determined by the Gundersen coefficient of error. The observer was blind to the experimental condition of the tissue. In some brains, the entire region of interest was not available for analysis (due to loss during processing); for those brains, only the regions in which tissue was fully intact were analyzed. This method allows for a precise estimate of the number of labelled cells within a known volume. Total immunoreactivity for each hemisphere of each region was quantified by stereological analysis as the total number of labelled cells counted in each region divided by the total volume of each counting frame

analyzed (added for each of the 4 serial sections) to obtain an estimate of the density of labelled-cells in each region of interest for each animal:

$$Density\ of\ labelled\ cells = \frac{\boldsymbol{Number\ labelled\ cells}}{\boldsymbol{Volume\ sampled}}$$

Statistical analysis. Two-, three-, or four-way ANOVAs were run for comparisons in intact rats. See Table 2 for all statistical tests performed. For brain measures, if no main effect or interactions of hemisphere was present, data were collapsed across hemisphere. The conservative Bonferroni test was used to correct for multiple tests to hold alpha at 0.05.

Experiment 2: Effect of social housing on female SPS response. The same experimental timeline from experiment 1 was followed in experiment 2 (Fig 1), with the addition of two measures. Because female rats exposed to SPS in experiment 1 showed a stress hormone response reminiscent of depression, we also enlisted new measures (sucrose preference and social interaction) not typically used to assess the effects of trauma.

Animals. 8wk old adult Sprague-Dawley female rats were housed in pairs on the day of arrival and handled 3min daily for one week before any testing or stress exposure. Rats were purchased from Charles River and housed with 12h reversed light-dark cycle, ad lib food and water. Cage bedding was changed weekly and no testing was conducted on days of cage changes. All behavior tests were conducted in the dark-phase > 2h of dark. Female rats were freely-cycling and assigned to treatment groups without regard to estrous cycle stage. All animal procedures and care met or exceeded the NIH guidelines, were approved by Michigan State University Institutional Animal Care and Use Committee.

ASR, DST, and IHC. The same outcome measures used in experiment 1 were assessed in experiment 2: ASR, DEX suppression test, and cFos and GR immunohistochemistry.

Sucrose preference test. To measure anhedonia, rats were given access to two bottles containing either 0.8% sucrose solution or tap water for 24h. To account for any circadian influences and side-preferences, the two water bottles were presented halfway through the rats' dark cycle and the bottle position switched after 12h, as previously described (293). The bottles were initially weighed and a final weight taken at 24 hrs. Sucrose preference was calculated as % total intake. To minimize the influence of metabolic factors and reduce the effect of acute stress, food and water were available *ad lib*.

Social interaction test. Each test rat was placed in a clear Plexiglas box at the end opposite to an empty wire enclosure. The test arena was a 60cm^3 clear Plexiglas box with an interaction zone measuring 8cm out from the edge of a circular wire enclosure. After 2.5 min, a probe rat matched for sex and age was placed inside the wire enclosure for an additional 2.5 min, as previously described (294). Videos were analyzed for measures of social interaction. Social interaction behaviors analyzed included latency to first enter the interaction zone, # of social interactions, and total time spent in the interaction zone. While total social interaction time is a general measure of anxiety in rats (295), latency to first contact is another valuable measure of social interaction that captures a different aspect of the stress response (296). As rats generally approach more quickly and spend more time interacting with a social target compared to a non-social target (in this case, the empty wire enclosure), the ratio of time spent in the interaction zone in the presence vs the absence of a social probe rat gives a readout of social interaction while accounting for individual variability in level of activity (297–299).

Statistical analysis. The same statistical analyses performed in experiment 1 were performed in experiment 2, except instead of using *sex* as a factor, *housing* (single or pair) was the independent variable. See Table 3 for all statistical tests performed.

Experiment 3: Conditioned fear in SPS. In a separate cohort of 48 rats (24 of each sex), the conditioned behavioral response to SPS was recorded by pairing the SPS procedure with a neutral tone. A neutral tone of 2kHz, 80dB 5-pulse beeps (500ms silence in between 750ms tone duration, NCH Tone Generator software) was played for the duration of the SPS paradigm. Control rats were exposed to the neutral tone for the same amount of time the SPS-exposed rats were exposed to the tone (2 hours 45 minutes). The only outcome measure of this experiment was the open field test, which was conducted starting on day 8 post-SPS.

The test arena was an empty white plastic box (122cm x 122cm) illuminated by a dim red light above and recorded by an overhead video camera. The floor of the arena was marked by a grid (individual grids were 20.3cm x 20.3cm) to demarcate entries into the center area. The center zone (approximately 20% the size of the open field chamber) was used to assess anxiety-related behavior. Each rat was placed into the corner of the empty arena and allowed to explore. After 5 minutes, the rat was returned to its home cage and the arena cleaned with 70% ethanol. After three minutes, the rat was

returned to the same corner of the arena, which now contained a novel object in the center of the chamber to assess behavioral response to a mild stressor, as previously described (292). An unconditioned tendency for “wall hugging” in an open field is a measure of anxiety-related behavior in rats (300). The duration of time spent in the center of the testing chamber, which is a measure of exploratory behavior and anxiety (with time spent in the center inversely related to anxiety behaviors), was recorded and analyzed from video recordings with Noldus software. The ratio of time spent in the center of the arena in the presence vs the absence of a novel object rat gives a readout of anxiety and exploratory behavior while accounting for individual variability in level of activity. Half of the rats were tested while the neutral tone from the SPS procedure was played while the other half were tested without the neutral tone playing. To account for any effect of noise alone, white noise background was continuously played throughout all testing sessions. This procedure was conducted for three consecutive days to asses if any contextual reminder-induced anxiety would be extinguished by repeated exposure.

Separate repeated measures three-way ANOVAs (SPS x day x sex) were run for comparisons in rats exploring the open field with no contextual reminder tone and for rats exploring the open field with the contextual reminder tone. Only day 1 and day 3 behavior were analyzed. The conservative Bonferroni test was used to correct for multiple tests to hold alpha at 0.05.

Results

Males and females respond differently to SPS. As expected, males exposed to SPS showed an enhanced ASR (Fig 2A), replicating a well-established effect of SPS (239). In females, on the other hand, SPS had no effect on ASR (Fig 2A). The DST also revealed the expected enhanced sensitivity to DEX in SPS-exposed males, blocking the restraint-induced increase in circulating CORT levels typical of control males (Fig 2B). In contrast, females exposed to SPS showed a *reduced* sensitivity—DEX pretreatment, which competes with endogenous CORT for GR binding, had no effect on stress-related CORT levels in SPS-exposed females (Fig 2B). In short, these data show the expected exaggerated DEX suppression of CORT in SPS-exposed males but not females. This sex difference in DEX sensitivity is due to trauma exposure *per se* and not because females are resistant to DEX, since DEX lowered post-restraint CORT levels in control females and drove *baseline* CORT levels down in both males and

females, SPS-exposed or not (Fig 2B). SPS did not affect baseline CORT levels in either sex, as previously shown for males (241) and control females showed significantly higher baseline and post-stress CORT levels than males (Fig 1B), as previously reported (211, 216). Additionally, body weight was not a factor (Fig 11).

These striking sex differences in response to SPS suggested that SPS might also affect cellular measures in the brain differently in males and females. This is indeed what we found when we examined GR and cFos expression in brain regions implicated in the traumatic stress response (159, 301). SPS moderately increased the number of GR-expressing neurons in the PVN of males (*P=0.06*), but significantly *decreased* their number in females (Fig 2C). GR expression in the PVN of control (unstressed) females was also higher than in control males (Fig 2C). SPS had surprisingly little effect on the cFos response to acute restraint stress of males, including in the PrL or IL sugbregions of the mPFC (Fig 2D), the basolateral amygdala (BLA) and medial amygdala (MeA; Fig 2E, F). On the other hand, SPS extensively affected cFos expression in females, increasing the number of cFos+ neurons more than a week after trauma in both the PrL and IL (Fig 2D), and in the *right* BLA (Fig 2E) compared to control females. The number of cFos+ neurons induced by acute stress was also sexually differentiated in the mPFC of control rats, with control females having fewer such neurons than control males (Fig 2D), as previously reported (220, 302). GR expression in the CA1/2 region of the dorsal hippocampus was differentially affected in males and females with SPS decreasing the number of GR+ neurons in males but increasing their number in females (Fig 2G), as previously reported (288). Taken together, we found sex differences in the response to SPS in every outcome measure examined.

Neither housing nor bright lights affect the female response to SPS. Females may have responded differently to SPS than males because of being singly housed, so we next compared the effects of SPS in females that were single- or pair-housed. Because our results based on single-housed males replicated those of group-housed males (240), we did not include a cohort of males in this next study. We also tested the effect of bright ambient light which can enhance acoustic startle for females (303), thinking that this might reveal an effect of SPS on the ASR in females that was masked in the dark. All other conditions were the same as in the first study. ASR in females, even under bright lights, was not

affected by SPS or housing condition (Fig 3A). Likewise, DEX failed to block a stress-induced CORT response in SPS-exposed females (Fig 3B), as seen previously (Fig 2B), although CORT levels overall were lower in this study. DEX again lowered baseline CORT levels to near zero in all groups, and neither housing condition nor SPS affected baseline CORT levels. Thus, female rats show a unique response to SPS that is distinct from the male phenotype; neither acoustic startle nor HPA negative feedback captured the effect of SPS in female rats.

Because SPS reduced the sensitivity to DEX for females in the first study, suggestive of DEX non-suppression, a marker of depression in humans (261) and rodents (304), we added two measures routinely used to assess a depressive phenotype in rodents, the social interaction and sucrose preference tests (295, 305). Contrary to expectation, SPS had no effect on sucrose preference in either housing condition (Fig 3C). On the other hand, social interaction was affected by SPS in females, although the direction of effect depended on housing. For pair-housed females, SPS increased the latency to approach a novel rat (social target), suggestive of an anxious phenotype, but for singly housed females, SPS decreased the latency to approach a novel rat, perhaps reflecting an inclination to seek social support (Fig 3D). Because neither housing nor SPS affected the latency to approach the empty enclosure (lacking a social target) (Fig 3E), the effect of SPS is on social interaction *per se*. These data indicate that social interaction is a sensitive readout of the traumatic stress response for female rats exposed to SPS. Body weight was not affected (Fig 12). Total time spent with a social target was not affected by housing or SPS (Fig 13). For experimental consistency, hereafter we continued to use single housing.

Traumatized rats are sensitive to contextual reminders of SPS. With no contextual reminder of the SPS, both unstressed controls and SPS+tone-exposed rats spent more time in the center of an open field when there was a novel object to explore relative to when the arena was empty, regardless of sex, and this behavior was consistent through all three days of testing (Fig 4A). When the contextual reminder tone was played during the OFT, unstressed control rats behaved similarly to when there was no tone; spending more time in the center of the open field when there was an object consistently through all three days of testing, regardless of sex (Fig 4B). On the other hand, both males and females that had been exposed to SPS+tone a week prior, spent significantly less time with the novel object on the third

day of testing compared to the first day of testing, suggesting that with repeated contextual reminders of SPS, they showed sensitized anxiety behavior in an exploratory task (Fig 4B).

Discussion

Sex differences in the traumatic stress response are among the most widely reported phenomena in epidemiological and clinical studies, but the neurobiological basis for these differences is unknown, largely due to an overwhelming male bias in the research (289). We find robust and comparable sex differences in the traumatic stress response to SPS that recapitulate sex differences in PTSD symptomology. Male rats showed a hyper-responsive phenotype (ASR and negative feedback control of CORT levels were both enhanced) typical of PTSD in men (160, 239) but female rats showed a unique phenotype with more affective behavioral changes (306). Trauma had no effect on the ASR or negative feedback control of CORT in female rats, aligning well with the increased likelihood for women with PTSD to show more “internalizing” characteristics. The only measure that did not show sex differences was the open field test, which interestingly indicated that both males and female anxiety behavior can become sensitized over time to otherwise innocuous situations when a neutral contextual reminder of their previous trauma exposure is repeatedly present. This evidence has implications for the use of prolonged exposure therapy, which has been shown useful in some studies but not in others.

These sex differences in behavior and physiology were accompanied by sex differences in how the brain responded to trauma, including increased activity in the mPFC and in the right amygdala only in females. The right amygdala is uniquely implicated in contextual fear-conditioning in rats (307) and humans with PTSD (308). Notably, a recent study also shows no apparent effect of trauma on cFos activation in these same brain regions of males, but a minority (15%) of trauma-exposed males show an anhedonic phenotype with increased cFos in *both* IL mPFC and BLA (309), similar to what we see after SPS (Figure 2D). Additionally, males and females recruit different aspects of the mPFC-amygdala during fear conditioning and extinction (283). These data are consistent with the idea that anhedonia associated with mPFC and amygdala activation may be relevant markers of trauma pathology for females but not males.

We also find divergent effects of trauma on GR expression in the PVN and in the CA1/2 region of the dorsal hippocampus, reflecting yet another sex-specific response that likely alters the sensitivity of the PVN to stress hormones, and may explain why trauma-exposed males show enhanced negative feedback control of the HPA axis while trauma-exposed females do not. Notably, PTSD patients have an increased number of GR and increased sensitivity to glucocorticoids, while depressed patients have a lower number of GR+ blood lymphocytes (153, 157), but the effects of stress on GR are likely region-specific in the brain, as our data indicate. Our discovery of such sex differences in the trauma response of rats aligns well with the female-biased internalizing and male-biased externalizing phenotypes identified in people with PTSD (188) and in the general population (310). Because non-stressed females have higher baseline and post-stress CORT levels than males, higher GR expression in the PVN, and lower cFos expression in the mPFC after acute restraint than males, complimenting previous findings (216, 220, 222, 311), such sex differences may well predispose males and females to respond differently to traumatic stress. These results align with other studies showing opposite effects of acute stress on males and females (312, 313).

Because major depression is highly comorbid with PTSD, which is female biased like PTSD (1, 183, 314, 315), we included standard measures of depression not typically used to assess the effects of traumatic stress on rodents. SPS affected social interaction in females; however, this effect depended on housing conditions, with SPS decreasing their latency to socially interact when single-housed but *increasing* latency when pair-housed, which is a measure of time spent evaluating safety cues (316). While the increased latency to approach a social target shown in SPS-exposed pair-housed females could be indicative of anxiety-like behavior or the social withdrawal symptom of PTSD, the decreased latency to approach a social target shown in SPS-exposed single-housed females may seem more perplexing, as social support in humans is a robust protective factor from developing PTSD. However, while *perceived* or *received* social support may impart resilience to PTSD, social support-seeking *behavior* as an early coping mechanism after trauma is actually a risk factor for PTSD, as the need for social support may be greater in individuals more adversely affected by trauma and they may or may not receive meaningful support (317, 318). So, social support seeking behavior in itself is not protective, and whether an individual seeks social support or withdraws from social support may depend on the social

context of their lives before trauma. Contrary to expectation, SPS had no effect on sucrose preference, suggesting that the female response to SPS may not be a depressive, anhedonic-like response.

The diametrically opposed responses to trauma exhibited by male and female rats conforms with the well-known opposing responses to acute and chronic stress of males and females (319, 320). While the same neural substrates may be enlisted to manage stress, the specific mechanisms or outcomes within these substrates seems fundamentally different in males and females (283, 321). This conclusion has wide reaching implications for therapeutics to treat PTSD in men and women.

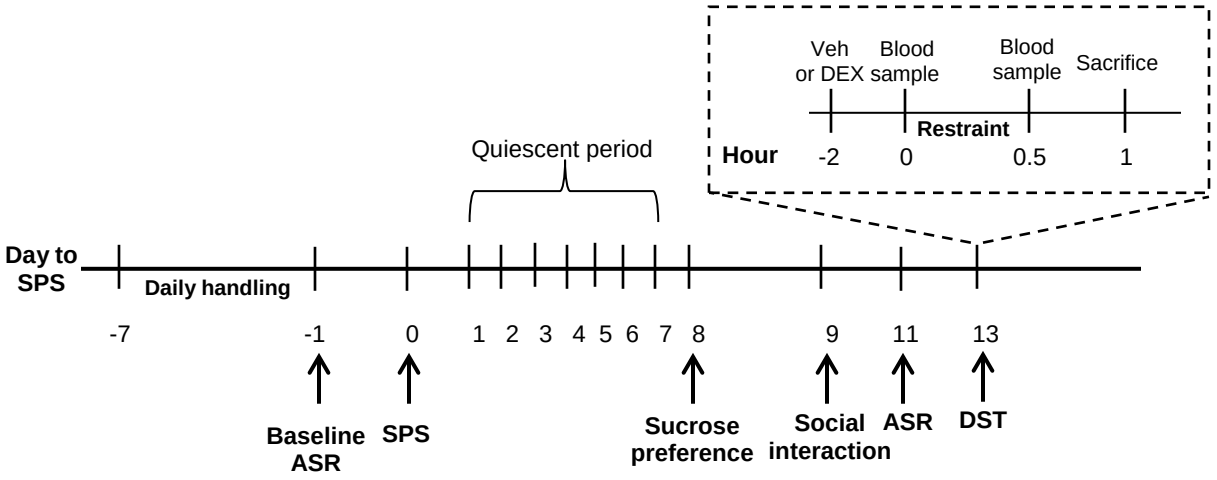

Figure 1. Timeline of procedures for experiments 1 and 2. Experimental timeline begins with daily handling one week before (-7) single prolonged stress (SPS) and baseline acoustic startle response (ASR) testing the day before (-1) SPS. In both experiments 1 and 2, post-stress ASR is conducted 11 days later and dexamethasone suppression test (DST) 13 days later. In experiment 2, sucrose preference is tested 8 days after SPS and social interaction 9 days after SPS.

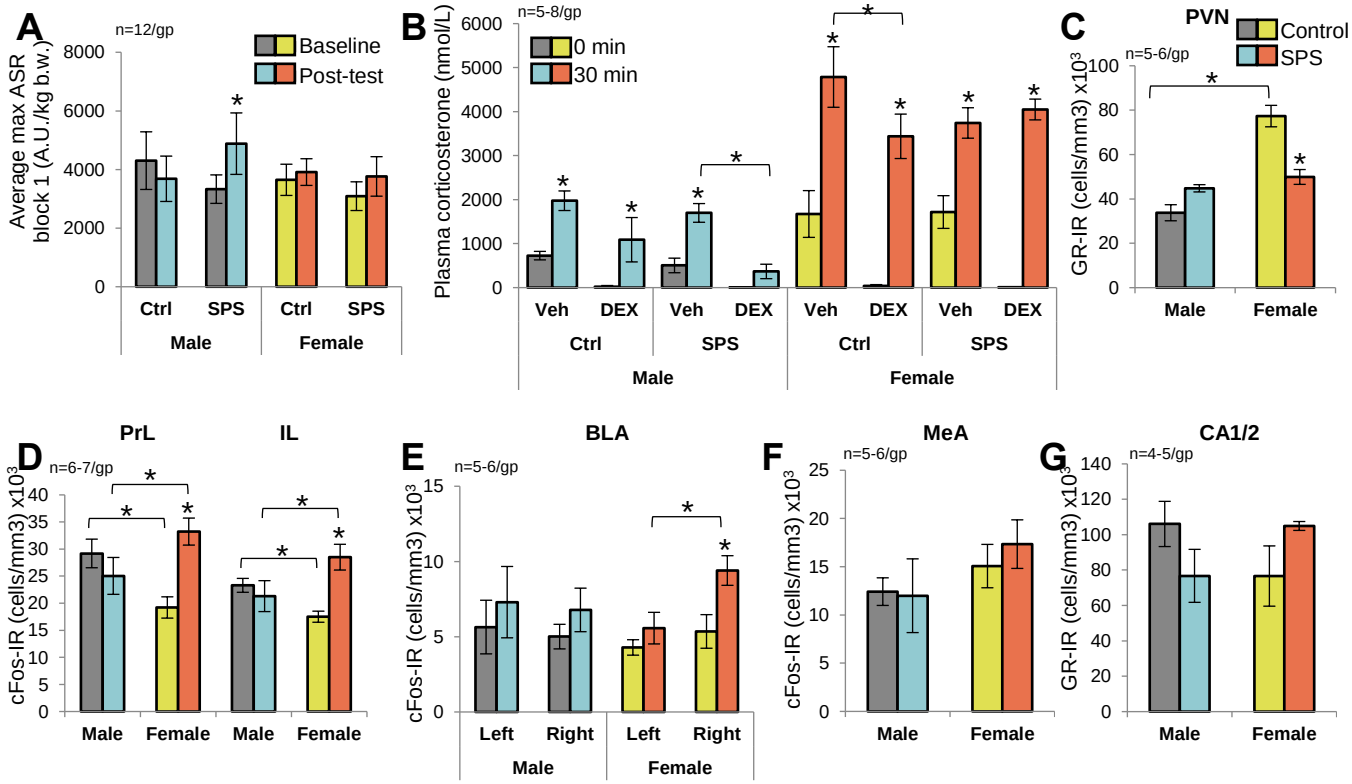

Figure 2. Single prolonged stress (SPS) affects males and females differently. (A) Males exposed to SPS one week earlier showed, as expected, a significant increase in the acoustic startle response (ASR), but females exposed to SPS showed no such increase. (B) Negative feedback control of the HPA axis was assessed by measuring corticosterone (CORT) response to acute stress in the presence or absence of a low dose dexamethasone (DEX) pretreatment. SPS males who received DEX had significantly lower CORT levels after 30 min of stress compared to SPS males who received only vehicle. Note that controls males were not as sensitive to the DEX treatment, failing to cause a significant reduction in CORT level, demonstrating the well-established enhancement DEX suppression induced by prior exposure to SPS for males. In contrast, SPS females showed comparable levels of CORT 30 min post-stress regardless of DEX treatment, suggesting DEX-nonsuppression that is more typical of depression. All females had significantly higher levels of both baseline and stress-induced CORT levels than males, as expected. DEX drove down baseline CORT levels (0 min) in all groups, demonstrating its effectiveness in both sexes. (C) SPS also had opposing actions on glucocorticoid receptor (GR) expression in the paraventricular nucleus of the hypothalamus (PVN), with a moderate increase (*P=.060*) in males but a significant *decrease* in females. Control females also had significantly higher GR expression in the PVN than control males, indicating that GR expression in the PVN is normally sexually differentiated. (D - F) Prior exposure to SPS had surprisingly little effect on the cFos response to restraint stress in males, with no effect of SPS in the prelimbic (PrL) or infralimbic (IL) subregions of the medial prefrontal cortex (mPFC), or in the basal lateral amygdala (BLA) or medial amygdala (MeA). On the other hand, SPS females showed significant increases in cFos in both the PrL and IL. SPS also increased the cFos response in the right BLA of females. Control females showed a lower cFos response to acute restraint stress in the mPFC than control males. (G) GR expression in the CA1/2 region of the dorsal hippocampus was also affected differently in males and females (sex*SPS interaction *P=.050*). Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 2 for full statistical results.

