ABSTRACT
Altered stress reactivity is a predominant feature of posttraumatic stress disorder (PTSD) and may reflect disease vulnerability, increasing the probability that an individual will develop PTSD following trauma exposure. Environmental factors, particularly prior stress history, contribute to the developmental programming of the hypothalamic-pituitary-adrenal stress axis. Critically, the consequences of stress experiences are transgenerational, with parental stress exposure impacting stress reactivity and PTSD risk in subsequent generations. Potential molecular mechanisms underlying this transmission have been explored in rodent models that specifically examine the paternal lineage, identifying epigenetic signatures in male germ cells as possible substrates of transgenerational programming. Here, we review the role of these germ cell epigenetic marks, including posttranslational histone modifications, DNA methylation, and populations of small noncoding RNAs, in the development of offspring stress axis sensitivity and disease risk.

Keywords: Animal models, Epigenetics, HPA axis, PTSD, Stress, Transgenerational

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Posttraumatic stress disorder (PTSD), an anxiety disorder triggered by exposure to a severe traumatic event, presents in only a subset of individuals who experience such a stressor (1). Individual differences in stress sensitivity, primarily regulated by the hypothalamic-pituitary-adrenal (HPA) neuroendocrine stress axis, may be an underlying component of this variability in disease risk (2). In fact, disruption of stress neurocircuity has been characterized as a pretrauma vulnerability that increases the probability of developing PTSD, rather than a symptom that develops in response to a stressful experience (3). In particular, PTSD has been associated with a unique stress response profile, including low levels of plasma cortisol and increased noradrenergic and adrenergic activity, although these findings are not universal (4). The development of stress reactivity, and its potential consequences on PTSD predisposition, occurs through a dynamic interplay of genetic and environmental factors (5). The role of the environment has been increasingly emphasized, with prior stress exposure highlighted as one of the strongest contributors to later stress sensitivity and PTSD presentation (6).

The behavioral and neuroendocrine consequences of stress exposure, as well as an increase in PTSD risk, have been observed not only in individuals directly exposed to stress but also in their children (7). Potential mechanisms of this experience-dependent transgenerational transmission or the reprogramming of offspring behavior and physiology in response to the parental environment have been investigated in rodent models (8). Studies investigating maternal or paternal experience occurring before offspring conception, so-called lifetime exposures, suggest that transmission occurs through an epigenetic reprogramming of germ cells (9–13). In this review, we focus on these parental lifetime stress experiences and their consequences on subsequent generations’ stress reactivity in both humans and animals, and we consider potential molecular mechanisms of transmission, highlighting the role of germ cell epigenetic marks and how these signatures may alter offspring development to confer disease risk or resilience. While maternal stress during pregnancy also impacts offspring stress responses, such experience likely does not rely on germ cell reprogramming and has been expertly reviewed elsewhere [see (14,15)] and thus will not be included in our discussion.

PARENTAL STRESS AND PTSD RISK
Epidemiological studies have offered strong evidence supporting altered offspring stress reactivity and neuropsychiatric disease risk following maternal or paternal lifetime stress exposure. For instance, adult children of Holocaust survivors were more likely to be diagnosed with a psychiatric disease such as depression, anxiety, and PTSD (16–18), and offspring disease risk was found to increase similarly following parental exposure to abuse or war-related trauma (19–22). In these studies of parental lifetime stress experience, offspring disease risk may have increased as a consequence of offspring HPA stress axis reprogramming (23–25). In fact, maternal PTSD among Holocaust survivors has been associated with increased offspring sensitivity to glucocorticoid stress

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Parental Stress and PTSD Risk

Parental stress and posttraumatic stress disorder (PTSD) risk have been widely investigated due to their potential impact on future generations. Notably, the transgenerational impact of parental lifetime stress is not restricted to the perinatal window and changes in offspring stress-related behavior and physiology have been reported following parental exposure stress through adolescence or in adulthood (12, 45, 46). For example, in our lab, male exposure to chronic variable stress either over the pubertal window or only in adulthood programmed a blunted HPA stress axis response in male and female offspring, a stress phenotype reflecting that observed in PTSD (11). While sex-specific effects reported in some rodent models offer the intriguing possibility that parental experience contributes to sex differences in stress responsivity and in humans, disease risk, the absence of these effects in other models contrasts this hypothesis. Further study of behavioral and physiological phenotypes in both male and female offspring will clarify potential sex-specific vulnerabilities as well as mechanisms by which they may be programmed.

Potential modes of transgenerational transmission have been investigated in rodent models specifically examining the paternal lineage, where the relative exclusion of behavioral and environmental factors affords the mechanistic evaluation of epigenetic marks in sperm, a readily accessible tissue (47). By contrast, transmission through the maternal lineage relies on the complex maternal-fetal/neonatal interaction, where changes in the intrauterine environment, parturition, lactation, and early maternal care may impact stress sensitivity in future generations (48). Few studies have investigated animal models of maternal stress exposure before offspring conception (12, 49), likely due to the confounding effects of the maternal milieu and behavior. Additionally, evaluation of potential epigenetic marks in these studies would require superovulation, a hormone-dependent process that may itself change marks in oocytes (50).