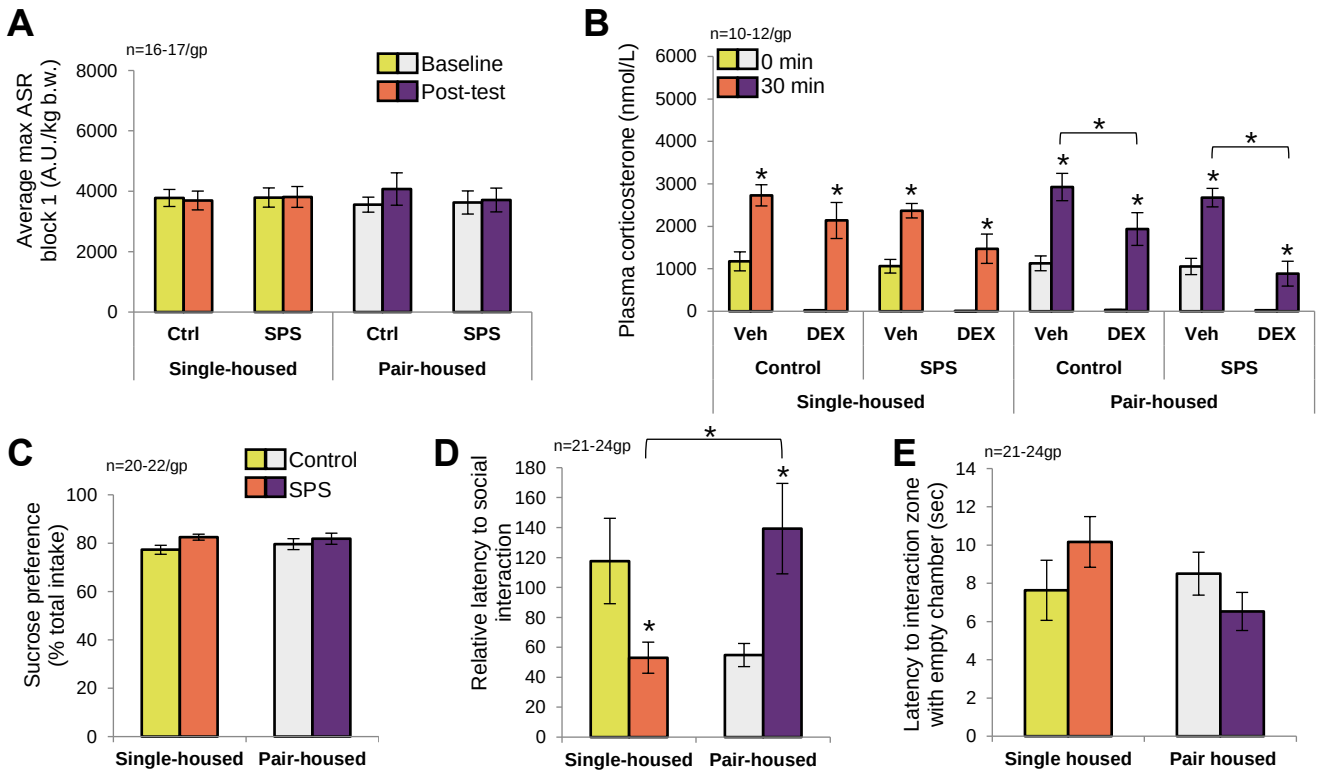

Figure 3. Housing conditions change how SPS affects social interaction in females. (A) The null effects of SPS on the ASR persisted regardless of housing condition (single v. paired) and under conditions of bright light. (B) Females exposed to SPS again did not show the enhancement of DEX suppression of CORT levels that is typical of SPS exposed males (Fig 1B). Note however, that pair-housing increased the sensitivity to DEX, and did so for both SPS and control females. Thus, this effect is due to housing and not SPS. (C) SPS did not affect sucrose preference in females, regardless of housing condition, inconsistent with a depressive-like phenotype. (D) SPS affected social interaction (based on latency to approach a novel female) but the direction of effect depended on the housing condition. SPS *decreased* the latency to approach when females were single-housed but *increased* the latency to approach when females were pair-housed. (E) Neither housing nor SPS affected the latency of females to approach the empty rat enclosure, indicating that the effect of SPS is on social interaction *per se* and not on general activity or exploration of the chamber. These data are consistent with the idea that the traumatic stress phenotype for females is distinctly different from that of males, and may share some traits of depression (given the consistent failure of SPS to enhance ASR or the effect of DEX on CORT) unlike the traumatic stress phenotype for males. Data are presented as mean±SEM. Significance set at $P<.05$ (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 3 for full statistical results.

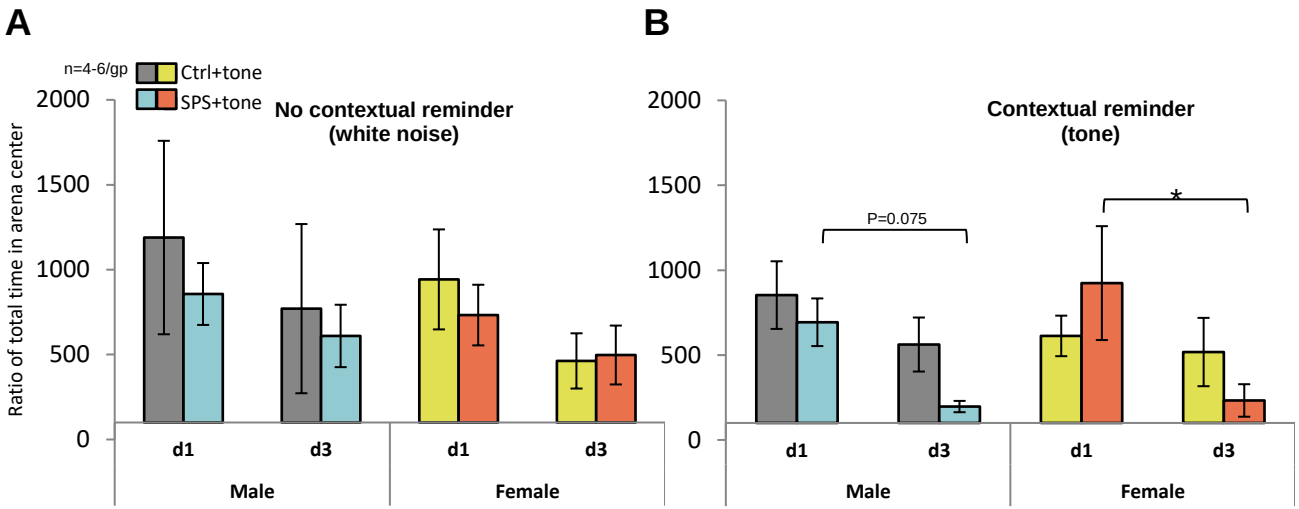

Figure 4. Contextual reminders to SPS sensitizes anxiety behavior over time. With no contextual SPS reminder (white noise), all rats spend more time in the center when it contains an object, regardless of sex or SPS or testing day (d1 or d3). After 3 days of testing with the contextual SPS reminder (white noise+neutral tone), both SPS males and SPS females spent less time in the center when it had an object, indicating that repeated exposure to contextual trauma reminders sensitized anxiety behavior over time. Data are presented as mean±SEM. Significance set at $P<.05$ (indicated by asterisk) for planned pairwise comparisons (Bonferroni).

CHAPTER 3: CHARACTERIZING SEX DIFFERENCES IN RESPONSE TO PREDX

Introduction

While we clearly identified sex differences in response to SPS, how generalizable these responses are to other types of traumatic stress is not yet known. Examining sex differences across different traumatic stress models is useful to determine whether males and females have fundamental differences in their underlying neurobiology that predisposes them to respond differently to traumatic stress, and to determine if the sex differences we observed in SPS were just an artifact of that particular paradigm. To our knowledge, no studies have used two traumatic stress models in the same lab, an approach that limits confounding variables to accurately compare the effects of trauma across models. We carefully chose a second model, PredX, that was different enough from SPS in stressor type to allow for inferences on the generalizability of effects but would also not introduce possible confounding effects of some types of stressors that include pain (i.e. footshock, immobilization) that may not reflect PTSD *per se*. There is a spectrum of PredX models ranging in stressor severity from predator odor exposure (low severity) to full-contact live predator exposure (high severity) (252). We chose a paradigm in the middle of the spectrum that exposes rats to a live predator (cat) but does not allow physical contact between the animals, which eliminates the chance of introducing pain if the cat should attack the rats. PredX is a well-validated and commonly used rodent model of PTSD, but as with SPS, only a handful of the >100 published PredX studies have examined sex differences (221, 322–327), and no studies have looked across models.

Methods and Materials

Experiment 4: Sex differences in the PredX model. The same experimental timeline from experiment 2 was followed in experiment 4 (Fig 5).

Animals. 8wk old adult Sprague-Dawley male and female rats were housed in same-sex pairs on the day of arrival and handled 3min daily for one week before any testing or stress exposure. Rats were purchased from Charles River and housed with 12h reversed light-dark cycle, ad lib food and water. Cage bedding was changed weekly and no testing was conducted on days of cage changes. All behavior tests were conducted in the dark-phase > 2h of dark. Female rats were freely-cycling and assigned to

treatment groups without regard to estrous cycle stage. All animal procedures and care met or exceeded the NIH guidelines, were approved by Michigan State University Institutional Animal Care and Use Committee.

Predator exposure. PredX rats were placed in individual wedge-shaped enclosures of a circular Plexiglas “pie restrainer” (Braintree Scientific) on which cat food was smeared and placed inside a Plexiglas arena (60cm3). This paradigm allows rats to be exposed to the cat without physical contact. Rats were exposed to a live female cat for 1h, as previously described (323). All rats were singly housed immediately before the PredX and control procedure. Control rats were removed from the vivarium for 1h but not put in the restrainer nor exposed to the cat, and housed in a separate room to prevent exposure to possible residual predator scent on PredX rats. Rats were left undisturbed for one week after PredX to allow the acute stress responses to resolve and long-term PTSD responses to develop.

Outcome measures. The same outcome measures used in experiments 1 and 2 were used in experiment 4: acoustic startle response, DEX suppression test, sucrose preference, social interaction, and cFos and GR IHC.

Statistical analysis. The same statistical analyses used in experiment 1 were used in experiment 4. See Table 4 for all statistical tests performed.

Results

Predator exposure induces comparable sex differences in ASR and DST. To test how general the sex difference is, rats were exposed to a live cat for 1h. Like SPS, PredX enhanced both ASR (Fig 6A) and DEX suppression of CORT for males but not females (Fig 6B). Indeed, the pattern of sex differences was remarkably similar following SPS and PredX, and occurred independent of effects on body weight (Fig 14). Specifically, DEX blocked an increase in CORT levels for PredX-exposed males, leading to a significant difference in CORT level between DEX and vehicle treated PredEx males not shown by control males, but failed to do so for PredX-exposed females, again showing that prior exposure to a traumatic event enhances sensitivity to DEX in males but not in females. Females again had higher baseline and post-restraint CORT levels compared to males (Fig 6B) and DEX drove baseline CORT levels down in all four groups (Fig 6B), confirming the effectiveness of the DEX treatment. Females

again had higher GR expression in the PVN than males, but unlike SPS, PredX did not affect GR expression in the PVN of either sex (Fig 6C).

Discussion

Regarding the ASR and DST tests, sex differences in PredX mirror sex differences in SPS and recapitulate sex differences in PTSD symptomology**.** While we replicated the sex difference that females normally have higher GR+ neurons in the PVN compared to males, PredX did not affect PVN GR as SPS did. The effects of SPS on GR expression may reflect a stressor-specific response to traumatic exposure, likely the ether component of SPS, since the replacement of ether with isoflurane abolishes the effect of SPS on hippocampal GR (328). Such dissociations in the stress response across different stressors help to identify core traits of the sex-specific phenotype that are independent of stressor type. Taken together, these data establish sex-specific responses to traumatic stress that are independent of the type of stressor and housing condition (ASR and DST), indicating fundamental sex differences in the neurobiology underlying the traumatic stress response. We are the first to recapitulate in two different animal models distinct subtypes of PTSD recognized in clinical studies (116, 120, 139, 140) that are linked to a single biological factor: sex. The next logical step is to examine what mediates these sex differences.

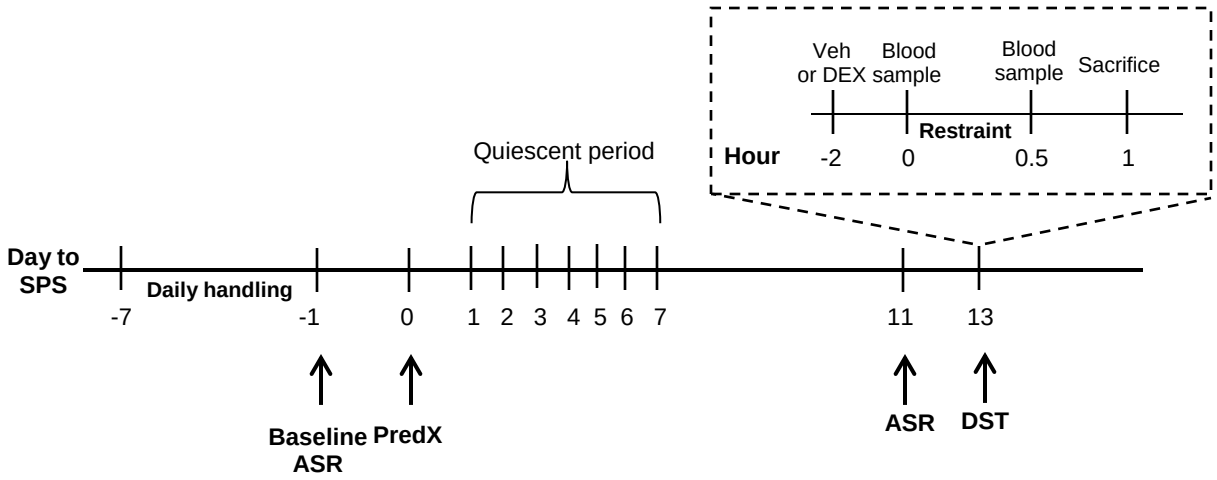

Figure 5. Timeline of procedures for experiment 4. Experimental timeline begins with daily handling one week before (-7) predator exposure (PredX) and baseline acoustic startle response (ASR) testing the day before (-1) PredX. Rats are left undisturbed for one week after PredX, and post-stress ASR is assessed 11 days later, and dexamethasone suppression test (DST) 13 days later.

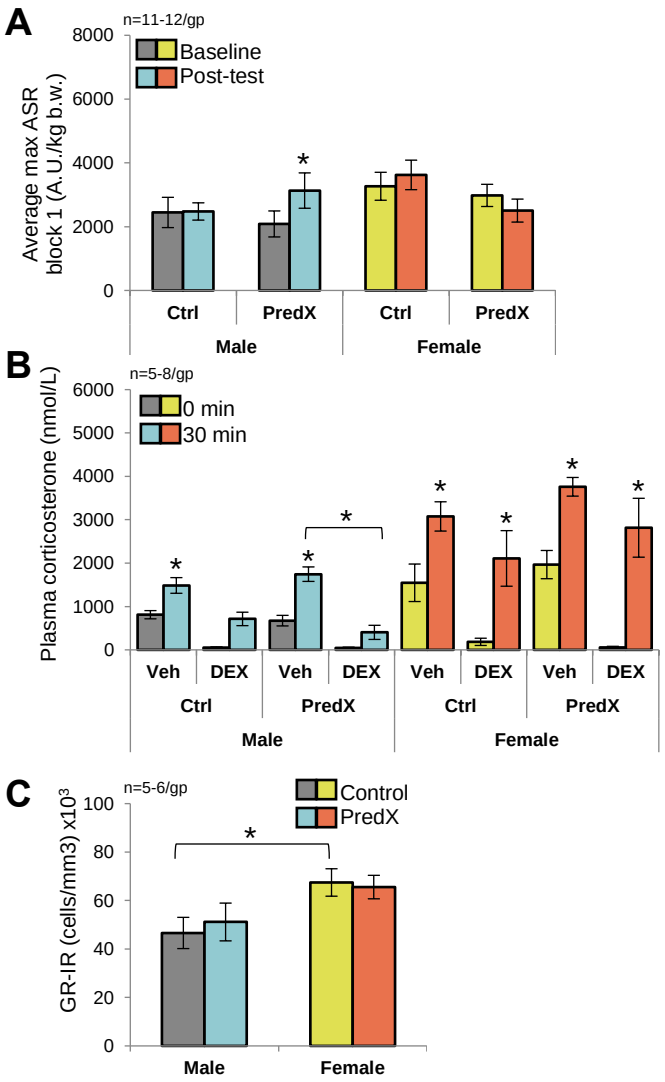

Figure 6. Predator exposure (PredX) leads to sex differences in the acoustic startle response (ASR) and HPA negative feedback comparable to SPS. (A) Only males and not females show an enhanced ASR after PredX exposure, replicating the sex difference found with SPS. (B) Likewise, PredX also enhanced HPA negative feedback in males but not in females, as indicated by the DEX suppression test. Again, DEX blocked an increase in acute stress-induced CORT levels in PredX males but not in PredX females, leading to a significant deficit in CORT levels of PredEx males treated with DEX compared to vehicle. This same difference is not evident for PredEx females, as in the SPS model (Fig 1B). CORT levels after 30 min of restraint stress were significantly higher than baseline for all female groups, independent of DEX or PredX. This pattern was not seen in males treated with DEX. CORT levels were significantly higher in females than males, replicating previous reports in rats. DEX lowered baseline CORT levels (0 min) to near zero in all groups, demonstrating that DEX was effective in both sexes. (C) Unlike SPS, PredX did not affect glucocorticoid receptor (GR) expression in the PVN of either sex, but we did replicate the sex difference in GR expression seen previously (see Figure 1C), with females having more GR+ neurons in the PVN than males. Because the sex difference in GR expression in the PVN was replicated across studies, it is likely some unique aspect of SPS that affected GR expression in the PVN and this response may be stressor-specific. Data are presented as mean±SEM. Significance set at $P<.05$ (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 4 for full statistical results.

CHAPTER 4: THE ROLE OF GONADAL HORMONES IN THE TRAUMATIC STRESS RESPONSE

Introduction

Given the novel sex differences we discovered in the traumatic stress response to SPS and PredX, we next tackled the question of whether such sex differences are related to sex differences in circulating levels of adult gonadal hormones. While some studies have found sex differences in these traumatic stress models, none have examined the possible role of gonadal hormones, despite the well-established role they serve in determining sex differences in brain morphology and function (329)

We find that the traumatic stress response in rats is highly sex-specific and recapitulates fundamental differences of PTSD in humans, with males exhibiting externalizing symptoms (e.g. hyperarousal, aggression, and risk-taking behaviors), while females exhibit internalizing symptoms (e.g. sadness, loss of pleasure, and social withdrawal) (117, 137–140). Because other sex-biased psychiatric disorders share this same divide between men and women (310), our studies of the underlying neurobiology of these sex differences in the traumatic stress response may lend insight into the neurobiological underpinnings of other psychiatric disorders.

Methods and Materials

Experiment 5: Effects of gonadectomy and testosterone replacement in SPS model. The same experimental timeline used in experiments 2 and 3 were used in experiment 5, with the addition of gonadectomy (GDX) surgery two weeks prior to any testing or SPS exposure (Fig 7).

Animals. 8wk old adult Sprague-Dawley male and female rats were housed in same-sex pairs on the day of arrival and handled 3min daily for one week before any testing or stress exposure. Rats were purchased from Charles River and housed with 12h reversed light-dark cycle, ad lib food and water. Cage bedding was changed weekly and no testing was conducted on days of cage changes. All behavior tests were conducted in the dark-phase > 2h of dark. Female rats were freely-cycling and assigned to treatment groups without regard to estrous cycle stage. All animal procedures and care met or exceeded the NIH guidelines, were approved by Michigan State University Institutional Animal Care and Use Committee.

Gonadectomy. The day after arrival, rats were randomly assigned to a treatment group (sham+blank, GDX+blank, GDX+T). Cagemates were placed in the same surgery treatment group. GDX was performed using sterile technique under isofluorane anesthesia. Ketoprofen analgesia was given at the onset of the procedure and the following day. For GDX, the outer layer of the scrotum was opened (for males) or the abdominal cavity opened (for females) and then closed with wound clips. Under the same about of anesthesia, Silastic capsules (3.2mm diameter, 40mm length) filled with T or blank were implanted s.c. at the back of the neck. Such implants provide a time-release of the hormone of interest for up to 4 wks without the stress of daily injections. The rationale for treating females, as well as males, with T was to see whether T might induce in females a male-like response to SPS. Rats recovered for 2 weeks, and were handled daily during the second week.

SPS paradigm. The SPS paradigm was followed as described in experiment 1, two weeks after GDX.

Outcome measures. The same outcome measures used in experiments 1 and 2 were used in experiment 5: ASR, DEX suppression test, sucrose preference, social interaction, and cFos and GR IHC.

Statistical analysis. Two-, three-, or four-way ANOVAs were run for comparisons in intact rats. See Tables 5-7 for all statistical tests performed. For brain measures, if no main effect or interactions of hemisphere was present, data were collapsed across hemisphere. In the GDX experiment, analysis was first conducted on only sham+blank rats to assess whether previously identified sex differences were replicated. Then, the effects of GDX were assessed within each sex, comparing GDX+blank to sham+blank. If there was a main effect or interaction with GDX, the effect of T treatment was assessed by comparing GDX+blank to GDX+T. The conservative Bonferroni test was used to correct for multiple tests to hold alpha at 0.05.

Results

Sex differences were replicated in the sham surgery group. We first compared sham operated males and females and found the same sex differences (Fig 8A,B,D), indicating that sham surgery itself did not alter the pattern of differences. SPS in this study replicated effects on social interaction latency in females, and notably, revealed a decrease in sucrose preference in females (Fig 8F,

G, Fig 15). These data indicate that both sucrose preference and social interaction are useful measures for capturing the effects of SPS on behavior in females. Interestingly, males appear *resilient* based on these particular measures.

Effects of SPS in males are largely independent of adult testicular hormones. Overall, we find that male rats castrated as adults respond to SPS much like gonadally intact male rats. To the extent that adult testicular hormones influenced our measures, they were independent of SPS. Specifically, gonadal status did not alter the effect of SPS on the ASR in males (Fig 9A). Likewise, the DST revealed the same pattern of differences in castrated (GDX) males as in gonadally intact (sham) males (Fig 9B). Both groups showed an exaggerated DEX suppression after exposure to SPS. Removing the testes did however significantly increase both baseline and restraint-induced CORT levels in control males, which was reversed by T treatment of castrated males (GDX+T), as previously reported (215). However, T treatment also seemed to mask an effect of SPS on HPA negative feedback, since both control and SPS males had comparably low, near baseline, CORT levels after restraint stress in the presence of DEX. This outcome may reflect the fact that exogenous T does not faithfully recapitulate normal levels of endogenous T, or that the low number of individuals in the GDX+T group (n=2-6) was insufficient to detect an effect of SPS. Regardless, these data indicate that the effect of SPS in males on the ASR and negative feedback control of stress-induced CORT levels does not depend on endogenous testicular hormones in adulthood.

SPS and castration each significantly increased the number of GR+ neurons in the PVN (Fig 9C). SPS increased GR expression in gonadally intact but not castrated males while castration increased GR expression in control but not SPS-exposed males, appearing to eliminate the effect of SPS in castrated males. These results indicate that testicular hormones normally regulate GR expression in the PVN, and depending on gonadal status, an effect of SPS on GR expression in the PVN may or may not be detected. However, T treatment did not reverse this effect of castration. Since other measures (e.g., Fig 9E-F, described below) confirm that the T capsules were effective, these data raise the question of whether other testicular factors regulate GR expression in the PVN.

While adult gonadal hormones had no effect on sucrose preference in males (Fig 9D), they did affect social interaction, in turn revealing a significant effect of SPS in GDX males. Castration of control males increased the latency to approach a novel male (Fig 9E) while decreasing the latency to approach the empty interaction chamber (Fig 9F), pushing both measures toward a more feminine phenotype in castrated males (Fig 9E, F). Consequently, an effect of SPS is detected in castrated males that is not apparent in gonadally intact males or castrates given T, suggesting that androgens in males may normally override an effect of SPS on social interaction. Body and adrenal weight in males were not affected by any treatment (Fig 16, 17).

Effects of SPS in females are largely independent of ovarian hormones. Regardless of ovarian hormone status, ASR is not influenced by SPS in females (Fig 10A). Adult T treatment also had no effect on the ASR in females, consistent with the lack of an effect of adult T on this measure in males (Fig 9A). The DEX suppression test revealed the same pattern of differences in CORT levels irrespective of gonadal status in females (Fig 10B). As expected, 30 min of acute restraint stress significantly increased the level of CORT in all vehicle-treated groups, although the level was significantly reduced in *both* groups of GDX control females (blank and T-treated) compared to sham control females. That T did not reverse the effect of ovariectomy suggests that ovarian hormones are involved in the blunted CORT response to restraint stress in GDX females. Importantly, the same low dose of DEX that blocked a significant increase in CORT in SPS-exposed males after 30 min of restraint stress did *not* block this increase in CORT in SPS-exposed females, whether GDX or treated with T. Nonetheless, DEX did significantly reduce baseline (0 min) CORT and the level of CORT induced by restraint stress in SPS-exposed females under all three hormonal conditions, indicating that DEX was working. As expected, T treatment significantly decreased baseline CORT levels in females (330). These data indicate that the enhanced CORT suppression characteristic of traumatized males is not a characteristic feature of the trauma response of females. This distinct sex difference in how trauma influences regulation of the negative feedback control of HPA axis of males and females is apparently independent of adult gonadal hormones in both sexes.

Interestingly, the effect of SPS on the number of GR+ neurons in the PVN depended on ovarian status. Without ovarian hormones, SPS had no effect on GR expression in the PVN as opposed to decreasing their number in gonadally-intact females (Fig 10C). Because ovariectomy *per se* does not affect the number of GR+ neurons in the PVN, ovarian hormones likely regulate how sensitive PVN neurons are to stress, with stress influencing GR expression in this brain region when ovarian hormones are present. SPS significantly and selectively reduced sucrose preference in sham operated females (Fig 10D), an effect that was reversed by ovariectomy. Because T did not reverse the effect of adult ovariectomy, estrogens and/or other ovarian factors likely mediate the effect of SPS on sucrose preference. On the other hand, the effect of SPS on social interaction did not depend on ovarian hormones, since SPS shortened the latency to approach a novel female, regardless of gonadal status (Fig 10E). T treatment, on the other hand, clearly reduced social interaction latency in general, eliminating the effect of SPS on this measure in GDX females. Latency to enter the interaction zone when it had an empty chamber was unaffected by SPS in females except in the presence of T, when approach time was increased by SPS compared to SPS-exposed females in the other two hormone groups (Fig 10F). Again, SPS did not affect body weight in females, but GDX led to significant increases in body weight (Fig 18), as expected (331). GDX had no effect on adrenal weight in females (Fig 19).

Discussion

Surprisingly, gonadal hormones had little role in these sex differences. This is particularly unexpected, given the current view that cycling estrogens robustly regulate stress susceptibility in females (332, 333). It seems these sex differences are determined earlier in life, predisposing individuals to certain endophenotypes of PTSD as adults. Indeed, adult gonadal hormones only partially modulate certain aspects of the stress response in rats, with neonatal organizational effects also at play (196). Individual differences in the stress response are apparent in rats at weaning and persist throughout life (334). We propose that such sex differences reflect differences in the underlying neurobiology, orchestrated earlier in life by sex hormones and/or genes.

Sex differences in the traumatic stress response were apparent at every level of analysis: traumatized males are hyper-responsive while traumatized females seem depressed. Traumatic stress

causes male rats to show an enhanced ASR while not affecting this same measure in females. Moreover, HPA negative feedback, which is enhanced in trauma-exposed males, is unaffected or even decreased by trauma in females, suggesting that the female trauma response may share common attributes with depression, characterized by a blunted ASR (335) and reduced HPA negative feedback (160, 261). We again included two common measures of depression, social interaction and sucrose preference. Each revealed effects of trauma in females but *not* males, further supporting the view that the female response to traumatic stress is distinct from that of males and is reminiscent of depression. While the overall pattern of effects of SPS in females points toward a depressive phenotype, the effects on sucrose preference was not consistent, indicating that this measure is only marginally sensitive to the effect of trauma in female rats and that the traumatic stress response in females is not equivalent to depression but is more affective in nature than in males.

Sex differences in the traumatic stress response were largely independent of adult circulating gonadal hormones. We find that increased ASR and exaggerated DEX suppression of CORT, characteristic of trauma-exposed males, do not depend on adult circulating gonadal hormones, as shown previously for ASR in males (336). These measures were also unaffected by gonadal hormones in females, further implicating the role for early life factors in laying the groundwork for sex differences in the trauma response. While sucrose preference was not affected by SPS or gonadal hormones in males, sucrose preference was affected by both in females. Because GDX did not alter sucrose preference in control rats, the effect of trauma on sucrose preference in females appears to be mediated by adult ovarian hormones. This could mean that fluctuating estrogen levels might influence the emergence and/or severity of depressive symptoms following exposure to trauma. However, whether ovarian hormones matter during and/or after experiencing trauma is entirely unanswered.

SPS affected social interaction only in females. Because stress effects on the latency to socially interact are sex-specific (337, 338), we chose this measure rather than the more typical measure of total social interaction time. Additionally, we normalized social interaction latency relative to latency to approach a non-social target, which serves the important function of controlling for individual differences in general inclination to move and explore, another sexually differentiated attribute (339), making this measure the more valid measure in assessing whether SPS affects social interaction in a sexually

differentiated manner. We are the first to test this measure in a traumatic stress paradigm and demonstrate that stress effects on this measure extend to traumatic stress, with effects only in females. This effect was also seen in GDX females, indicating that the effect of SPS on this measure is independent of adult ovarian hormones.

While SPS had no apparent effect on social interaction in gonadally intact males, castration did increase social interaction latency in control males, introducing an apparent effect of SPS to reduce latency in castrated males (Fig 9E). Whether the decreased latency to approach a novel rat reflects a genuine effect of SPS in castrates or an artifact of the effect of castration in control males is unclear. Likewise, SPS increased GR expression in the PVN of intact males, an effect lost in castrated males because castration increased GR expression in the PVN only of control males (Fig 9C). Thus, castration may have protected GR expression from trauma in the PVN of males, or may have simply masked the effect of trauma due to a ceiling effect. On the other hand, the effect of SPS to decrease GR expression in the PVN of females was not apparent after ovariectomy, but ovariectomy alone did not change the number of GR+ neurons in the PVN of non-traumatized females, indicating that the effect of SPS on this measure, like sucrose preference, depends on ovarian hormones in females. These data raise questions about the potential importance of gonadal hormones at the time of trauma versus at the time of assessment, an entirely unexplored problem in trauma research. It is clear that differences in gonadal hormones change the response to acute stress. Hence, such changes could also potentially reveal or mask an effect of *prior* trauma. While the effect of trauma on the ASR and DEX suppression of CORT appear independent of adult gonadal hormones, other measures like social interaction and sucrose preference may well depend on hormonal status, and could potentially lead to different conclusions about whether trauma exposure led to pathology.

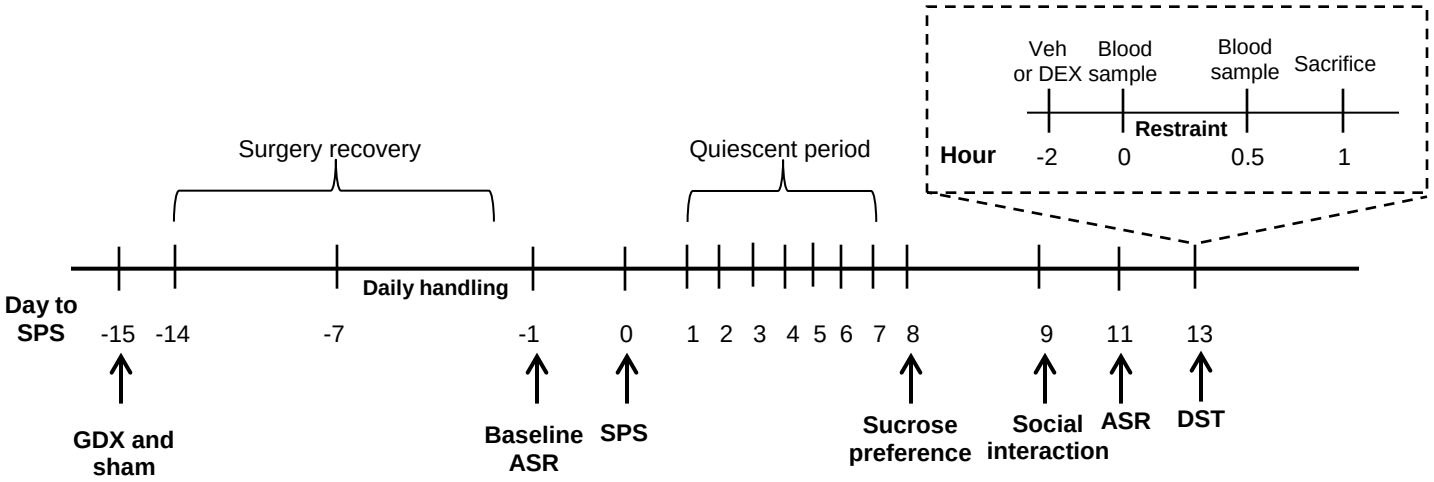

Figure 7. Timeline of procedures for experiment 5. Experimental timeline begins with GDX or sham surgery performed fifteen days before single prolonged stress (SPS), with daily handling one week before (-7) SPS and baseline acoustic startle response (ASR) testing the day before (-1) SPS. Rats are left undisturbed for one week after SPS, and sucrose preference is assessed 8 days later, social interaction 9 days later, post-stress ASR 11 days later, and dexamethasone suppression test (DST) 13 days later.

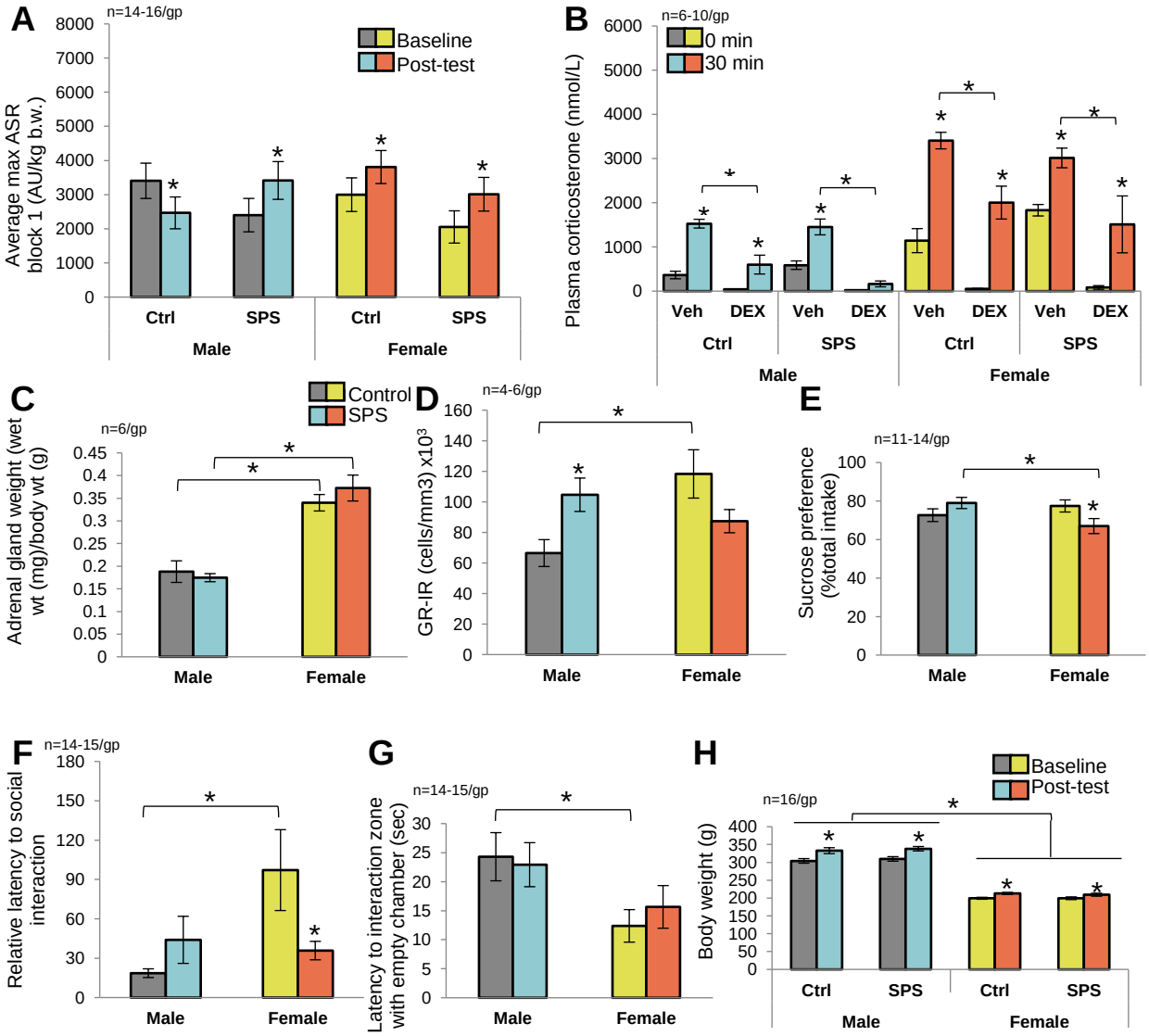

Figure 8. SPS elicits comparable sex differences in sham gonadectomized rats. (A) Despite prior exposure to anesthesia and sham gonadectomy, SPS enhanced the acoustic startle response (ASR) only in males, not females. While females showed a significant increase in the ASR in the post-test compared to baseline, this effect was not specific to SPS exposure as it is for males. Control males showed a habituation to the acoustic stimulus in the post-test compared to baseline, which was not seen in the previous study (Figure 1A). (B) As before, SPS enhanced the negative feedback control of the HPA axis in males, but not in females. Note that while DEX completely blocked the increase in CORT levels induced by acute restraint stress in SPS-exposed males, it did not block this increase in SPS-exposed females, nor did DEX block this increase in control males and females. SPS did increase *baseline* CORT levels in females but had no effect on baseline CORT in males. Again, our dose of DEX drove baseline CORT levels to near zero in all four groups, confirming its overall efficacy in both sexes. (C) The significantly higher CORT level in females than males was paralleled by a sex difference in adrenal weight, as females had significantly heavier adrenal glands than males. Note that SPS had no effect on adrenal weight in either sex. (D) The opposing effects of SPS on glucocorticoid receptor (GR) expression in the paraventricular nucleus of the hypothalamus (PVN) in males versus females was comparable to that seen in the previous study (Fig 1C), but this time, the effect of increasing GR expression in males was significant while the effect of decreasing GR expression in females fell short of significance (*P=.056*). As in the first study, control females had significantly more GR+ PVN neurons than control males. (E - G) SPS affected sucrose preference and social interaction *only* in females, significantly decreasing their preference for sucrose and latency to approach a novel conspecific, while having no effect on these measures in males. Control females also took longer than control males to approach a novel rat and this sex difference flipped for latency to approach the interaction zone. (H) SPS did not affect body weight, but females weighed less than males. Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 5 for full statistical results.

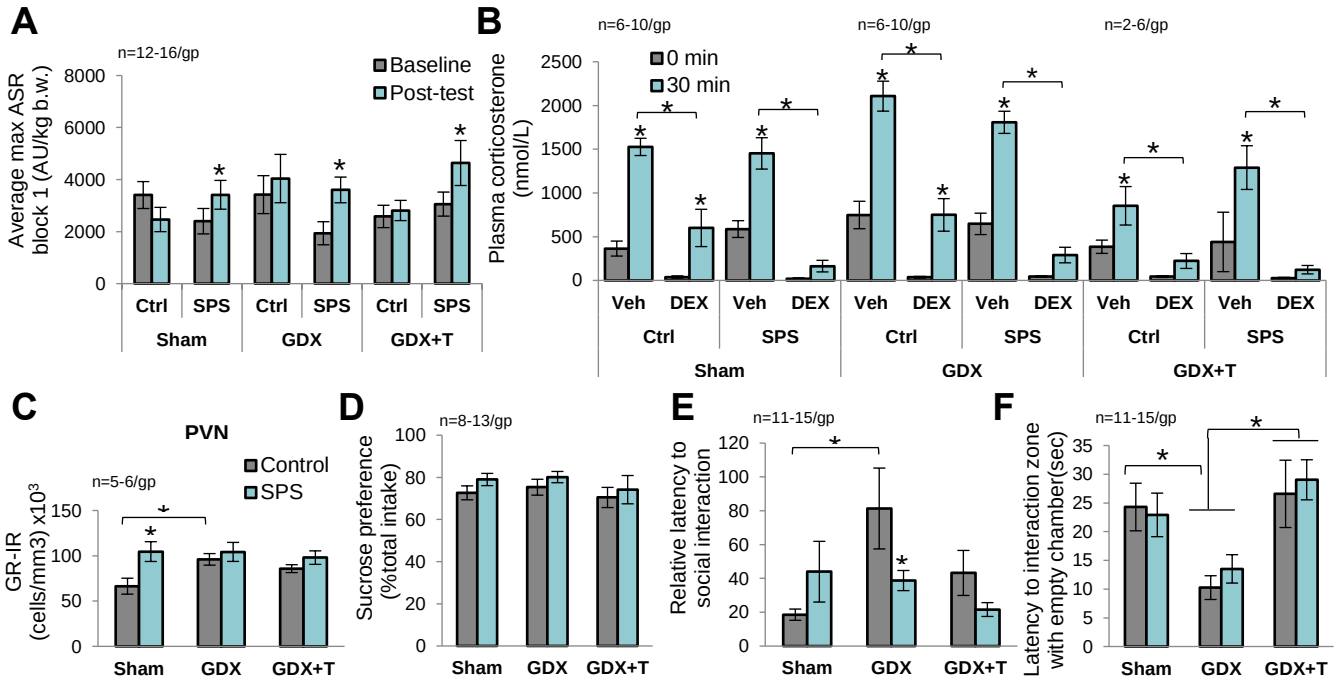

Figure 9. Effects of SPS in males are largely independent of adult testicular hormones. (A, B) The ability of SPS to enhance the ASR and negative feedback control of CORT levels was unaffected by gonadal hormone status in males. In each hormonal condition, DEX inhibited the rise in CORT levels after 30 min of restraint stress only for SPS males, with the single exception of control males that were gonadectomized and treated with testosterone (GDX+T), which may reflect the low number of individuals in the GDX+T group (n=2-6). Importantly, removal of the testes (GDX) did not change the pattern of differences seen in gonadally intact males (shams). (C) While SPS increased GR expression in the PVN of males, this effect was not apparent in GDX males because the number of GR+ PVN neurons increased by GDX in sham control males, presumably masking the effect of SPS. This effect was not reversed by T treatment, suggesting other testicular hormones may normally inhibit GR expression in the PVN. (D) Neither SPS nor gonadal hormone status affected sucrose preference in males. (E) Castration (GDX) of control males increased their latency to approach a novel rat, without similar influences on SPS males. Thus, SPS may have no genuine effect on this measure in males. These data highlight the difficult issue of gonadal hormones possibly masking real effects of trauma or spuriously introducing apparent effects of trauma, simply because baseline measures are sensitive to gonadal hormone levels independent of the trauma experience itself. The effect of GDX on this measure was reversed by T treatment. (F) SPS had no effect on latency to enter the interaction zone when it had an empty chamber, regardless of gonadal status, but GDX reduced this latency for both control and SPS-exposed males, an effect reversed with T replacement. These results suggest that T normally affects this measure independent of SPS exposure. Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 6 for full statistical results.

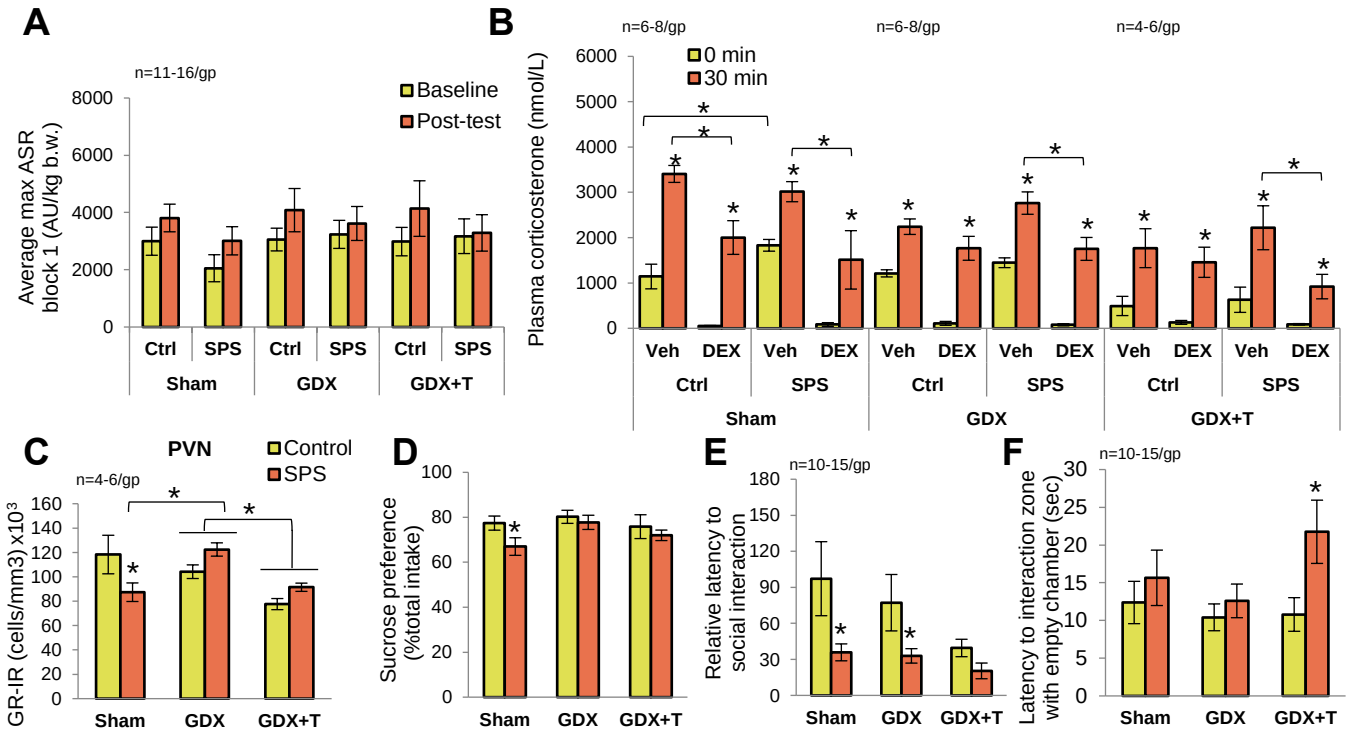

Figure 10. Effects of SPS in females are largely independent of ovarian hormones. (A, B) SPS again had no effect on ASR or negative feedback control of CORT levels in females, and this was true regardless of gonadal hormone status, neither enhancing ASR nor negative feedback of CORT levels, in contrast to the effects of SPS on males (Fig 4A,B). GDX did significantly decrease restraint-induced CORT levels in vehicle-treated control females, but T treatment did not reverse this effect, implicating ovarian hormones other than androgens. (C) SPS decreased GR expression in the PVN of gonadally intact (sham) females, an effect that was abolished by GDX. Given that this effect was specific to SPS exposure, ovarian hormones may mediate this response to trauma in the brain. However, what this means for the functional response to stress is not clear since corresponding changes were not seen in the DEX suppression test. T treatment lowered the number of GR+ neurons overall in the PVN, but did not restore the effect of SPS on GR expression in the PVN, suggesting that the effect of ovarian hormones on this measure is via estrogen or progesterone receptors and not androgen receptors. (D) While decreased sucrose preference was decreased by SPS in sham females, the effect of SPS was abolished by GDX implicating ovarian hormones in this female-specific effect of SPS. That T did not reverse the effect of GDX on sucrose preference suggests that ovarian hormones may act via estrogen and/or progesterone receptors to modulate the effect of SPS on sucrose preference. (E) SPS decreased the latency to approach a novel rat in both sham and GDX females, indicating that the effect of SPS on social interaction is independent of adult ovarian hormones. However, T treatment lowered the latency of control females to approach a social target, eliminating an apparent effect of SPS on this measure. Perhaps T acted as an anxiolytic to have this effect. (F) Latency to enter the interaction zone when it had an empty chamber was affected only by the combination of SPS and T treatment to significantly increase latency. Data are presented as mean±SEM. Significance set at $P<.05$ (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 7 for full statistical results.

CHAPTER 5: CONCLUSIONS

Factors that mediate differences in how individuals adjust after traumatic stress are attractive targets for the prevention and treatment of PTSD. At least 75% of people in the United States experience a traumatic event in their lifetime and do *not* develop PTSD (6), begging the question of *why some people who experience trauma develop PTSD while others do not?* The significant sex differences in the prevalence of stress-related disorders in humans is a call for inquiries of the factors behind such differences, which undoubtedly offer insight into the brain regions and mechanisms that underlie individual differences in the response to trauma stress. While psychological theories of PTSD give great insight into how trauma is experienced by people and how to address the effects of traumatic stress with psychotherapeutic approaches (340), underlying all psychological experiences are neurobiological substrates. The experiments described here indicate that neurobiological factors mediating the traumatic stress response are fundamentally different in males and females, challenging the assumption that what is known about PTSD in males can be readily applied to females.

This is not to suggest that there is a dichotomous “male PTSD” and “female PTSD,” but rather that sex accounts for some of the variability we see in the traumatic stress response with males more likely to exhibit certain phenotypes and females more likely to exhibit other phenotypes. The extent that adult circulating gonadal hormones mediate these sex differences is likely limited to specific aspects of the traumatic stress response such as social or affective symptoms, while the greatest effects are likely organized prior to adulthood. Activational effects of steroid hormones do not themselves regulate physiology or behavior, but rather they have “permissive, preparative, stimulating, and suppressive actions” that make certain outcomes more likely in certain contexts (e.g. “Testosterone does not cause aggression, it simply exaggerates the pre-existing pattern and response to environmental triggers of aggression.”) (341). That neither sex differences in PTSD nor any traumatic stress responses are dichotomously determined has implications for the identification of resilience vs. susceptibility factors.

Do sex differences in the traumatic stress response reflect differences in resilience?

Based on the ASR and DST, females might appear more resilient to the effects of traumatic stress than

males, but as we looked further, it became clear that females were not more resilient but rather, responded *differently* to trauma. Similarly, in response to uncontrollable footshocks, females appear more resilient than males to the negative effects of stress on learning that involves operant conditioning (342), but if classical conditioning is involved, females are less resilient than males (20), suggesting that sex *per se* may not be a factor that confers susceptibility or resilience to stress but simply leads to different responses, so that the choice of outcome measures may profoundly shape the conclusions drawn. Our data suggest that the line between resiliency and susceptibility may not be clear enough to separate individuals into these two groups as other stress studies have done (343), because perceived susceptibility can change depending on the measures used and the gonadal hormonal status of the individual (Fig 20). Resilience likely exists on a continuum that also depends on other factors, such that an individual may be resilient in one domain of functioning but not in another, or at one time during the lifespan but not another (344). While our data seem to question the heuristic value of the terms "resilience" and "susceptibility," these terms remain useful and valid. Not only does the epidemiological data tell us that only a small fraction of people exposed to trauma develop PTSD, indicating that differences in susceptibility are real, but also a growing list of gene polymorphisms and epigenetic states have been identified that appear to bias the nervous system toward more or less resilience to the negative effects of stress.

Future directions: developmental effects of trauma. The evidence presented here indicates that robust sex differences in rodent models of PTSD are largely independent of adult circulating gonadal hormones, which undoubtedly points to organizational effects earlier in development that predisposed males and females to respond differently to trauma. Indeed, most adult women with PTSD do not show exaggerated HPA negative feedback unless trauma occurred before puberty (290, 345, 346), and the increased risk for PTSD associated with low cortisol at the time of trauma is reversed in children (increased cortisol imparts increased risk in children) but only in boys, not girls (347). That the *DSM-5* recognized the child subtype of PTSD is a start to acknowledging the heterogeneity in the traumatic stress response across development and across sexes. Converging at the intersection of sex and nervous system development will undoubtedly lie valuable answers to questions regarding the

heterogeneity of the traumatic stress response that will advance efforts in the prevention, diagnosis, and treatment of PTSD.

APPENDIX

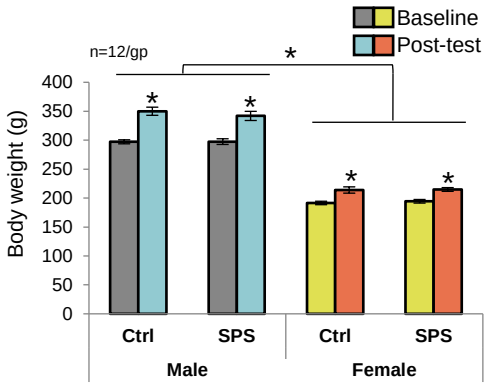

Figure 11. SPS did not affect body weight. Rats in all groups showed a significant gain in body weight between the two time points regardless of traumatic stress exposure. As expected, females weighed less than males. Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 2 for full statistical results.

Table 2. Statistical results for data shown in Figures 2 and 11. All pairwise comparisons use Bonferroni adjustment for multiple comparisons. RM denotes repeated measure; otherwise assume between group measures. Only statistically significant results are shown**.**

Outcome measure	*Statistical test*	*Significant effects*	*p value*	*Power (α=0.05)*
ASR (Fig. 2A)	RM 3-way ANOVA (stress*sex*time)	Interaction: stress*time	0.043	0.529
		Pairwise: SPS male baseline v. post-test	0.017	0.683
DST (Fig. 2B)	RM 4-way ANOVA (stress*sex*time*DEX)	Main effect: time	<0.0001	1.000
		Main effect: DEX	<0.0001	1.000
		Main effect: sex	<0.0001	1.000
		Interaction: sex*time	<0.0001	1.000
		Interaction: sex*time*DEX	0.004	0.835
		Interaction: sex*time*DEX*stress	0.037	0.557
		Pairwise: SPS male time 2 DEX v. veh	0.022	0.639
		Pairwise: SPS female time 1 DEX v. veh	<0.0001	0.999
		Pairwise: control male time 1 DEX v. veh	0.05	0.506
		Pairwise: control female time 1 DEX v. veh	<0.0001	0.995
		Pairwise: control female time 2 DEX v. veh	0.019	0.663
		Pairwise: SPS DEX time 2 male v. female	<0.0001	1.000
		Pairwise: SPS vehicle time 1 male v. female	0.001	0.953
		Pairwise: SPS vehicle time 2 male v. female	<0.0001	0.974
		Pairwise: control DEX time 2 male v. female	<0.0001	0.977
		Pairwise: control vehicle time 1 male v. female	0.006	0.802
		Pairwise: control vehicle time 2 male v. female	<0.0001	1.000
		Pairwise: SPS DEX female time 1 v. 2	<0.0001	1.000
		Pairwise: SPS vehicle male time 1 v. 2	0.002	0.886
		Pairwise: SPS vehicle female time 1 v. 2	<0.0001	1.000
		Pairwise: control DEX male time 1 v. 2	0.019	0.665
		Pairwise: control DEX female time 1 v. 2	<0.0001	1.000
		Pairwise: control vehicle male time 1 v. 2	0.001	0.943
		Pairwise: control vehicle female time 1 v. 2	<0.0001	1.000

Table 2 (cont'd).

PVN GR (Fig. 2C)	2-way ANOVA (stress*sex)	Main effect: stress	0.040	0.553
		Main effect: sex	<0.0001	1.000
		Interaction: stress*sex	<0.0001	0.998
		Pairwise: control male v. female	<0.0001	1.000
		Pairwise: female SPS v. control	<0.0001	0.999
		Pairwise: male SPS v. control	0.060	0.477
PrL cFos (Fig. 2D)	2-way ANOVA (stress*sex)	Interaction: stress*sex	0.002	0.916
		Pairwise: SPS male v. female	0.040	0.552
		Pairwise: control male v. female	0.011	0.754
		Pairwise: female SPS v. control	0.001	0.960
IL cFos (Fig. 2D)	2-way ANOVA (stress*sex)	Main effect: stress	0.030	0.603
		Interaction: stress*sex	0.003	0.891
		Pairwise: SPS male v. female	0.018	0.688
		Pairwise: control male v. female	0.042	0.542
		Pairwise: female SPS v. control	0.001	0.974
BLA cFos (Fig. 2E)	3-way ANOVA (stress*sex*side)	Main effect: stress	0.015	0.697
		Pairwise: female SPS left v. right	0.029	0.602
		Pairwise: female right SPS v. control	0.021	0.650
MeA cFos (Fig. 2F)	2-way ANOVA (stress*sex)	None		
CA1/2 GR (Fig. 2G)	2-way ANOVA (stress*sex)	Interaction: sex*group	0.050	0.517
Body wt. (Fig. 10)	RM 3-way ANOVA (stress*sex*time)	Main effect: sex	<0.0001	1.000
		Main effect: time	<0.0001	1.000
		Interaction: time*sex	<0.0001	1.000
		Pairwise: SPS time 1 male v. female	<0.0001	1.000
		Pairwise: SPS time 2 male v. female	<0.0001	1.000
		Pairwise: Control time 1 male v. female	<0.0001	1.000
		Pairwise: Control time 2 male v. female	<0.0001	1.000
		Pairwise: SPS male time 1 v. 2	<0.0001	1.000
		Pairwise: Control male time 1 v. 2	<0.0001	1.000
		Pairwise: SPS female time 1 v. 2	<0.0001	1.000
		Pairwise: Control female time 1 v. 2	<0.0001	1.000

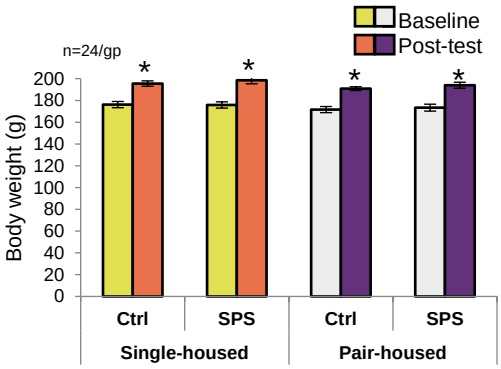

Figure 12. Neither SPS nor housing affected female body weight. Rats in all groups showed a significant gain in body weight between the two time points regardless of traumatic stress exposure. Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 3 for full statistical results.

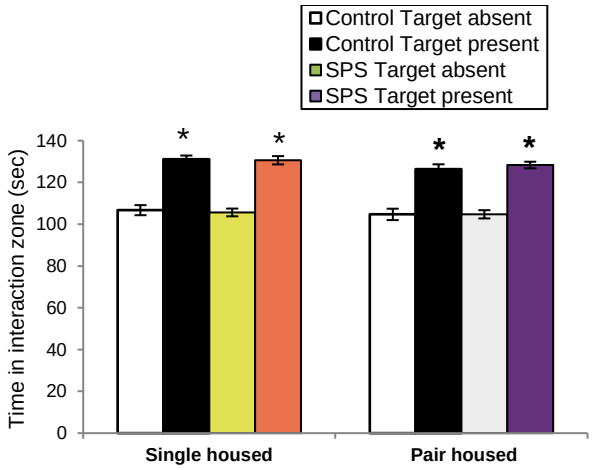

Figure 13. Females spent more total time in the interaction zone when there was a target rat present compared to an empty chamber, regardless of SPS exposure or housing. Main effect of target ($p<0.0001$). Data are presented as mean±SEM. Significance set at $P<.05$ (indicated by asterisk) for planned pairwise comparisons (Bonferroni).

Table 3. Statistical results for data shown in Figures 3 and 12. All pairwise comparisons use Bonferroni adjustment for multiple comparisons. RM denotes repeated measure; otherwise assume between group measures. Only statistically significant results are shown.

Outcome measure	*Statistical test*	*Significant effects*	*p value*	*Power (α=0.05)*
ASR (Fig. 3A)	RM 3-way ANOVA (stress*housing*time)	None		
DST (Fig. 3B)	RM 4-way ANOVA (stress*housing*time*DEX)	Main effect: time	<0.0001	1.000
		Main effect: stress	0.013	0.712
		Main effect: DEX	<0.0001	1.000
		Interaction: time*stress	0.024	0.623
Because there was no effect of housing on DST, a separate 3-way ANOVA was run for single- and pair-housed females				
DST, single-housed (Fig. 3B)	RM 3-way ANOVA (stress*time*DEX)	Main effect: time	<0.0001	1.000
		Main effect: DEX	<0.0001	0.999
		Pairwise: SPS time 1 DEX v. veh	<0.0001	0.999
		Pairwise: control time 1 DEX v. veh	<0.0001	1.000
		Pairwise: SPS DEX time 1 v. 2	<0.0001	0.984
		Pairwise: SPS vehicle time 1 v. 2	<0.0001	0.980
		Pairwise: control DEX time 1 v. 2	<0.0001	1.000
		Pairwise: control vehicle time 1 v. 2	<0.0001	0.995
DST, pair-housed (Fig. 3B)	RM 3-way ANOVA (stress*time*DEX)	Main effect: time	<0.0001	1.000
		Main effect: DEX	<0.0001	1.000
		Pairwise: DEX time 2 SPS v. control	0.020	0.654
		Pairwise: SPS time 1 DEX v. veh	<0.0001	1.000
		Pairwise: SPS time 2 DEX v. veh	<0.0001	0.984
		Pairwise: control time 1 DEX v. veh	<0.0001	1.000
		Pairwise: control time 2 DEX v. veh	0.032	0.585
		Pairwise: SPS DEX time 1 v. 2	0.007	0.788
		Pairwise: SPS vehicle time 1 v. 2	<0.0001	0.999
		Pairwise: control DEX time 1 v. 2	<0.0001	1.000
		Pairwise: control vehicle time 1 v. 2	<0.0001	1.000

Table 3 (cont'd).

Sucrose pref. (Fig. 3C)	2-way ANOVA (stress*housing)	None		
Social interaction (Fig. 3D)	2-way ANOVA (stress*housing)	Interaction: stress*housing	0.001	0.902
		Pairwise: pair-housed SPS v. control	0.009	0.751
		Pairwise: single-housed SPS v. control	0.049	0.505
		Pairwise: SPS pair-housed v. single-housed	0.009	0.758
Latency/empty zone (Fig. 3E)	2-way ANOVA (stress*housing)	None		
Body wt. (Fig. 11)	RM 3-way ANOVA (stress*housing*time)	Main effect: time	<0.0001	1.000
		Pairwise: SPS single-housed time 1 v. 2	<0.0001	1.000
		Pairwise: Control single-housed time 1 v. 2	<0.0001	1.000
		Pairwise: SPS pair-housed time 1 v. 2	<0.0001	1.000
		Pairwise: Control pair-housed time 1 v. 2	<0.0001	1.000

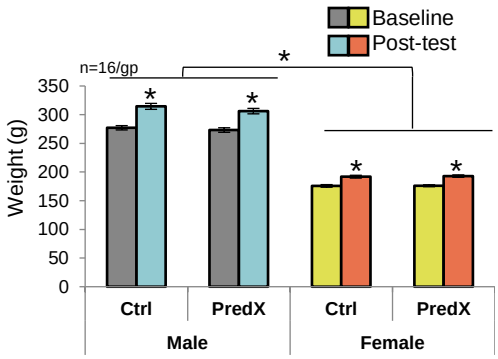

Figure 14. As with SPS, predator exposure (PredX) did not affect body weight, but females weighed less than males. Likewise, rats in all groups showed a significant gain in body weight regardless of traumatic stress exposure or sex. Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 4 for full statistical results.

Table 4. Statistical results for data shown in Figures 6 and 14. All pairwise comparisons use Bonferroni adjustment for multiple comparisons. RM denotes repeated measure; otherwise assume between group measures. Only statistically significant results are shown.

Outcome measure	*Statistical test*	*Significant effects*	*p value*	*Power (α=0.05)*
ASR (Fig. 6A)	RM 3-way ANOVA (stress*sex*time)	Interaction: stress*sex*time	0.034	0.570
		Pairwise: SPS male time 1 v. 2	0.016	0.688
DST (Fig. 6B)	RM 4-way ANOVA (stress*sex*time*DEX)	Main effect: time	<0.0001	1.000
		Main effect: sex	<0.0001	1.000
		Main effect: DEX	<0.0001	1.000
		Interaction: sex*time	<0.0001	0.998
		Pairwise: PredX DEX time 2 male v. female	<0.0001	0.995
		Pairwise: PredX vehicle time 1 male v. female	<0.0001	0.992
		Pairwise: PredX vehicle time 2 male v. female	<0.0001	0.984
		Pairwise: control DEX time 2 male v. female	0.007	0.784
		Pairwise: control vehicle time 1 male v. female	0.012	0.730
		Pairwise: control vehicle time 2 male v. female	0.001	0.918
		Pairwise: Male PredX time 1 DEX v. veh	0.042	0.537
		Pairwise: Male PredX time 2 DEX v. veh	0.010	0.752
		Pairwise: Male control time 1 DEX v. veh	0.010	0.755
		Pairwise: female PredX time 1 DEX v. veh	<0.0001	1.000
		Pairwise: female control time 1 DEX v. veh	<0.0001	0.994
		Pairwise: Male PredX veh time 1 v. 2	0.006	0.801
		Pairwise: Male control vehicle time 1 v. 2	0.043	0.532
		Pairwise: female PredX DEX time 1 v. 2	<0.0001	1.000
		Pairwise: female PredX vehicle time 1 v. 2	<0.0001	0.999
		Pairwise: female control DEX time 1 v. 2	<0.0001	0.999
		Pairwise: female control vehicle time 1 v. 2	<0.0001	0.979
PVN GR (Fig. 6C)	2-way ANOVA (stress*sex)	Main effect: sex	0.012	0.756
		Pairwise: Control male v. female	0.028	0.621
Body wt. (Fig. 13)	RM 3-way ANOVA (stress*sex*time)	Main effect: sex	<0.0001	1.000

Table 4 (cont'd).

		Main effect: time	<0.0001	1.000
		Interaction: time*sex	<0.0001	1.000
		Pairwise: PredX time 1 male v. female	<0.0001	1.000
		Pairwise: PredX time 2 male v. female	<0.0001	1.000
		Pairwise: Control time 1 male v. female	<0.0001	1.000
		Pairwise: Control time 2 male v. female	<0.0001	1.000
		Pairwise: PredX male time 1 v. 2	<0.0001	1.000
		Pairwise: Control male time 1 v. 2	<0.0001	1.000
		Pairwise: PredX female time 1 v. 2	<0.0001	1.000
		Pairwise: Control female time 1 v. 2	<0.0001	1.000

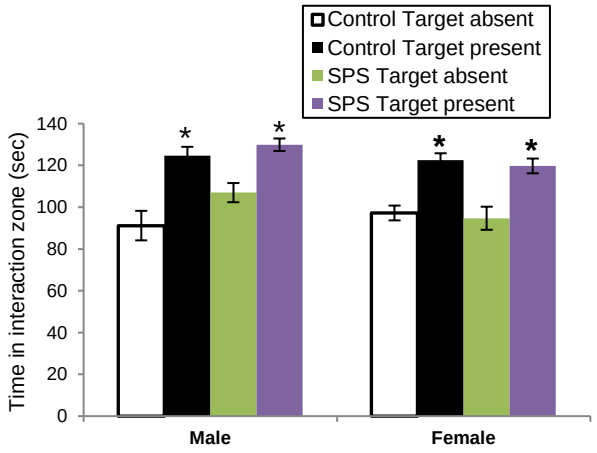

Figure 15. Rats spent more total time in the interaction zone when there was a target rat present compared to an empty chamber, regardless of SPS exposure or sex. Main effect of target ($p<0.0001$). Data are presented as mean±SEM. Significance set at $P<.05$ (indicated by asterisk) for planned pairwise comparisons (Bonferroni).

Table 5. Statistical results for data shown in Figure 8. All pairwise comparisons use Bonferroni adjustment for multiple comparisons. RM denotes repeated measure; otherwise assume between group measures. Only statistically significant results are shown.

Outcome measure	*Statistical test*	*Significant effects*	*p value*	*Power (α=0.05)*
ASR (Fig. 8A)	RM 3-way ANOVA (stress*sex*time)	Main effect: time	0.020	0.655
		Interaction: stress*time	0.008	0.767
		Interaction: sex*time	0.032	0.582
		Interaction: stress*sex*time	0.022	0.639
		Pairwise: SPS male time 1 v. 2	0.008	0.767
		Pairwise: SPS female time 1 v. 2	0.019	0.660
		Pairwise: control male time 1 v. 2	0.021	0.644
		Pairwise: control female time 1 v. 2	0.033	0.575
DST (Fig. 8B)	RM 4-way ANOVA (stress*sex*time*DEX)	Main effect: time	<0.0001	1.000
		Main effect: DEX	<0.0001	1.000
		Main effect: sex	<0.0001	1.000
		Interaction: stress*time	0.005	0.814
		Interaction: sex*time	<0.0001	0.999
		Interaction: sex*DEX	0.004	0.840
		Pairwise: veh female time 1 SPS v. control	<0.0001	0.969
		Pairwise: SPS male time 1 DEX v. veh	0.002	0.887
		Pairwise: SPS male time 2 DEX v. veh	0.001	0.914
		Pairwise: SPS female time 1 DEX v. veh	<0.0001	1.000
		Pairwise: SPS female time 2 DEX v. veh	0.001	0.950
		Pairwise: control male time 1 DEX v. veh	0.048	0.512
		Pairwise: control male time 2 DEX v. veh	0.010	0.746
		Pairwise: control female time 1 DEX v. veh	<0.0001	1.000
		Pairwise: control female time 2 DEX v. veh	0.001	0.951
		Pairwise: SPS DEX time 2 male v. female	0.003	0.875
		Pairwise: SPS vehicle time 1 male v. female	<0.0001	1.000

Table 5 (cont'd).

		Pairwise: SPS vehicle time 2 male v. female	<0.0001	0.988
		Pairwise: control DEX time 2 male v. female	<0.0001	0.960
		Pairwise: control vehicle time 1 male v. female	<0.0001	0.996
		Pairwise: control vehicle time 2 male v. female	<0.0001	0.999
		Pairwise: SPS DEX female time 1 v. 2	<0.0001	0.993
		Pairwise: SPS vehicle male time 1 v. 2	0.001	0.935
		Pairwise: SPS vehicle female time 1 v. 2	<0.0001	0.978
		Pairwise: control DEX male time 1 v. 2	0.034	0.571
		Pairwise: control DEX female time 1 v. 2	<0.0001	1.000
		Pairwise: control vehicle male time 1 v. 2	<0.0001	0.993
		Pairwise: control vehicle female time 1 v. 2	<0.0001	1.000
Adrenal wt (Fig. 8C)	2-way ANOVA (stress*sex)	Main effect: sex	<0.0001	1.000
		Pairwise: SPS male v. female	<0.0001	1.000
		Pairwise: control male v. female	<0.0001	0.998
PVN GR (Fig. 8D)	2-way ANOVA (stress*sex)	Interaction: stress*sex	0.005	0.869
		Pairwise: control male v. female	0.004	0.876
		Pairwise: male SPS v. control	0.020	0.490
		Pairwise: female SPS v. control	0.056	0.682
Sucrose pref. (Fig. 8E)	2-way ANOVA (stress*sex)	Interaction: stress*sex	0.015	0.699
		Pairwise: female SPS v. control	0.034	0.570
		Pairwise: SPS male v. female	0.017	0.675
Social interaction (Fig. 8F)	2-way ANOVA (stress*sex)	Interaction: stress*sex	0.023	0.632
		Pairwise: female SPS v. control	0.023	0.632
		Pairwise: control female v. male	0.004	0.849
Latency/empty zone (Fig. 8G)	2-way ANOVA (stress*sex)	Main effect: sex	0.011	0.736
		Pairwise: control male v. female	0.022	0.639
Body weight (Fig. 8H)	RM 3-way ANOVA (stress*sex*time)	Main effect: sex	<0.0001	1.000
		Main effect: time	<0.0001	1.000
		Interaction: time*sex	<0.0001	1.000

Table 5 (cont'd).

		Pairwise: SPS time 1 male v. female	<0.0001	1.000
		Pairwise: SPS time 2 male v. female	<0.0001	1.000
		Pairwise: Control time 1 male v. female	<0.0001	1.000
		Pairwise: Control time 2 male v. female	<0.0001	1.000
		Pairwise: SPS male time 1 v. 2	<0.0001	1.000
		Pairwise: Control male time 1 v. 2	<0.0001	1.000
		Pairwise: SPS female time 1 v. 2	<0.0001	0.911
		Pairwise: Control female time 1 v. 2	<0.0001	0.994

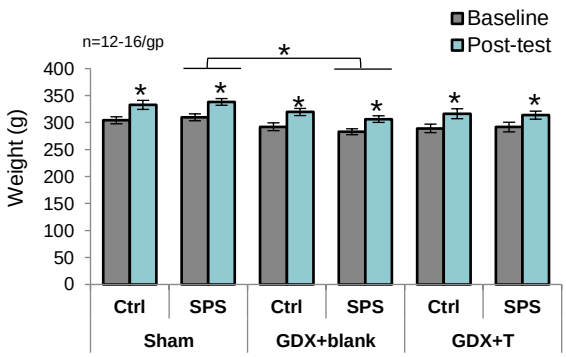

Figure 16. Neither SPS nor castration (GDX) affected body weight of males. Behind a main effect of GDX was a significant lower body weight in SPS castrated males compared to SPS sham males, but this effect was present at baseline before any testing, indicating that particular group had smaller males independently of surgery or SPS. Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 6 for full statistical results.

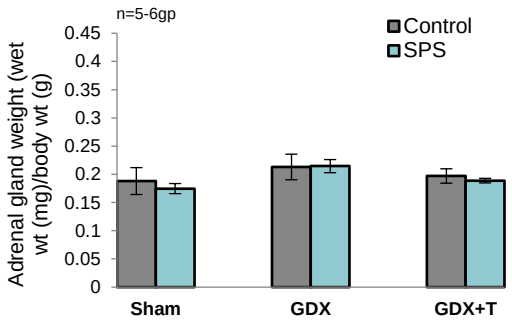

Figure 17. Neither SPS nor GDX affected adrenal weight of males. Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 6 for full statistical results.

Table 6. Statistical results for data shown in Figures 9 and 16-17. All pairwise comparisons use Bonferroni adjustment for multiple comparisons. RM denotes repeated measure; otherwise assume between group measures. Only statistically significant results are shown.

Outcome measure	*Statistical test*	*Significant effects*	*p value*	*Power (α=0.05)*
ASR (Fig. 9A)	RM 3-way ANOVA (stress*GDX*time) sham v. GDX	Main effect: time	0.016	0.684
		Interaction: stress*time	0.003	0.874
		Interaction: GDX*time	0.024	0.627
		Pairwise: SPS sham time 1 v. time 2	0.027	0.607
		Pairwise: SPS GDX time 1 v. time 2	0.001	0.910
	RM 3-way ANOVA (stress*GDX*time) GDX v. GDX+T	Main effect: time	0.001	0.93
		Interaction: stress*time	0.046	0.52
		Pairwise: SPS GDX time 1 v. time 2	0.005	0.817
		Pairwise: SPS GDX+T time 1 v. time 2	0.011	0.741
DST (Fig. 9B)	RM 4-way ANOVA stress*GDX*time*DEX sham v. GDX	Main effect: time	<0.0001	1.000
		Main effect: stress	0.055	0.485
		Main effect: DEX	<0.0001	1.000
		Main effect: GDX	0.006	0.795
		Interaction: stress*time	0.007	0.783
		Interaction: DEX*time	<0.0001	1.000
		Pairwise: SPS sham time 1 DEX v. veh	<0.0001	0.974
		Pairwise: SPS sham time 2 DEX v. veh	<0.0001	0.999
		Pairwise: SPS GDX time 1 DEX v. veh	<0.0001	0.982
		Pairwise: SPS GDX time 2 DEX v. veh	<0.0001	1.000
		Pairwise: control sham time 1 DEX v. veh	0.015	0.689
		Pairwise: control sham time 2 DEX v. veh	<0.0001	0.980
		Pairwise: control GDX time 1 DEX v. veh	<0.0001	1.000
		Pairwise: control GDX time 2 DEX v. veh	<0.0001	1.000
		Pairwise: control vehicle time 1 sham v. GDX	0.004	0.842

Table 6 (cont'd).

		Pairwise: control vehicle time 2 sham v. GDX	0.011	0.733
		Pairwise: SPS vehicle sham time 1 v. time 2	<0.0001	1.000
		Pairwise: SPS vehicle GDX time 1 v. time 2	<0.0001	1.000
		Pairwise: control DEX sham time 1 v. time 2	0.001	0.909
		Pairwise: control DEX GDX time 1 v. time 2	<0.0001	0.993
		Pairwise: control vehicle sham time 1 v. time 2	<0.0001	1.000
		Pairwise: control vehicle GDX time 1 v. time 2	<0.0001	1.000
	RM 4-way ANOVA stress*GDX*time*DEX GDX v. GDX+T	Main effect: time	<0.0001	1.000
		Main effect: DEX	<0.0001	1.000
		Main effect: GDX	<0.0001	0.979
		Interaction: DEX*time	<0.0001	0.989
		Interaction: GDX*time	0.003	0.867
		Interaction: DEX*GDX	0.033	0.574
		Pairwise: SPS GDX time 1 DEX v. veh	<0.0001	0.970
		Pairwise: SPS GDX time 2 DEX v. veh	<0.0001	1.000
		Pairwise: SPS GDX+T time 2 Dex v. veh	0.004	0.835
		Pairwise: control GDX time 1 DEX v. veh	<0.0001	1.000
		Pairwise: control GDX time 2 DEX v. veh	<0.0001	1.000
		Pairwise: control GDX+T time 2 DEX v. veh	0.025	0.621
		Pairwise: control DEX time 2 GDX v. GDX+T	0.038	0.553
		Pairwise: control veh time 1 GDX v. GDX+T	0.020	0.657
		Pairwise: control veh time 2 GDX v. GDX+T	<0.0001	1.000
		Pairwise: SPS veh GDX time 1 v. time 2	<0.0001	1.000
		Pairwise: SPS veh GDX+T time 1 v. time 2	0.015	0.697
		Pairwise: control DEX GDX time 1 v. time 2	<0.0001	0.996
		Pairwise: control veh GDX time 1 v. time 2	<0.0001	1.000

Table 6 (cont'd).

		Pairwise: control veh GDX+T time 1 v. time 2	0.020	0.656
PVN (Fig. 9C)	2-way ANOVA (stress*GDX) sham v. GDX	Main effect: stress	0.021	0.670
		Pairwise: control GDX v. sham	0.031	0.601
		Pairwise: sham SPS v. control	0.010	0.780
	2-way ANOVA (stress*GDX) GDX v. GDX+T	None		
Sucrose (Fig. 9D)	2-way ANOVA (stress*GDX) sham v. GDX	None		
	2-way ANOVA (stress*GDX) GDX v. GDX+T	None		
Social int. (Fig. 9E)	2-way ANOVA (stress*GDX) sham v. GDX	Main effect: GDX	0.056	0.485
		Interaction: stress*GDX	0.025	0.623
		Pairwise: GDX SPS v. control	0.049	0.507
		Pairwise: control sham v. GDX	0.004	0.842
	2-way ANOVA (stress*GDX) GDX v. GDX+T	Main effect: stress	0.034	0.573
		Main effect: GDX	0.066	0.454
		Pairwise: GDX SPS v. control	0.035	0.564
Latency/empty zone (Fig. 9F)	2-way ANOVA (stress*GDX) sham v. GDX	Main effect: GDX	0.001	0.934
		Pairwise: control sham v. GDX	0.050	0.503
	2-way ANOVA (stress*GDX) GDX v. GDX+T	Main effect: GDX	<0.0001	0.992
		Pairwise: SPS GDX v. GDX+T	0.003	0.861
		Pairwise: control GDX v. GDX+T	0.002	0.883
Body weight (Fig. 15)	RM 3-way ANOVA (stress*GDX*time) sham v. GDX	Main effect: GDX	0.002	0.887
		Main effect: time	<0.0001	1.000
		Pairwise: SPS time 1 sham v. GDX	0.005	0.815
		Pairwise: SPS time 2 sham v. GDX	0.002	0.887
		Pairwise: SPS sham time 1 v. 2	<0.0001	1.000

Table 6 (cont'd).

		Pairwise: SPS GDX time 1 v. 2	<0.0001	1.000
		Pairwise: control sham time 1 v. 2	<0.0001	1.000
		Pairwise: control GDX time 1 v. 2	<0.0001	1.000
	RM 3-way ANOVA (stress*GDX*time) GDX v. GDX+T	Main effect: time	<0.0001	1.000
		Pairwise: SPS GDX time 1 v. 2	<0.0001	1.000
		Pairwise: SPS GDX+T time 1 v. 2	<0.0001	1.000
		Pairwise: control GDX time 1 v. 2	<0.0001	1.000
		Pairwise: control GDX+T time 1 v. 2	<0.0001	1.000
Adrenal weight (Fig 17)	RM 3-way ANOVA (stress*GDX*time) sham v. GDX	None		
	RM 3-way ANOVA (stress*GDX*time) GDX v. GDX+T	None		

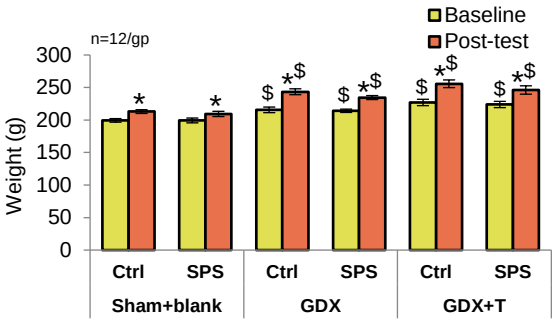

Figure 18. SPS did not affect the body weight of females, but as expected, GDX did by increasing the body weight of ovariectomized females. Interestingly, the effect of GDX was not reversed by testosterone (T) treatment. Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by * or $) for planned pairwise comparisons (Bonferroni). *, vs baseline same group; $, vs sham same group. Refer to Table 7 for full statistical results.

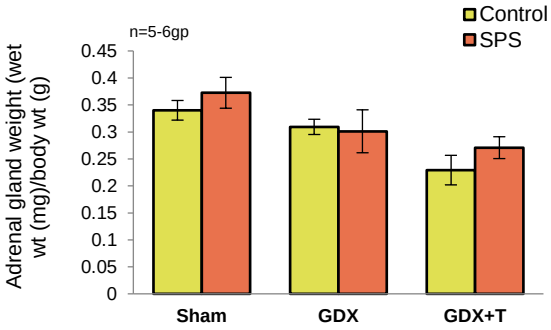

Figure 19. Neither SPS nor GDX affected adrenal weight of females. Data are presented as mean±SEM. Significance set at *P*<.05 (indicated by asterisk) for planned pairwise comparisons (Bonferroni). Refer to Table 7 for full statistical results.

Table 7. Statistical results for data shown in Figures 10 and 18-19. All pairwise comparisons use Bonferroni adjustment for multiple comparisons. RM denotes repeated measure; otherwise assume between group measures. Only statistically significant results are shown.

Outcome measure	*Statistical test*	*Significant effects*	*p value*	*Power (α=0.05)*
ASR (Fig. 10A)	RM 3-way ANOVA (stress*GDX*time) sham vs GDX	Main effect: time	<0.0001	0.972
		Pairwise: SPS sham time 1 v. 2	0.024	0.625
		Pairwise: Control sham time 1 v. 2	0.040	0.541
		Pairwise: Control GDX time 1 v. 2	0.013	0.715
	RM 3-way ANOVA (stress*GDX*time) GDX v. GDX+T	Main effect: time	<0.0001	0.705
		Pairwise: Control GDX time 1 v. 2	0.037	0.555
		Pairwise: Control GDX+T time 1 v. 2	0.045	0.524
DST (Fig. 10B)	RM 4-way ANOVA (stress*GDX*time*DEX) sham v. GDX	Main effect: time	<0.0001	1.000
		Main effect: DEX	<0.0001	1.000
		Interaction: stress*time*GDX	0.044	0.526
		Pairwise: vehicle sham time 1 SPS v. control	<0.0001	0.962
		Pairwise: SPS sham time 1 DEX v. veh	<0.0001	1.000
		Pairwise: SPS sham time 2 DEX v. veh	0.002	0.897
		Pairwise: SPS GDX time 1 DEX v. veh	<0.0001	1.000
		Pairwise: SPS GDX time 2 DEX v. veh	0.027	0.608
		Pairwise: control sham time 1 DEX v. veh	<0.0001	1.000
		Pairwise: control sham time 2 DEX v. veh	0.002	0.899
		Pairwise: control GDX time 1 DEX v. veh	<0.0001	1.000
		Pairwise: SPS vehicle time 1 sham v. GDX	0.038	0.550
		Pairwise: control vehicle time 2 sham v. GDX	0.012	0.730
		Pairwise: SPS DEX sham time 1 v. time 2	<0.0001	0.979
		Pairwise: SPS DEX GDX time 1 v. time 2	<0.0001	0.997
		Pairwise: SPS vehicle sham time 1 v. time 2	0.001	0.946

Table 7 (cont'd).

		Pairwise: SPS vehicle GDX time 1 v. time 2	<0.0001	0.989
		Pairwise: control DEX sham time 1 v. time 2	<0.0001	1.000
		Pairwise: control DEX GDX time 1 v. time 2	<0.0001	1.000
		Pairwise: control vehicle sham time 1 v. time 2	<0.0001	1.000
		Pairwise: control vehicle GDX time 1 v. time 2	0.005	0.821
	RM 4-way ANOVA stress*GDX*time*DEX GDX vs GDX+T	Main effect: time	<0.0001	1.000
		Main effect: DEX	<0.0001	1.000
		Main effect: GDX	0.001	0.926
		Interaction: time*DEX*GDX	0.041	0.539
		Pairwise: SPS GDX time 1 DEX v. veh	<0.0001	1.000
		Pairwise: SPS GDX time 2 DEX v. veh	0.015	0.697
		Pairwise: SPS GDX+T time 1 Dex v. veh	0.004	0.850
		Pairwise: SPS GDX+T time 2 Dex v. veh	0.008	0.775
		Pairwise: control GDX time 1 DEX v. veh	<0.0001	1.000
		Pairwise: control GDX+T time 1 Dex v. veh	0.051	0.502
		Pairwise: SPS vehicle time 1 GDX v. GDX+1	<0.0001	0.999
		Pairwise: control veh time 1 GDX v. GDX+T	<0.0001	0.974
		Pairwise: SPS DEX GDX time 1 v. time 2	<0.0001	1.000
		Pairwise: SPS DEX GDX+T time 1 v. time 2	0.009	0.764
		Pairwise: SPS veh GDX time 1 v. time 2	<0.0001	1.000
		Pairwise: SPS veh GDX+T time 1 v. time 2	<0.0001	0.999
		Pairwise: control DEX GDX time 1 v. time 2	<0.0001	1.000
		Pairwise: control DEX GDX+T time 1 v. time 2	<0.0001	0.997
		Pairwise: control veh GDX time 1 v. time 2	0.001	0.953
		Pairwise: control veh GDX+T time 1 v. time 2	0.001	0.957

Table 7 (cont'd).

PVN (Fig. 10C)	2-way ANOVA (stress*GDX) sham v. GDX	Interaction: stress*GDX	0.010	0.780
		Pairwise: SPS GDX v. sham	0.006	0.848
		Pairwise: sham SPS v. control	0.023	0.654
	2-way ANOVA (stress*GDX) GDX v. GDX+T	Main effect: GDX	<0.0001	0.999
		Main effect: stress	0.007	0.834
		Pairwise: SPS GDX v. GDX+T	0.001	0.977
		Pairwise: control GDX v. GDX+T	0.002	0.934
		Pairwise: GDX SPS v. control	0.017	0.701
Sucrose (Fig. 10D)	2-way ANOVA (stress*GDX) sham v. GDX	Main effect: GDX	0.046	0.518
		Main effect: stress	0.057	0.480
		Pairwise: sham SPS v. control	0.029	0.596
		Pairwise: SPS sham v. GDX	0.027	0.608
	2-way ANOVA (stress*GDX) GDX v. GDX+T	None		
Social int. (Fig. 10E)	2-way ANOVA (stress*GDX) sham v. GDX	Main effect: stress	0.014	0.702
		Pairwise: sham SPS v. control	0.039	0.549
	2-way ANOVA (stress*GDX) GDX v. GDX+T	Main effect: stress	0.044	0.528
		Pairwise: GDX SPS v. control	0.031	0.585
Latency/empty zone (Fig. 10F)	2-way ANOVA (stress*GDX) sham v. GDX	None		
	2-way ANOVA (stress*GDX) GDX v. GDX+T	Main effect: stress	0.016	0.690
		Pairwise: GDX+T SPS v. control	0.008	0.771
		Pairwise: SPS GDX v. GDX+T	0.018	0.670
Body weight (Fig. 17)	RM 3-way ANOVA (stress*GDX*time) sham v. GDX	Main effect: GDX	0.002	0.887
		Main effect: time	<0.0001	1.000
		Pairwise: SPS time 1 sham v. GDX	0.005	0.815
		Pairwise: SPS time 2 sham v. GDX	0.002	0.887

Table 7 (cont'd).

		Pairwise: SPS sham time 1 v. 2	<0.0001	1.000
		Pairwise: SPS GDX time 1 v. 2	<0.0001	1.000
		Pairwise: control sham time 1 v. 2	<0.0001	1.000
		Pairwise: control GDX time 1 v. 2	<0.0001	1.000
	RM 3-way ANOVA (stress*GDX*time) GDX v. GDX+T	Main effect: time	<0.0001	1.000
		Pairwise: SPS GDX time 1 v. 2	<0.0001	1.000
		Pairwise: SPS GDX+T time 1 v. 2	<0.0001	1.000
		Pairwise: control GDX time 1 v. 2	<0.0001	1.000
		Pairwise: control GDX+T time 1 v. 2	<0.0001	1.000
Adrenal weight (Fig. 18)	RM 3-way ANOVA (stress*GDX*time) Sham v. GDX	None		
	RM 3-way ANOVA (stress*GDX*time) GDX v. GDX+T	None		

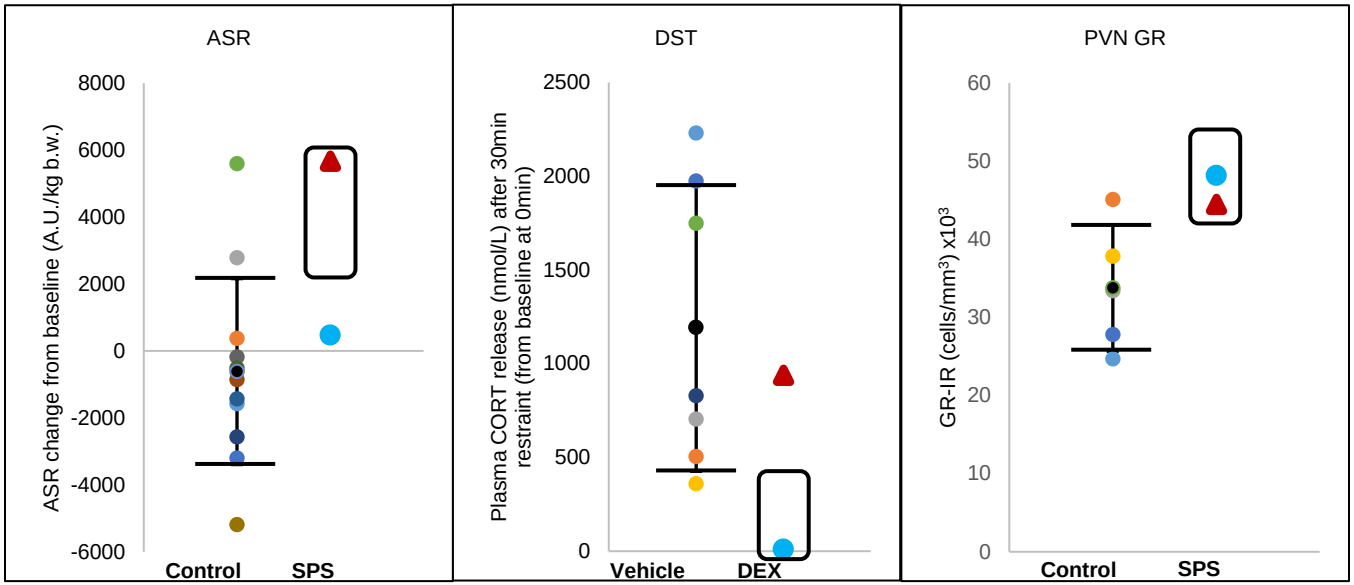

Figure 20. Individuals are not uniformly susceptible or resilient across measures. SPS affects one individual male (red triangle) differently than another (blue circle) across measures of acoustic startle response (ASR), dexamethasone (DEX) suppression test (DST), and glucocorticoid receptor (GR) expression in the paraventricular nucleus of the hypothalamus (PVN). All individuals in the control groups are shown with the mean (black circle) and standard deviation (black bars). Responses that lie outside the standard deviation are considered “affected” by SPS and are denoted with a black box, an approach used to infer susceptibility in chronic unpredictable and acute stress paradigms in rodents (343). While one individual is affected by SPS in terms of ASR, that rat is unaffected in terms of DST—and vice versa for another individual. Both individuals, however, are equally affected by SPS in terms of GR expression in the PVN, which challenges the notion that an individual is either resilient or susceptible to trauma.

REFERENCES

REFERENCES

1. Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson C (1995) Posstraumatic Stress Disorder in the National Comorbidity Survey. *Arch Gen Psychiatry* 52:1048–1060.

2. Kessler RC, et al. (2005) Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 62(June 2005):593–602.

3. Breslau N, Davis GC, Andreski P, Peterson E (1991) Traumatic Events and Posttraumatic Stress Disorder in an Urban Population of Young Adults. *Arch Gen Psychiatry* 48:216–222.

4. Cifu DX, et al. (2014) Traumatic brain injury, posttraumatic stress disorder, and pain diagnoses in OIF/OEF/OND Veterans. *J Rehabil Res Dev* 50(9):1169–76.

5. Orr SP, Pitman RK, Lasko NB, Herz LR (1993) Psychophysiological assessment of posttraumatic stress disorder imagery in World War II and Korean combat veterans. *J Abnorm Psychol* 102(1):152–9.

6. Sledjeski EM, Speisman B, Dierker LC (2008) Does number of lifetime traumas explain the relationship between PTSD and chronic medical conditions? Answers from the National Comorbidity Survey-Replication (NCS-R). *J Behav Med* 31(4):341–9.

7. Seyle H (1936) A Syndrome Produced by Diverse Nocuous Agents. *Nature* 138:32.

8. Pacák K, Palkovits M (2001) Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. *Endocr Rev* 22(4):502–48.

9. Boccia M, et al. (2016) Different neural modifications underpin PTSD after different traumatic events: an fMRI meta-analytic study. *Brain Imaging Behav* 10:226–237.

10. Freed S, D'Andrea W (2015) Autonomic Arousal and Emotion in Victims of Interpersonal Violence : Shame Proneness But Not Anxiety Predicts Vagal Tone Autonomic Arousal and Emotion in Victims of Interpersonal Violence : Shame Proneness. *J trauma dissociation* 16(September):367–383.

11. Foa EB, Zinbarg R, Rothbaum BO (1992) Uncontrollability and unpredictability in post-traumatic stress disorder: an animal model. *Psychol Bull* 112(2):218–38.

12. Clinchy M, et al. (2010) The Neurological Ecology of Fear: Insights Neuroscientists and Ecologists Have to Offer one Another. *Front Behav Neurosci* 4(April):21.

13. Hawlena D, Schmitz OJ (2010) Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *Am Nat* 176(5):537–56.

14. Creel S, Christianson D (2008) Relationships between direct predation and risk effects. *Trends Ecol Evol* 23(4):194–201.

15. Parker KJ, Maestripieri D (2011) Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates. *Neurosci Biobehav Rev* 35(7):1466–1483.

16. Huether G (1996) The central adaptation syndrome: psychosocial stress as a trigger for adaptive modifications of brain structure and brain function. *Prog Neurobiol* 48(6):569–612.

17. Bracha HS (2006) Human brain evolution and the “Neuroevolutionary Time-depth Principle:” Implications for the Reclassification of fear-circuitry-related traits in DSM-V and for studying resilience to warzone-related posttraumatic stress disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 30(5):827–53.

18. Tedstone JE, Tarrier N (2003) Posttraumatic stress disorder following medical illness and treatment. *Clin Psychol Rev* 23(3):409–448.

19. Amat J, et al. (2005) Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. *Nat Neurosci* 8(3):365–71.

20. Leuner B, Mendolia-Loffredo S, Shors TJ (2004) Males and females respond differently to controllability and antidepressant treatment. *Biol Psychiatry* 56(12):964–970.

21. Turnbull GJ (1998) A review of post-traumatic stress disorder. Part I: Historical development and classification. *Injury* 29(2):87–91.

22. Dimauro J, Carter S, Folk JB, Kashdan TB (2014) A historical review of trauma-related diagnoses to reconsider the heterogeneity of PTSD. *J Anxiety Disord* 28(8):774–786.

23. Birmes P, Hatton L, Brunet A, Schmitt L (2003) Early historical literature for post-traumatic symptomatology. *Stress Heal* 19(1):17–26.

24. Shay BJ (1991) Learning About Combat Stress from Homer ‘ s Iliad. *J Trauma Stress* 4(4):561–579.

25. Jones E, et al. (2013) Flashbacks and post-traumatic stress disorder : the genesis of a 20th-century diagnosis Flashbacks and post-traumatic stress disorder : the genesis of a 20th-century diagnosis. *Br J Psychiatry* 182:158–163.

26. Lasiuk GC, Hegadoren KM (2006) Posttraumatic stress disorder part I: historical development of the concept. *Perspect Psychiatr Care* 42(1):13–20.

27. van der Kolk BA, Herron N, Hostetler A (1994) The History of Trauma of Psychiatry. *Pyschiatric Clin North Am* 17(3):583–600.

28. Erichsen JE (1866) THE CLASSIC: Injuries of the Nervous System. *Clin Orthop Relat Res* 458:47–51.

29. Page HW (1891) Railway Injuries: In their Medico-Legal and Clinical Aspects.

30. Freud S (1896) The Aetiology of Hysteria. 170–189.

31. Myers CS (1915) A Contribution to the Study of Shell Shock. *Lancet* 188:316–320.

32. Hartshorne H (1864) Summary of the transactions of the College of Physicians of Philadelphia. 1863. June 3. On heart disease in the army. *Am J Med Sci* 48(7):59–63.

33. Chodoff P (1963) Late Effects of the Concentration Camp Syndrome. *Arch Gen Psychiatry* 8(4):323–333.

34. Burgess AW, Holmstrom LL (1974) Rape Trauma Syndrome. *Am J Psychiatry* 131(9):981–986.

35. Friedman MJ (2013) Finalizing PTSD in DSM-5 : Getting Here From There and Where to Go Next. *J Trauma Stress* (26):548–556.

36. Pitman RK (1989) Post-Traumatic Stress Disorder, Hormones, and Memory. *Biol Psychiatry* 26:221–223.

37. Rausch S, et al. (2016) Women with exposure to childhood interpersonal violence without psychiatric diagnoses show no signs of impairment in general functioning, quality of life and sexuality. *Borderline Personal Disord Emot Dysregulation* 3(1):13.

38. Copeland WE, Keeler G, Angold A, Costello EJ (2007) Traumatic Events and Posttraumatic Stress in Childhood. *Arch Gen Psychiatry* 64(5):577.

39. Lowe SR, Galea S, Uddin M, Koenen KC (2014) Trajectories of Posttraumatic Stress Among Urban Residents. *Am J Community Psychol* 53(0):159–172.

40. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER (2013) The three-hit concept of vulnerability and resilience: Toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* 38(9):1858–1873.

41. True WR, et al. (1993) A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. *Arch Gen Psychiatry* 50:257–264.

42. Stein MB, Jang K, Taylor S, Vernon P, Livesley WJ (2002) Genetic and environmental influences on trauma exposure and ... *Am J Psychiatry* 159(10):1675–1681.

43. Xie P, et al. (2009) Interactive Effect of Stressful Life Events and the Serotonin Transporter 5-HTTLPR Genotype on Posttraumatic Stress Disorder Diagnosis in 2 Independent Populations. *Arch Gen Psychiatry* 66(11):1201.

44. Walsh K, Uddin M, Soliven R, Wildman DE, Bradley B (2014) Associations between the SS variant of 5-HTTLPR and PTSD among adults with histories of childhood emotional abuse: Results from two African American independent samples. *J Affect Disord* 161:91–96.

45. Zhang L, et al. (2013) PTSD risk is associated with BDNF Val66Met and BDNF overexpression. *Mol Psychiatry*:1–2.

46. Frielingsdorf H, et al. (2010) Variant brain-derived neurotrophic factor Val66Met endophenotypes: implications for posttraumatic stress disorder. *Ann N Y Acad Sci* 1208:150–7.

47. Ressler KJ, et al. (2011) Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* 470(7335):492–7.

48. Uddin M, et al. (2013) ADCYAP1R1 GENOTYPE, POSTTRAUMATIC STRESS DISORDER, AND DEPRESSION AMONG WOMEN EXPOSED TO CHILDHOOD MALTREATMENT. *Depress Anxiety* 30(3):251–258.

49. Gillespie CF, et al. (2013) Sex dependent influence of a functional polymorphism in steroid 5-α-reductase type 2 (SRD5A2) on post-traumatic stress symptoms. *Am J Med Genet Part B, Neuropsychiatr Genet* 162B(3):283–92.

50. Lowe SR, et al. (2015) RORA and posttraumatic stress trajectories: Main effects and interactions with childhood physical abuse history. *Brain Behav* 5(4):1–11.

51. Wingo AP, et al. (2015) DICER1 and microRNA regulation in post-traumatic stress disorder with comorbid depression. *Nat Commun* 6:10106.

52. Norrholm SD, Ressler KJ (2009) Genetics of anxiety and trauma-related disorders. *Neuroscience* 164(1):272–87.

53. Bird A (2002) DNA methylation patterns and epigenetic memory DNA methylation patterns and epigenetic memory. *Genes Dev*:6–21.

54. Uddin M, et al. (2010) Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci* 107(20).

55. Perroud N, et al. (2011) Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Transl Psychiatry* 1(12):e59.

56. Smith AK, et al. (2011) Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *Am J Med Genet Part B, Neuropsychiatr Genet* 156B(6):700–8.

57. Sipahi L, et al. (2014) Longitudinal epigenetic variation of DNA methyltransferase genes is associated with vulnerability to post-traumatic stress disorder. *Psychol Med* (2014):1–15.

58. Labonté B, Azoulay N, Yerko V, Turecki G, Brunet a (2014) Epigenetic modulation of glucocorticoid receptors in posttraumatic stress disorder. *Transl Psychiatry* 4(November 2013):e368.

59. Yehuda R, et al. (2014) Influences of Maternal and Paternal PTSD on Epigenetic Regulation of the Glucocorticoid Receptor Gene in Holocaust Survivor Offspring. *Am J Psychiatry* 171:872–880.

60. Yehuda R, Bell A, Bierer LM, Schmeidler J (2008) Maternal, not paternal, PTSD is related to increased risk for PTSD in offspring of Holocaust survivors. *J Psychiatr Res* 42(13):1104–11.

61. Schechter DS, et al. (2015) Methylation of NR3C1 is related to maternal PTSD, parenting stress and maternal medial prefrontal cortical activity in response to child separation among mothers with histories of violence exposure. *Front Psychol* 6(May):1–12.

62. Rodgers AB, Bale TL (2015) Germ Cell Origins of Posttraumatic Stress Disorder Risk: The Transgenerational Impact of Parental Stress Experience. *Biol Psychiatry* 78(5):307–314.

63. Bader HN, et al. (2014) Maternal Age at Holocaust Exposure and Maternal PTSD Independently Influence Urinary Cortisol Levels in Adult Offspring. *Front Endocrinol (Lausanne)* 5(July):103.

64. McFarlane AC, Atchison M, Yehuda R (1997) The acute stress response following motor vehicle accidents and its relation to PTSD. *Psychobiol Posttraumatic Stress Disord* 821:437–441.

65. Delahanty DL, Raimonde AJ, Spoonster E (2000) Initial posttraumatic urinary cortisol levels predict subsequent PTSD symptoms in motor vehicle accident victims. *Biol Psychiatry* 48(9):940–947.

66. Gilbertson MW, et al. (2002) Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat Neurosci* 5(11):1242–1247.

67. Andrews JA, Neises KD (2012) Cells, biomarkers, and post-traumatic stress disorder: evidence for peripheral involvement in a central disease. *J Neurochem* 120:26–36.

68. Yehuda R, et al. (2000) Low cortisol and risk for PTSD in adult offspring of holocaust survivors. *Am J Psychiatry* 157(8):1252–9.

69. Karam EG, et al. (2014) Cumulative Traumas and Risk Thresholds: 12-Month PTSD in the World Mental Health (WMH) Surveys. *Depress Anxiety* 31(2):130–142.

70. Frans O, Rimmö P, Aberg L, Fredrikson M (2005) Trauma exposure and post-traumatic stress disorder in the general population. *Acta Psychiatr Scand* 111(4):291–9.

71. McEwen BS (1998) Stress, Adaptation, and Disease: Allostasis and Allostatic Load. *Ann N Y Acad Sci* 840(1):33–44.

72. Kilpatrick DG, et al. (2013) National Estimates of Exposure to Traumatic Events and PTSD Prevalence Using DSM-IV and DSM-5 Criteria. *J Trauma Stress* 26:537–547.

73. Ozer EJ, Best SR, Lipsey TL, Weiss DS (2003) Predictors of posttraumatic stress disorder and symptoms in adults: A meta-analysis. *Psychol Bull* 129(1):52–73.

74. Ullman SE, Relyea M, Peter-Hagene L, Vasquez AL (2013) Trauma histories, substance use coping, PTSD, and problem substance use among sexual assault victims. *Addict Behav* 38(6):2219–2223.

75. Molnar BE, Buka SL, Kessler RC (2001) Child sexual abuse and subsequent psychopathology: results from the National Comorbidity Survey. *Am J Public Health* 91(5):753–60.

76. Freyd JJ (1994) Betrayal Trauma: Traumatic Amnesia as an Adaptive Response to Childhood Abuse. *Ethics Behav* 4(4):307–329.

77. Goldsmith RE, Freyd JJ, DePrince a. P (2012) Betrayal Trauma: Associations With Psychological and Physical Symptoms in Young Adults. *J Interpers Violence* 27:547–567.

78. Spindler H, Elklit A, Christiansen D (2010) Risk factors for posttraumatic stress disorder following an industrial disaster in a residential area: A note on the origin of observed gender differences. *Gend Med* 7(2):156–65.

79. Ehlers A, Mayou R, Bryant B (1998) Psychological predictors of chronic posttraumatic stress disorder after motor vehicle accidents. *J Abnorm Psychol* 107(3):508–519.

80. Lee CS, et al. (2009) Acculturation, psychiatric comorbidity and posttraumatic stress disorder in a Taiwanese aboriginal population. *Soc Psychiatry Psychiatr Epidemiol* 44(1):55–62.

81. Marsella AJ (2010) Ethnocultural Aspects of PTSD: An Overview of Concepts, Issues, and Treatments. *Traumatology (Tallahass Fla)* 16(4):17–26.

82. Hinton DE, Lewis-Fernández R (2011) The cross-cultural validity of posttraumatic stress disorder: implications for DSM-5. *Depress Anxiety* 28(9):783–801.

83. Algeria M, et al. (2013) Prevalence, Risk, and Correlates of Posttraumatic Stress Disorder across Ethnic and Racial Minority Groups in the U.S. *Med Care* 51(12):1114–1123.

84. Marshall GN, Orlando M (2002) Acculturation and peritraumatic dissociation in young adult Latino survivors of community violence. *J Abnorm Psychol* 111(1):166–174.

85. Fortuna LR, Porche M V., Alegria M (2008) Political violence, psychosocial trauma, and the context of mental health services use among immigrant Latinos in the United States. *Ethn Health* 13(5):435–463.

86. Hobfoll SE (2012) Conservation of resources and disaster in cultural context: the caravans and passageways for resources: commentary on "a conceptual framework for understanding the mental health impacts of oil spills: lessons from the Exxon Valdez oil spill. *Psychiatry* 75(3):227–232.

87. Smid GE, Mooren TTM, Van Der Mast RC, Gersons BPR, Kleber RJ (2009) Delayed posttraumatic stress disorder: Systematic review, meta-analysis, and meta-regression analysis of prospective studies. *J Clin Psychiatry* 70(11):1572–1582.

88. Shively C, Kaplan J (1984) Effects of social factors on adrenal weight and related physiology of Macaca fascicularis. *Physiol Behav* 33(5):777–782.

89. Abbott DH, et al. (2003) Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. *Horm Behav* 43(1):67–82.

90. Golub MS, Sassenrath EN, Goo GP (1979) Plasma cortisol levels and dominance in peer groups of rhesus monkey weanlings. *Horm Behav* 12(1):50–59.

91. Russo SJ, Murrough JW, Han M-H, Charney DS, Nestler EJ (2012) Neurobiology of resilience. *Nat Neurosci* 15(11):1475–84.

92. Caspi A, Moffitt TE (2006) Gene – environment interactions in psychiatry : joining forces with neuroscience. 7(July):583–590.

93. Belsky J, Beaver KM (2011) Cumulative-genetic plasticity, parenting and adolescent self-regulation. *J Child Psychol Psychiatry* 52(5):619–626.

94. Ogle CM, Rubin DC, Siegler IC (2013) The Impact of the Developmental Timing of Trauma Exposure on PTSD Symptoms and Psychosocial Functioning Among Older Adults. *Dev Psychol* 49(11):1–18.

95. Ehring T, Quack D (2010) Emotion Regulation Difficulties in Trauma Survivors: The Role of Trauma Type and PTSD Symptom Severity. *Behav Ther* 41(4):587–598.

96. Stevens NR, et al. (2013) Emotion Regulation Difficulties, Low Social Support, and Interpersonal Violence Mediate the Link Between Childhood Abuse and Posttraumatic Stress Symptoms. *Behav Ther* 44(1):152–161.

97. Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP (2002) Developmental neurobiology of childhood stress and trauma. *Psychiatr Clin North Am* 25(2):397–426.

98. Widom CS, DuMont K, Czaja SJ (2007) A Prospective Investigation of Major Depressive Disorder and Comorbidity in Abused and Neglected Children Grown Up. *Arch Gen Psychiatry* 64:49–56.

99. Molnar BE, Berkman LF, Buka SL (2001) Psychopathology, childhood sexual abuse and other childhood adversities: relative links to subsequent suicidal behaviour in the US. *Psychol Med* 31(6):965–977.

100. Brewin CR, Andrews B, Valentine JD (2000) Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. *J Consult Clin Psychol* 68(5):748–766.

101. Anda RF, et al. (2006) The enduring effects of abuse and related adverse experiences in childhood: A convergence of evidence from neurobiology and epidemiology. *Eur Arch Psychiatry Clin Neurosci* 256(3):174–186.

102. Felitti VJ, et al. (1998) Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study.[see comment]. *Am J Prev Med* 14(4):245–258.

103. Cloitre M, et al. (2009) A Developmental Approach to Complex PTSD: Childhood and Adult Cumulative Trauma as Predictors of Symptom Complexity. *J Trauma Stress* 22(5):399–408.

104. Bustamante AC, et al. (2016) Glucocorticoid receptor DNA methylation, childhood maltreatment and major depression. *J Affect Disord* 206:181–188.

105. Nugent NR, Goldberg A, Uddin M (2015) Topical Review: The Emerging Field of Epigenetics: Informing Models of Pediatric Trauma and Physical Health. *J Pediatr Psychol* 41(March 2015):1–8.

106. Teicher MH, et al. (2003) The neurobiological consequences of early stress and childhood maltreatment. *Neurosci Biobehav Rev* 27(1–2):33–44.

107. Sapolsky RM, Krey LC, McEwen BS (1986) The Neuroendocrinology of Stress and Aging: The Glucorticoid Cascade Hypothesis. *Endocr Rev* 7(3):284–301.

108. Zoladz PR, Diamond DM (2013) Current status on behavioral and biological markers of PTSD: A search for clarity in a conflicting literature. *Neurosci Biobehav Rev* 37(5):860–95.

109. Mcfarlane AC (2000) Posttraumatic Stress Disorder : A Model of the Longitudinal Course and the Role of Risk Factors r py Co ig a du es r Co ht ig In. *J Clin Psychiatry* 61(suppl 5):15–20.

110. Yehuda R, McFarlane AC, Shalev AY (1998) Predicting the development of posttraumatic stress disorder from the acute response to a traumatic event. *Biol Psychiatry* 44(12):1305–1313.

111. Andrews B, Brewin CR, Rose S (2003) Gender, social support, and PTSD in victims of violent crime. *J Trauma Stress* 16(4):421–427.

112. Morris MC, Rao U (2013) Psychobiology of PTSD in the acute aftermath of trauma: Integrating research on coping, HPA function and sympathetic nervous system activity. *Asian J Psychiatr* 6(1):3–21.

113. Galatzer-Levy IR, Bryant RA (2013) 636,120 Ways To Have Posttraumatic Stress Disorder. *Perspect Psychol Sci* 8(6):651–662.

114. van der Kolk BA, Van der Hart O (1989) Pierre Janet and the breakdown of adaption in psychological trauma. *Am J Psychiatry* 146(12):1530–1540.

115. Janet P (1889) *L'automatisme Psychologique* ed Alcan F (Ancienne Librairie Germer Bailliere et Cie, Paris) doi:10.1176/ajp.148.12.1730.

116. Steuwe C, Lanius RA, Frewen PA (2012) Evidence for a dissociative subtype of PTSD by latent profile and confirmatory factor analyses in a civilian sample. *Depress Anxiety* 29(8):689–700.

117. Wolf EJ, Miller MW, Harrington KM, Reardon A (2012) Personality-based latent classes of posttraumatic psychopathology: personality disorders and the internalizing/externalizing model. *J Abnorm Psychol* 121(1):256–62.

118. Wolf EJ, et al. (2012) A Latent Class Analysis of Dissociation and PTSD: Evidence for a Dissociative Subtype. *Arch Gen Psychiatry* 69(7):698–705.

119. Stein DJ, et al. (2013) Dissociation in Posttraumatic Stress Stress Disorder: Evidence from the World Mental Health Surveys. *Biol Psychiatry* 73(4):302–312.

120. Wolf EJ, et al. (2012) The dissociative subtype of PTSD: A replication and extension. *Depress Anxiety* 29(8):679–688.

121. Lanius RA, et al. (2005) Functional connectivity of dissociative responses in posttraumatic stress disorder: A functional magnetic resonance imaging investigation. *Biol Psychiatry* 57(8):873–884.

122. Lanius RA, et al. (2010) Emotion Modulation in PTSD: Clinical Neurobiological Evidence for a Dissociative Subtype. *Am J Psychiatry* 167(6):640–647.

123. Lanius RA, Brand B, Vermetten E, Frewen PA, Spiegel D (2012) The dissociative subtype of posttraumatic stress disorder: rationale, clinical and neurobiological evidence, and implications. *Depress Anxiety* 29(8):701–8.

124. Hopper JW, Frewen PA, Lanius RA (2007) Neural Correlates of Reexperiencing , Avoidance , and Dissociation in PTSD : Symptom Dimensions and Emotion Dysregulation in Responses to Script-Driven Trauma Imagery. *J Trauma Stress* 20(5):713–725.

125. Simeon D, et al. (2007) Hypothalamic-Pituitary-Adrenal Axis Function in Dissociative Disorders, Post-Traumatic Stress Disorder, and Healthy Volunteers. *Biol Psychiatry* 61(8):966–973.

126. Griffin MG, Resick PA, Mechanic MB (1997) Objective Assessment of Peritraumatic Dissociation: Psychophysiological Indicators. *Am J Psychiatry* 154(8):1081–1088.

127. Bernstein RE, Delker BC, Knight JA, Freyd JJ (2015) Hypervigilance in college students : Associations with betrayal and dissociation and psychometric properties in a brief hypervigilance scale. *Psychol Trauma* 7(5):448–455.

128. Smith CP, Freyd JJ (2013) Dangerous Safe Havens: Institutional Betrayal Exacerbates Sexual Trauma. *J Trauma Stress* 26(February):119–127.

129. Mehta D, et al. (2013) Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proc Natl Acad Sci* 110(20):8302–8307.

130. Schmid M, Petermann F, Fegert JM (2013) Developmental trauma disorder: pros and cons of including formal criteria in the psychiatric diagnostic systems. *BMC Psychiatry* 13(1):3.

131. Rahim M (2014) Developmental trauma disorder: An attachment-based perspective. *Clin Child Psychol Psychiatry* 19(4):548–560.

132. Ford JD, et al. (2013) Clinical significance of a proposed developmental trauma disorder diagnosis: Results of an international survey of clinicians. *J Clin Psychiatry* 74(8):841–849.

133. Stolbach BC, et al. (2013) Complex Trauma Exposure and Symptoms in Urban Traumatized Children: A Preliminary Test of Proposed Criteria for Developmental Trauma Disorder. *J Trauma Stress* 26:483–491.

134. Andrews B, Brewin CR, Philpott R, Stewart L (2007) Delayed-onset posttraumatic stress disorder: a systematic review of the evidence. *Am J Psychiatry* 164(9):1319–26.

135. Utzon-Frank N, et al. (2014) Occurrence of delayed-onset post-traumatic stress disorder: A systematic review and meta-analysis of prospective studies. *Scand J Work Environ Health* 40(3):215–229.

136. Friedman MJ, Resick PA, Bryant RA, Brewin CR (2011) Considering PTSD for DSM-5. *Depress Anxiety* 28(9):750–769.

137. Forbes D, Elhai JD, Miller MW, Creamer M (2010) Internalizing and Externalizing Classes in Posttraumatic Stress Disorder : A Latent Class Analysis. *J Trauma Stress* 23(3):340–349.

138. Miller MW, Resick PA (2007) Internalizing and externalizing subtypes in female sexual assault survivors: implications for the understanding of complex PTSD. *Behav Ther* 38(1):58–71.

139. Castillo DT, et al. (2014) Externalizing and Internalizing Subtypes of Posttraumatic Psychopathology and Anger Expression. *J Trauma Stress* 27:108–111.

140. Rielage JK, Hoyt T, Renshaw K (2010) Internalizing and Externalizing Personality Styles and Psychopathology in OEF – OIF Veterans. *J Trauma Stress* 23(3):350–357.

141. Herman JL (1992) Complex PTSD: A syndrome in survivors of prolonged and repeted trauma. *J Trauma Stress* 5(3):377–392.

142. Resick PA, et al. (2012) A Critical Evaluation of the Complex PTSD Literature: Implications for DSM-5. *J Trauma Stress* 25:241–251.

143. Herman JL (2012) CPTSD is a Distinct Entity: Comment on Resick et al. (2012). *J Trauma Stress* 25:256–257.

144. Shin LM, et al. (2009) Resting Metabolic Activity in the Cingulate Cortex and Vulnerability to Posttraumatic Stress Disorder. *Arch Gen Psychiatry* 66(10):1099–1107.

145. Yehuda R (1997) Sensitization of the hypothalamic-pituitary-adrenal axis in posttraumatic stress disorder. *Ann N Y Acad Sci* 821(7 18):57–75.

146. Rodrigues SM, Ledoux JE, Sapolsky RM (2009) The Influence of Stress Hormones on Fear Circuitry. *Annu Rev neurosience* 32:289–313.

147. Cahill L, Prins B, Weber M, McGaugh JL (1994) β-Adrenergic activation and memory for emotional events. *Nature* 371(6499):702–704.

148. O'Donnell T, Hegadoren KM, Coupland NC (2004) Noradrenergic mechanisms in the pathophysiology of post-traumatic stress disorder. *Neuropsychobiology* 50(4):273–283.

149. Southwick SM, et al. (1997) Noradrenergic and Serotonergic Function in Posttraumatic Stress Disorder. *Arch Gen Psychiatry* 54:749–758.

150. Keller-Wood ME, Dallman MF (1984) Corticosteroid inhibition of ACTH secretion. *Endocr Rev* 5(1):1–24.

151. Jacobson L, Sapolsky RM (1991) The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr Rev* 12(2):118–34.

152. Yehuda R, et al. (1993) Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. *Am J Psychiatry* 150(1):83–6.

153. Rohleder N, Joksimovic L, Wolf JM, Kirschbaum C (2004) Hypocortisolism and increased glucocorticoid sensitivity of pro-Inflammatory cytokine production in Bosnian war refugees with posttraumatic stress disorder. *Biol Psychiatry* 55(7):745–51.

154. Yehuda R, Bierer LM (2008) Transgenerational transmission of cortisol and PTSD risk. *Prog Brain Res* 167(7):121–35.

155. Yehuda R, Halligan SL, Golier JA, Grossman R, Bierer LM (2004) Effects of trauma exposure on the cortisol response to dexamethasone administration in PTSD and major depressive disorder. *Psychoneuroendocrinology* 29(3):389–404.

156. Grossman R, et al. (2003) Dexamethasone Suppression Test Findings in Subjects With Personality Disorders : Associations With Posttraumatic Stress Disorder and Major Depression. *Am J Psychiatry* 160(July):1291–1297.

157. Yehuda R, Boisoneau D, Mason JW, Giller EL (1993) Glucocorticoid receptor number and cortisol excretion in mood, anxiety, and psychotic disorders. *Biol Psychiatry* 34(1–2):18–25.

158. Wahbeh H, Oken BS (2013) Salivary Cortisol Lower in Posttraumatic Stress Disorder. *J Trauma Stress* 26:1–8.

159. Pitman RK, et al. (2012) Biological studies of post-traumatic stress disorder. *Nat Rev Neurosci* 13(11):769–87.

160. Yehuda R (2002) Post-Traumatic Stress Disorder. *N Engl J Med* 346(2):108–114.

161. Pacak K, Palkovits M, Kopin IJ, Goldstein DS (1995) Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympathoadrenal activity: in vivo microdialysis studies. *Front Neuroendocr* 16(2):89–150.

162. Schelling G, et al. (2004) Stress doses of hydrocortisone, traumatic memories, and symptoms of posttraumatic stress disorder in patients after cardiac surgery: a randomized study. *Biol Psychiatry* 55(6):627–33.

163. Zohar J, et al. (2011) High dose hydrocortisone immediately after trauma may alter the trajectory of PTSD: interplay between clinical and animal studies. *Eur Neuropsychopharmacol* 21(11):796–809.

164. Shin LM, Rauch SL, Pitman RK (2006) Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Ann N Y Acad Sci* 1071:67–79.

165. Hughes KC, Shin LM (2011) Functional neuroimaging studies of post-traumatic stress disorder. *Expert Rev Neurother* 11(2):275–285.

166. Weber M, et al. (2013) Voxel-Based Morphometric Gray Matter Correlates of Posttraumatic Stress Disorder. *J Anxiety Disord* 27(4):413–419.

167. Mahan A, Ressler KJ (2012) Fear conditioning, synaptic plasticity, and the amygdala: implications for posttraumatic stress disorder. *Trends Neurosci* 35(1):24–35.

168. Hayes JP, Hayes SM, Mikedis AM (2012) Quantitative meta-analysis of neural activity in posttraumatic stress disorder. *Biol Mood Anxiety Disord* 2(1):9.

169. Etkin A, Wager T (2007) Functional Neuroimaging of Anxiety: A Meta-Analysis of Emotional Processing in PTSD, Social Anxiety Disorder, and Specific Phobia. *Am J Psychiatry* 164(10):1476–1488.

170. Kasai K, et al. (2008) Evidence for acquired pregenual anterior cingulate gray matter loss from a twin study of combat-related posttraumatic stress disorder. *Biol Psychiatry* 63(6):550–6.

171. Rauch SL, Shin LM, Phelps EA (2006) Neurocircuitry Models of Posttraumatic Stress Disorder and Extinction: Human Neuroimaging Research-Past, Present, and Future. *Biol Psychiatry* 60(4):376–382.

172. Kühn S, Gallinat J (2013) Gray matter correlates of posttraumatic stress disorder: A quantitative meta-analysis. *Biol Psychiatry* 73(1):70–74.

173. Gilboa A, et al. (2004) Functional connectivity of the prefrontal cortex and the amygdala in posttraumatic stress disorder. *Biol Psychiatry* 55(3):263–72.

174. Garfinkel SN, et al. (2014) Impaired Contextual Modulation of Memories in PTSD: An fMRI and Psychophysiological Study of Extinction Retention and Fear Renewal. *J Neurosci* 34(40):13435–13443.

175. Christova P, James LM, Engdahl BE, Lewis SM, Georgopoulos AP (2015) Diagnosis of posttraumatic stress disorder (PTSD) based on correlations of prewhitened fMRI data: outcomes and areas involved. *Exp Brain Res* 233(9):2695–2705.

176. Uddin M, Sipahi L, Li J, Koenen KC (2013) Sex differences in DNA methylation may contribute to risk of PTSD and depression: A review of existing evidence. *Depress Anxiety* 30(12):1151–1160.

177. Vukojevic V, et al. (2014) Epigenetic Modification of the Glucocorticoid Receptor Gene Is Linked to Traumatic Memory and Post-Traumatic Stress Disorder Risk in Genocide Survivors. *J Neurosci* 34(31):10274–10284.

178. Tolin DF, Foa EB (2006) Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research. *Psychol Bull* 132(6):959–92.

179. Zlotnick C, Zimmerman M, Wolfsdorf B a, Mattia JI (2001) Gender differences in patients with posttraumatic stress disorder in a general psychiatric practice. *Am J Psychiatry* 158(11):1923–5.

180. Yehuda R, et al. (2015) Post-traumatic stress disorder. *Nat Rev Dis Prim* 1(October):1–22.

181. Stevens JS, Hamann S (2012) Sex differences in brain activation to emotional stimuli: a meta-analysis of neuroimaging studies. *Neuropsychologia* 50(7):1578–93.

182. Woon F, Hedges DW (2011) Gender does not moderate hippocampal volume deficits in adults with posttraumatic stress disorder: a meta-analysis. *Hippocampus* 21(3):243–52.

183. Metzger LJ, et al. (2008) Basal and suppressed salivary cortisol in female vietnam nurse veterans with and without PTSD. *Psychiatry Interpers Biol Process* 161(3):330–335.

184. Rasmusson a M, et al. (2001) Increased pituitary and adrenal reactivity in premenopausal women with posttraumatic stress disorder. *Biol Psychiatry* 50(12):965–77.

185. Resnick HS, Yehuda R, Pitman RK, Foy DW (1995) Effect of previous trauma on acute plasma cortisol level following rape. *Am J Psychiatry* 152(11):1675–1677.

186. Medina AM, Mejia VY, Schell a M, Dawson ME, Margolin G (2001) Startle reactivity and PTSD symptoms in a community sample of women. *Psychiatry Res* 101(2):157–69.

187. Breslau N (2002) Gender Differences in Trauma and Posttraumatic Stress Disorder. *J Gend Specif Med* 5(1):34–40.

188. Kucharska J (2016) Sex Differences in the Appraisal of Traumatic Events and Psychopathology. *Psychol Trauma Theory , Res , Pract , Policy*. doi:http://dx.doi.org/10.1037/tra0000244.

189. Kamkwalala A, et al. (2012) Dark-enhanced startle responses and heart rate variability in a traumatized civilian sample: putative sex-specific correlates of posttraumatic stress disorder. *Psychosom Med* 74(2):153–9.

190. Shvil E, et al. (2014) Sex Differences in Extinction Recall in Posttraumatic Stress Disorder: A Pilot fMRI Study. *Neurobiol Learn Mem* 113:101–108.

191. Inslicht SS, et al. (2013) Sex differences in fear conditioning in posttraumatic stress disorder. *J Psychiatr Res* 47(1):64–71.

192. Hawk LW, Dougall a L, Ursano RJ, Baum a (2000) Urinary catecholamines and cortisol in recent-onset posttraumatic stress disorder after motor vehicle accidents. *Psychosom Med* 62(3):423–434.

193. Shin LM, et al. (2004) Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD. *Arch Gen Psychiatry* 61:168–176.

194. Stevens JS, et al. (2013) Disrupted amygdala-prefrontal functional connectivity in civilian women with posttraumatic stress disorder. *J Psychiatr Res* 47(10):1469–1478.

195. Arnold AP (2009) The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Horm Behav* 55(5):570–578.

196. Seale J V, Wood S a, Atkinson HC, Lightman SL, Harbuz MS (2005) Organizational role for testosterone and estrogen on adult hypothalamic-pituitary-adrenal axis activity in the male rat. *Endocrinology* 146(4):1973–82.

197. Mccarthy MM (2016) Multifaceted origins of sex differences in the brain Multifaceted origins of sex differences in the brain. *Philos Trans R Soc London B* (FEBRUARY). doi:10.1098/rstb.2015.0106.

198. Joel D, et al. (2015) Sex beyond the genitalia : The human brain mosaic. *Proc Natl Acad Sci*:1–6.

199. Forger NG (2016) Epigenetic mechanisms in sexual differentiation of the brain and behaviour. *Philos Trans R Soc London B Biol Sci* 371(1688). doi:10.1098/rstb.2015.0114.

200. Arnold AP (2012) The end of gonad-centric sex determination in mammals. *Trends Genet* 28(2):55–61.

201. Sterrenburg L, et al. (2012) Sex-dependent and differential responses to acute restraint stress of corticotropin-releasing factor-producing neurons in the rat paraventricular nucleus, central amygdala, and bed nucleus of the stria terminalis. *J Neurosci Res* 90(1):179–92.

202. Goel N, Bale TL (2010) Sex differences in the serotonergic influence on the hypothalamic-pituitary-adrenal stress axis. *Endocrinology* 151(4):1784–94.

203. Young EA, Altemus M, Parkison V, Shastry S (2001) Effects of estrogen antagonists and agonists on the ACTH response to restraint stress in female rats. *Neuropsychopharmacology* 25(6):881–91.

204. Young EA (1996) Sex differences in response to exogenous corticosterone: a rat model hypercortisolemia. *Mol Psychiatry* 1:313–319.

205. Williamson M, Bingham B, Gray M, Innala L, Viau V (2010) The medial preoptic nucleus integrates the central influences of testosterone on the paraventricular nucleus of the hypothalamus and its extended circuitries. *J Neurosci* 30(35):11762–70.

206. Lund TD, Munson DJ, Haldy ME, Handa RJ (2004) Androgen inhibits, while oestrogen enhances, restraint-induced activation of neuropeptide neurones in the paraventricular nucleus of the hypothalamus. *J Neuroendocrinol* 16(3):272–8.

207. Handa RJ, Kudwa AE, Donner NC, McGivern RF, Brown R (2013) Central 5-alpha reduction of testosterone is required for testosterone's inhibition of the hypothalamo-pituitary-adrenal axis response to restraint stress in adult male rats. *Brain Res* 1529:74–82.

208. Larkin JW, Binks SL, Li Y, Selvage D (2010) The role of oestradiol in sexually dimorphic hypothalamic-pituitary-adrena axis responses to intracerebroventricular ethanol administration in the rat. *J Neuroendocrinol* 22(1):24–32.

209. Handa RJ, Weiser MJ (2013) Gonadal steroid hormones and the hypothalamo-pituitary-adrenal axis. *Front Neuroendocrinol*. doi:10.1016/j.yfrne.2013.11.001.

210. Burgess L, Handa RJ (1992) Chronic Estrogen-Induced Alterations in Adrenocorticotropin and Corticosterone Secretion, and Glucocorticoid Receptor-Mediated Functions in Female Rats. *Endocrinology* 131(3):1261–1269.

211. Handa RJ, Burgess L, Kerr J, O'Keefe J (1994) Gonadal Steroid Hormone Receptors and Sex Differences in the Hypothalamic-Pituitary-Adrenal Axis. *Horm Behav* 28:464–476.

212. Handa RJ, et al. (1994) Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. *Physiol Behav* 55(1):117–24.

213. Dart DA, Waxman J, Aboagye EO, Bevan CL (2013) Visualising androgen receptor activity in male and female mice. *PLoS One* 8(8):e71694.

214. Fernández-Guasti A, Kruijver FP, Fodor M, Swaab DF (2000) Sex differences in the distribution of androgen receptors in the human hypothalamus. *J Comp Neurol* 425(3):422–35.

215. Seale J V, Wood S a, Atkinson HC, Harbuz MS, Lightman SL (2004) Gonadal steroid replacement reverses gonadectomy-induced changes in the corticosterone pulse profile and stress-induced hypothalamic-pituitary-adrenal axis activity of male and female rats. *J Neuroendocrinol* 16(12):989–98.

216. Seale J V, et al. (2004) Gonadectomy reverses the sexually diergic patterns of circadian and stress-induced hypothalamic-pituitary-adrenal axis activity in male and female rats. *J Neuroendocrinol* 16(6):516–24.

217. Turner BB, Weaver DA (1985) Sexual dimorphism of glucocorticoid binding in rat brain. *Brain Res* 343:16–23.

218. Spinedi E, Suescun MO, Hadid R, Gaillard RC (1992) Effects of Gonadectomy and Sex Hormone Therapy on the Endotoxin-Stimulated Hypothalamo-Pituitary-Adrenal Axis: Evidence for a Neuroendocrine-Immunological Sexual Dimorphism EDUARDO. *Endocrinology* 131(5):2430–36.

219. Bale TL, Epperson CN (2016) Sex As a Biological Variable: Who, What, When, Why and How. *Neuropsychopharmacology*:1–11.

220. Bland ST, et al. (2005) Expression of c-fos and BDNF mRNA in subregions of the prefrontal cortex of male and female rats after acute uncontrollable stress. *Brain Res* 1051(1–2):90–9.

221. Mazor A, et al. (2009) Gender-related qualitative differences in baseline and post-stress anxiety responses are not reflected in the incidence of criterion-based PTSD-like behaviour patterns. *World J Biol Psychiatry* 10(4):856–869.

222. Babb JA, Masini CV, Day HEW, Campeau S (2013) Sex differences in activated corticotropin-releasing factor neurons within stress-related neurocircuitry and hypothalamic-pituitary-adrenocortical axis hormones following restraint in rats. *Neuroscience* 234:40–52.

223. Mirshekar M, Abrari K, Goudarzi I, Rashidy-Pour A (2013) Systemic administrations of β-estradiol alleviate both conditioned and sensitized fear responses in an ovariectomized rat model of post-traumatic stress disorder. *Neurobiol Learn Mem* 102:12–9.

224. McDermott CM, Liu D, Schrader L a (2012) Role of gonadal hormones in anxiety and fear memory formation and inhibition in male mice. *Physiol Behav* 105(5):1168–74.

225. Garrett JE, Wellman CL (2009) Chronic stress effects on dendritic morphology in medial prefrontal cortex: sex differences and estrogen dependence. *Neuroscience* 162(1):195–207.

226. McCarthy MM, Konkle ATM (2005) When is a sex difference not a sex difference? *Front Neuroendocrinol* 26(2):85–102.

227. McCarthy MM, Arnold AP, Ball GF, Blaustein JD, De Vries GJ (2012) Sex differences in the brain: the not so inconvenient truth. *J Neurosci* 32(7):2241–7.

228. Bale TL, Epperson CN (2015) Sex differences and stress across the lifespan. *Nat Neurosci* 18(10):1413–20.

229. Joel D, McCarthy MM (2016) Incorporating Sex As a Biological Variable in Neuropsychiatric Research: Where Are We Now and Where Should We Be? *Neuropsychopharmacology* (May):1–23.

230. Becker JB, et al. (2005) Strategies and methods for research on sex differences in brain and behavior. *Endocrinology* 146(4):1650–1673.

231. Zuloaga DG, Puts D a, Jordan CL, Breedlove SM (2008) The role of androgen receptors in the masculinization of brain and behavior: what we've learned from the testicular feminization mutation. *Horm Behav* 53(5):613–26.

232. Yehuda R, Antelman SM (1993) Criteria for rationally evaluating animal models of posttraumatic stress disorder. *Biol Psychiatry* 33(7):479–86.

233. Siegmund A, Wotjak CT (2006) Toward an animal model of posttraumatic stress disorder. *Ann N Y Acad Sci* 1071:324–34.

234. Yamamoto S, et al. (2009) Single prolonged stress: toward an animal model of posttraumatic stress disorder. *Depress Anxiety* 26(12):1110–7.

235. Serova LI, et al. (2013) Single intranasal neuropeptide Y infusion attenuates development of PTSD-like symptoms to traumatic stress in rats. *Neuroscience* 236:298–312.

236. Brand L, Groenewald I, Stein DJ, Wegener G, Harvey BH (2008) Stress and re-stress increases conditioned taste aversion learning in rats: possible frontal cortical and hippocampal muscarinic receptor involvement. *Eur J Pharmacol* 586(1–3):205–11.

237. Wang W, et al. (2008) A modified single-prolonged stress model for post-traumatic stress disorder. *Neurosci Lett* 441(2):237–41.

238. Imanaka A, Morinobu S, Toki S, Yamawaki S (2006) Importance of early environment in the development of post-traumatic stress disorder-like behaviors. *Behav Brain Res* 173(1):129–37.

239. Kohda K, et al. (2007) Glucocorticoid receptor activation is involved in producing abnormal phenotypes of single-prolonged stress rats: a putative post-traumatic stress disorder model. *Neuroscience* 148(1):22–33.

240. Khan SA, Liberzon I (2004) Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. *Psychopharmacology (Berl)* 172(2):225–9.

241. Liberzon I, Krstov M, Young EA (1997) Stress-restress: effects on ACTH and fast feedback. *Psychoneuroendocrinology* 22(6):443–53.

242. Liberzon I, López JF, Flagel SB, Vázquez DM, Young EA (1999) Differential regulation of hippocampal glucocorticoid receptors mRNA and fast feedback: relevance to post-traumatic stress disorder. *J Neuroendocrinol* 11(1):11–7.

243. Knox D, et al. (2012) Single prolonged stress disrupts retention of extinguished fear in rats. *Learn Mem* 19(2):43–9.

244. Schneier FR, et al. (2012) Combined Prolonged Exposure Therapy and Paroxetine for Posttraumatic Stress Disorder Related to the World Trade Center Attacks: A Randomized Controlled Trial. *Am J Psychiatry* 169(1):80–88.

245. Corchs F, Nutt DJ, Hood S, Bernik M (2009) Serotonin and sensitivity to trauma-related exposure in selective serotonin reuptake inhibitors-recovered posttraumatic stress disorder. *Biol Psychiatry* 66(1):17–24.

246. Wang J-W, David DJ, Monckton JE, Battaglia F, Hen R (2008) Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *J Neurosci* 28(6):1374–84.

247. Takahashi T, Morinobu S, Iwamoto Y, Yamawaki S (2006) Effect of paroxetine on enhanced contextual fear induced by single prolonged stress in rats. *Psychopharmacology (Berl)* 189(2):165–73.

248. Peng Z, et al. (2013) Ziprasidone ameliorates anxiety-like behaviors in a rat model of PTSD and up-regulates neurogenesis in the hippocampus and hippocampus-derived neural stem cells. *Behav Brain Res* 244C:1–8.

249. Adamec R, Holmes A, Blundell J (2008) Vulnerability to Lasting Anxiogenic Effects of Brief Exposure to Predator Stimuli: Sex, Serotonin and Other Factors - Relevance to PTSD. *Neurosci Biobehav Rev* 32(7):1287–1292.

250. Campos AC, Ferreira FR, da Silva WA, Guimarães FS (2013) Predator threat stress promotes long lasting anxiety-like behaviors and modulates synaptophysin and CB1 receptors expression in brain areas associated with PTSD symptoms. *Neurosci Lett* 533:34–8.

251. Cohen H, et al. (2006) Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. *Biol Psychiatry* 59(12):1208–18.

252. Cohen H, et al. (2004) Setting apart the affected: the use of behavioral criteria in animal models of post traumatic stress disorder. *Neuropsychopharmacology* 29(11):1962–70.

253. Cohen H, Zohar J, Matar MA (2003) The Relevance of Differential Response to Trauma in an Animal Model of Posttraumatic Stress Disorder. *Biol Psychiatry* 53:463–473.

254. Mackenzie L, Nalivaiko E, Beig MI, Day T a, Walker FR (2010) Ability of predator odour exposure to elicit conditioned versus sensitised post traumatic stress disorder-like behaviours, and forebrain deltaFosB expression, in rats. *Neuroscience* 169(2):733–42.

255. Adamec R, Muir C, Grimes M, Pearcey K (2007) Involvement of noradrenergic and corticoid receptors in the consolidation of the lasting anxiogenic effects of predator stress. *Behav Brain Res* 179(2):192–207.

256. Matar MA, Cohen H, Kaplan Z, Zohar J (2006) The effect of early poststressor intervention with sertraline on behavioral responses in an animal model of post-traumatic stress disorder. *Neuropsychopharmacology* 31(12):2610–8.

257. Fountoulakis K, et al. (2004) Relationship among Dexamethasone Suppression Test, personality disorders and stressful life events in clinical subtypes of major depression: An exploratory study. *Ann Gen Hosp Psychiatry* 3(1):15.

258. Cole M a, Kim PJ, Kalman B a, Spencer RL (2000) Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. *Psychoneuroendocrinology* 25(2):151–67.

259. Masi G, Brovedani P (2011) The hippocampus, neurotrophic factors and depression: possible implications for the pharmacotherapy of depression. *CNS Drugs* 25(11):913–31.

260. Watson S, Gallagher P, Smith MS, Ferrier IN, Young AH (2006) The dex/CRH test--is it better than the DST? *Psychoneuroendocrinology* 31(7):889–94.

261. Jokinen J, Nordström P (2009) HPA axis hyperactivity and attempted suicide in young adult mood disorder inpatients. *J Affect Disord* 116(1–2):117–20.

262. Jokinen J, Mårtensson B, Nordström A-L, Nordström P (2008) CSF 5-HIAA and DST non-suppression -independent biomarkers in suicide attempters? *J Affect Disord* 105(1–3):241–5.

263. Menke A, et al. (2012) Dexamethasone stimulated gene expression in peripheral blood is a sensitive marker for glucocorticoid receptor resistance in depressed patients. *Neuropsychopharmacology* 37(6):1455–64.

264. Young EA, et al. (1993) Dissociation Between Pituitary and Adrenal Suppression to Dexamethasone in Depression. *Arch Gen Psychiatry* 50:395–403.

265. Grillon C, Morgan C a, Southwick SM, Davis M, Charney DS (1996) Baseline startle amplitude and prepulse inhibition in Vietnam veterans with posttraumatic stress disorder. *Psychiatry Res* 64(3):169–78.

266. Shalev a Y, Orr SP, Peri T, Schreiber S, Pitman RK (1992) Physiologic responses to loud tones in Israeli patients with posttraumatic stress disorder. *Arch Gen Psychiatry* 49(11):870–5.

267. Charney DS, Deutch AY, Krystal JH, Southwick SM, Davis M (1993) Psychobiologic Mechanisms of Posttraumatic Stress Disorder. *Arch Gen Psychiatry* 50:294–305.

268. Pitman RK (1997) Overview of biological themes in PTSD. *Ann N Y Acad Sci* 821(603):1–9.

269. Young EA, Korszun A (2010) Sex, trauma, stress hormones and depression. *Mol Psychiatry* 15(1):23–8.

270. Altemus M, et al. (1997) Reduced Sensitivity to Glucocorticoid Feedback and Reduced Glucocorticoid Receptor mRNA Expression in the Luteal Phase of the Menstrual Cycle. *Neuropsychopharmacology* 17:100–109.

271. Kudielka BM, Kirschbaum C (2005) Sex differences in HPA axis responses to stress: a review. *Biol Psychol* 69(1):113–32.

272. Glover EM, et al. (2012) Estrogen levels are associated with extinction deficits in women with posttraumatic stress disorder. *Biol Psychiatry* 72(1):19–24.

273. Glover EM, et al. (2013) Inhibition of fear is differentially associated with cycling estrogen levels in women. *J psychiatry Neurosci* 38(5):341–348.

274. Goldstein JM, et al. (2005) Hormonal cycle modulates arousal circuitry in women using functional magnetic resonance imaging. *J Neurosci* 25(40):9309–16.

275. Zeidan MA, et al. (2011) Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. *Biol Psychiatry* 70(10):920–7.

276. Manuck SB, et al. (2010) Salivary testosterone and a trinucleotide (CAG) length polymorphism in the androgen receptor gene predict amygdala reactivity in men. *Psychoneuroendocrinology* 35(1):94–104.

277. Hermans EJ, Putman P, Baas JM, Koppeschaar HP, van Honk J (2006) A Single Administration of Testosterone Reduces Fear-Potentiated Startle in Humans. *Biol Psychiatry* 59(9):872–874.

278. Hermans EJ, et al. (2007) Exogenous testosterone attenuates the integrated central stress response in healthy young women. *Psychoneuroendocrinology* 32(8–10):1052–1061.

279. King MW, Street A, Gradus JL, Vogt DS, Resick PA (2013) Gender Differences in Posttraumatic Stress Symptoms Among OEF/OIF Veterans: An Item Response Theory Analysis. *J Trauma Stress* 26:175–183.

280. Cohen H, Kozlovsky N, Alona C, Matar MA, Joseph Z (2012) Animal model for PTSD: from clinical concept to translational research. *Neuropharmacology* 62(2):715–24.

281. Farrell MR, Sengelaub DR, Wellman CL (2013) Sex differences and chronic stress effects on the neural circuitry underlying fear conditioning and extinction. *Physiol Behav* 122:208–215.

282. Leuner B, Shors TJ (2013) Stress, anxiety, and dendritic spines: What are the connections? *Neuroscience* 251:108–119.

283. Gruene TM, Roberts E, Thomas V, Ronzio A, Shansky RM (2015) Sex-specific neuroanatomical correlates of fear expression in prefrontal-amygdala circuits. *Biol Psychiatry* 78(3):186–193.

284. Zer-Aviv TM, Akirav I (2016) Sex differences in hippocampal response to endocannabinoids after exposure to severe stress. *Hippocampus* 26(7):947–957.

285. He Y-Q, et al. (2013) PKCγ Receptor Mediates Visceral Nociception and Hyperalgesia following Exposure to PTSD-Like Stress in the Spinal Cord of Rats. *Mol Pain* 9(1):1744-8069-9–35.

286. Yu H, Watt H, Kesavan C, Mohan S (2013) The negative impact of single prolonged stress (SPS) on bone development in mice. *Stress* 16(5):564–570.

287. Arai A, et al. (2016) A single prolonged stress paradigm produces enduring impairments in social bonding in monogamous prairie voles. *Behav Brain Res* 315:83–93.

288. Keller SM, Schreiber WB, Staib JM, Knox D (2015) Sex differences in the single prolonged stress model. *Behav Brain Res* 286:29–32.

289. Shansky RM (2015) Sex differences in PTSD resilience and susceptibility: Challenges for animal models of fear learning. *Neurobiol Stress* 1(1):60–65.

290. Zaba M, et al. (2015) Identification and characterization of HPA-axis reactivity endophenotypes in a cohort of female PTSD patients. *Psychoneuroendocrinology* 55:102–115.

291. Jia M, et al. (2014) Corticosterone mitigates the stress response in an animal model of PTSD. *J Psychiatr Res*:1–11.

292. Zuloaga DG, Poort JE, Jordan CL, Breedlove SM (2011) Male rats with the testicular feminization mutation of the androgen receptor display elevated anxiety-related behavior and corticosterone response to mild stress. *Horm Behav* 60(4):380–8.

293. Strekalova T, Spanagel R, Bartsch D, Henn FA, Gass P (2004) Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacology* 29(11):2007–2017.

294. Walsh JJ, et al. (2013) Stress and CRF gate neural activation of BDNF in the mesolimbic reward pathway. *Nat Neurosci* 17(1):27–29.

295. Louvart H, Maccari S, Ducrocq F, Thomas P, Darnaudéry M (2005) Long-term behavioural alterations in female rats after a single intense footshock followed by situational reminders. *Psychoneuroendocrinology* 30(4):316–24.

296. Rex A, Voigt JP, Gustedt C, Beckett S, Fink H (2004) Anxiolytic-like profile in Wistar, but not Sprague-Dawley rats in the social interaction test. *Psychopharmacology (Berl)* 177(1–2):23–34.

297. Vialou V, et al. (2010) DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat Neurosci* 13(6):745–752.

298. Krishnan V, et al. (2007) Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* 131(2):391–404.

299. Dong X, Li Y (2014) Peritraumatic startle response predicts the vulnerability to develop PTSD-like behaviors in rats: a model for peritraumatic dissociation. *Front Behav Neurosci* 8(January):14.

300. Eagle AL, et al. (2013) Single prolonged stress enhances hippocampal glucocorticoid receptor and phosphorylated protein kinase B levels. *Neurosci Res* 75(2):130–7.

301. Myers B, McKlveen JM, Herman JP (2014) Glucocorticoid actions on synapses, circuits, and behavior: Implications for the energetics of stress. *Front Neuroendocrinol* 35(2):180–196.

302. Figueiredo HF, Dolgas CM, Herman JP (2002) Stress activation of cortex and hippocampus is modulated by sex and stage of estrus. *Endocrinology* 143(7):2534–40.

303. Toufexis D, Davis C, Hammond A, Davis M (2005) Sex differences in hormonal modulation of anxiety measured with light-enhanced startle: possible role for arginine vasopressin in the male. *J Neurosci* 25(39):9010–6.

304. Pisu MG, et al. (2016) Sex differences in the outcome of juvenile social isolation on HPA axis function in rats. *Neuroscience* 320:172–182.

305. Vialou V, et al. (2014) Prefrontal Cortical Circuit for Depression- and Anxiety-Related Behaviors Mediated by Cholecystokinin: Role of ΔFosB. *J Neurosci* 34(11):3878–87.

306. Nestler EJ, et al. (2002) Neurobiology of Depression. *Neuron* 34:13–25.

307. Baker KB, Kim JJ (2004) Amygdalar lateralization in fear conditioning: evidence for greater involvement of the right amygdala. *Behav Neurosci* 118(1):15–23.

308. Georgopoulos AP, et al. (2010) The synchronous neural interactions test as a functional neuromarker for post-traumatic stress disorder (PTSD): a robust classification method based on the bootstrap. *J Neural Eng* 7(1):16011.

309. Ritov G, Boltyansky B, Richter-Levin G (2016) A novel approach to PTSD modeling in rats reveals alternating patterns of limbic activity in different types of stress reaction. *Mol Psychiatry* 21:630–641.

310. Kramer MD, Krueger RF, Hicks BM (2008) The role of internalizing and externalizing liability factors in accounting for gender differences in the prevalence of common psychopathological syndromes. *Psychol Med* 38(1):51–61.

311. Zavala JK, Fernandez AA, Gosselink KL (2011) Female responses to acute and repeated restraint stress differ from those in males. *Physiol Behav* 104(2):215–21.

312. Shors TJ, Beylin A V., Wood GE, Gould E (2000) The modulation of Pavlovian memory. *Behav Brain Res* 110(1–2):39–52.

313. Wood GE, Shors TJ (1998) Stress facilitates classical conditioning in males, but impairs classical conditioning in females through activational effects of ovarian hormones. *Proc Natl Acad Sci* 95(7):4066–4071.

314. Kessler RC (2003) Epidemiology of women and depression. *J Affect Disord* 74:5–13.

315. Horesh D, Lowe SR, Galea S, Uddin M, Koenen KC (2014) Gender Differences in the Long-Term Associations Between Posttraumatic Stress Disorder and Depression Symptoms: Findings From the Detroit Neighborhood Health Study. *Depress Anxiety* 11:1–11.

316. van Hasselt FN, et al. (2012) Within-litter variation in maternal care received by individual pups correlates with adolescent social play behavior in male rats. *Physiol Behav* 106(5):701–706.

317. Silver RC, Alison Holman E, McIntosh DN, Poulin M, Gil-Rivas V (2002) Nationwide longitudinal study of psychological responses to September 11. *J Am Med Assoc* 288(10):1235–1244.

318. McFarlane AC (1989) The Aetiology of Post-traumatic Precipitating Morbidity : Factors. *Br J Psychiatry* 154:221–228.

319. Maeng LY, Milad MR (2015) Sex differences in anxiety disorders: Interactions between fear, stress, and gonadal hormones. *Horm Behav* 76:106–117.

320. Shors TJ (2016) A trip down memory lane about sex differences in the brain. *Philos Trans R Soc B Biol Sci* 371(1688):20150124.

321. Wood GE, Beylin A V, Shors TJ (2001) The contribution of adrenal and reproductive hormones to the opposing effects of stress on trace conditioning in males versus females. *Behav Neurosci* 115(1):175–187.

322. Koresh O, et al. (2016) Distinctive cardiac autonomic dysfunction following stress exposure in both sexes in an animal model of PTSD. *Behav Brain Res* 308:128–142.

323. Burke HM, et al. (2013) Sex-specific impairment of spatial memory in rats following a reminder of predator stress. *Stress* 16(4):469–476.

324. Cozzoli D, Tanchuck-Nipper M, Kaufman M, Horowitz C, Finn D (2015) Environmental stressors influence limited-access ethanol consumption by C57BL/6J mice in a sex-dependent manner. *Alcohol* 48(8):741–754.

325. Adamec R, Head D, Blundell J, Burton P, Berton O (2006) Lasting anxiogenic effects of feline predator stress in mice: sex differences in vulnerability to stress and predicting severity of anxiogenic response from the stress experience. *Physiol Behav* 88(1–2):12–29.

326. Daskalakis NP, Cohen H, Cai G, Buxbaum JD, Yehuda R (2014) Expression profiling associates blood and brain glucocorticoid receptor signaling with trauma-related individual differences in both sexes. *Proc Natl Acad Sci* 111(37):1–6.

327. Toth M, et al. (2015) Overexpression of Forebrain CRH During Early Life Increases Trauma Susceptibility in Adulthood. *Neuropsychopharmacology* 41(6):1–10.

328. Knox D, Nault T, Henderson C, Liberzon I (2012) Glucocorticoid receptors and extinction retention deficits in the single prolonged stress model. *Neuroscience* 223:163–73.

329. Farrell MR, Gruene TM, Shansky RM (2015) The influence of stress and gonadal hormones on neuronal structure and function. *Horm Behav* 76:118–124.

330. Goel N, Bale TL (2008) Organizational and activational effects of testosterone on masculinization of female physiological and behavioral stress responses. *Endocrinology* 149(12):6399–405.

331. Serova LI, Harris HA, Maharjan S, Sabban EL (2010) Modulation of responses to stress by estradiol benzoate and selective estrogen receptor agonists. *J Endocrinol* 205(3):253–262.

332. Shors TJ, Leuner B (2003) Estrogen-mediated effects on depression and memory formation in females. *J Affect Disord* 74(1):85–96.

333. Shansky RM, et al. (2010) Estrogen promotes stress sensitivity in a prefrontal cortex-amygdala pathway. *Cereb cortex* 20(11):2560–7.

334. Cavigelli SA, McClintock MK (2003) Fear of novelty in infant rats predicts adult corticosterone dynamics and an early death. *Proc Natl Acad Sci U S A* 100(26):16131–6.

335. Mcteague LM, et al. (2010) Aversive Imagery in Posttraumatic Stress Disorder : Trauma Recurrence , Comorbidity , and Physiological. *Biol Psychiatry* 67(4):346–356.

336. Fenchel D, et al. (2015) Beyond the HPA-axis: the role of the gonadal steroid hormone receptors in modulating stress related responses in an animal model of PTSD. *Eur J Psychotraumatol* 3(0):1–14.

337. Farrell MR, Holland FH, Shansky RM, Brenhouse HC (2016) Sex-specific effects of early life stress on social interaction and prefrontal cortex dendritic morphology in young rats. *Behav Brain Res* 310:119–125.

338. Holland FH, Ganguly P, Potter DN, Chartoff EH, Brenhouse HC (2014) Early life stress disrupts social behavior and prefrontal cortex parvalbumin interneurons at an earlier time-point in females than in males. *Neurosci Lett* 566:131–136.

339. Archer J (1975) Rodent Sex Differences in Emotional and Related Behavior I Ethology and Neurophysiology Group , School of Biological Sciences , University of Sussex ,. *Neurophysiology* 479(4298):451–479.

340. Brewin CR, Holmes E a (2003) Psychological theories of posttraumatic stress disorder. *Clin Psychol Rev* 23(3):339–376.

341. Korte SM, Koolhaas JM, Wingfield JC, McEwen BS (2005) The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. *Neurosci Biobehav Rev* 29(1):3–38.

342. Shors TJ, et al. (2007) Neurogenesis and helplessness are mediated by controllability in males but not in females. *Biol Psychiatry* 62(5):487–95.

343. Nasca C, Bigio B, Zelli D, Nicoletti F, McEwen BS (2015) Mind the gap: glucocorticoids modulate hippocampal glutamate tone underlying individual differences in stress susceptibility. *Mol Psychiatry* 20(6):755–763.

344. Southwick SM, Bonanno GA, Masten AS, Panter-brick C, Yehuda R (2014) Resilience definitions, theory, and challenges: interdisciplinary perspectives. *Eur J Psychotraumatol* 1:1–14.

345. Newport DJ, Heim C, Bonsall R, Miller AH, Nemeroff CB (2004) Pituitary-adrenal responses to standard and low-dose dexamethasone suppression tests in adult survivors of child abuse. *Biol Psychiatry* 55(1):10–20.

346. Stein MB, Yehuda R, Koverola C, Hanna C (1997) Enhanced dexmethasone suppression of plasma cortisol in adult women traumatized by childhood sexual abuse. *Biol Psychiatry* 42(96):680–686.

347. Delahanty DL, Nugent NR, Christopher NC, Walsh M (2005) Initial urinary epinephrine and cortisol levels predict acute PTSD symptoms in child trauma victims. *Psychoneuroendocrinology* 30(2):121–128.