In paternal stress studies, epigenetic signatures in sperm have been highlighted as a likely substrate of offspring reprogramming (11, 13, 51), supported by evidence of altered patterns of retained histone modifications, DNA methylation, and/or populations of small noncoding RNAs in germ cells following diverse paternal insults (52–58). Though behaviorally mediated mechanisms of transmission have been proposed in paternal studies, such as potential shifts in maternal investment in response to a perception of mate quality or the role of paternal behavior (59, 60), laboratory rodents typically are not bi-parental. Male rodents do not participate in rearing offspring, and male-female interactions can be limited to defined breeding windows to control for confounding effects of the male rodent’s impact on the dam (47). Further, artificial reproductive techniques including in vitro fertilization and zygote microinjection have been used to directly assess epigenetic transmission through the male germ line, demonstrating the role of sperm epigenetic marks in transgenerational reprogramming (13, 45, 55). Recent development of enzymes capable of site-specific epigenetic modification may offer additional opportunities to investigate the role of specific epigenetic signatures in the sperm in the transgenerational transmission of paternal stress experience (61, 62).

**ANIMAL MODELS OF LIFETIME STRESS EXPOSURES**

The first evidence of the transgenerational effects of parental lifetime stress experience in an animal model was reported nearly half a century ago, where exposure of adult female rats to stress before mating altered offspring behavioral stress responses, increasing exploratory behavior in a novel environment through two subsequent generations (41). In the years since, significant progress has been made in understanding mechanisms by which parental experience reprograms offspring stress-related behaviors and physiology, afforded by extensive evidence of offspring reprogramming in response to parental lifetime stress. For example, in our model of early prenatal stress, exposure to chronic variable stress in utero increased male HPA stress axis reactivity and altered male stress coping behaviors, including increased immobility in the tail suspension test, and these phenotypes transmitted to the next generation through the male lineage (9, 42). Postnatal stress has also been shown to induce stress dysregulation in subsequent generations, including observations of behavioral deficits on the forced swim task and decreased blood glucose in response to acute restraint in first and second generation offspring of male mice exposed postnatally to unpredictable maternal separation with maternal stress (10, 13, 43, 44).

Notably, the transgenerational impact of parental lifetime stress is not restricted to the perinatal window and changes in offspring stress-related behavior and physiology have been reported following parental exposure stress through adolescence or in adulthood (12, 45, 46). For example, in our lab, male exposure to chronic variable stress either over the pubertal window or only in adulthood programmed a blunted HPA stress axis response in male and female offspring, a stress phenotype reflecting that observed in PTSD (11). While sex-specific effects reported in some rodent models offer the intriguing possibility that parental experience contributes to sex differences in stress responsivity and in humans, disease risk, the absence of these effects in other models contrasts this hypothesis. Further study of behavioral and physiological phenotypes in both male and female offspring will clarify potential sex-specific vulnerabilities as well as mechanisms by which they may be programmed.

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RNA populations—have been proposed as likely substrates of transgenerational transmission, noting their potential ability to both respond to paternal stress experience and reprogram offspring stress reactivity (63). Evidence suggests that these germ cell marks are continuously vulnerable to environmental perturbations, as altered offspring phenotypes following an initial paternal stress experience prenatally, postnatally, in adolescence, or in adulthood have been reported (9,11,13,45,51). However, the effects of stress experience on the germ line likely depend on the timing and duration of exposure. Gestational or early life exposures likely reprogram primordial germ cells in the embryonic gonadal ridge or immature postnatal testis, and insults later in life may instead impact germ cells further along in spermatogenesis (64). Once sperm are fully mature, they are stored in the epididymis, where epigenetic marks are thought to be largely impervious to change or to the environment (65), although limited evidence of intercellular communication between mature sperm and the epididymal epithelial cells suggests that small noncoding RNA populations in mature sperm may still be altered (66,67). In the following sections, we explore evidence of transgenerational transmission through the three classes of epigenetic marks in sperm cells, examining their regulation through spermatogenesis and highlighting potential consequences on offspring reprogramming.

**Posttranslational Histone Modifications**

The basic unit of chromatin is the nucleosome, which consists of DNA tightly bound to a core of eight histone proteins. Covalent modifications, such as acetylation, methylation, or ubiquitination, of the N-terminal tails of these histone proteins determine the accessibility of chromatin and broadly regulate transcription (68). Until recently, the code of posttranslational modifications on paternal histone proteins was considered to be lost during the process of spermatogenesis, when chromatin compaction is achieved through the incorporation of the unique nucleosome proteins known as protamines, suggesting therefore that these epigenetic marks could not directly mediate transgenerational transmission (69,70). However, studies in C. elegans and Drosophila found that a subset of histone modifications persists through spermatogenesis and fertilization and may act as a substrate of epigenetic inheritance (71,72). Notably, a small percentage of histone proteins and their modifications have also been observed in condensed mammalian sperm, with approximately 1% of histone proteins retained in rodents and 10% in humans (73). Histone retention was found to occur preferentially at genomic regions critical to embryogenesis, including developmental gene promoters, microRNA clusters, and imprinted loci, supporting the potential of posttranslational histone marks to affect paternal control over early offspring development (73–75). Future studies will need to examine the mechanisms by which paternal stress experience may influence posttranslational modifications of retained histone proteins and their ability to alter their offspring’s phenotype.

**DNA Methylation**

Methylation of cytosine residues at cytosine-phosphate-guanine dinucleotides, as well as non-cytosine-phosphate-guanine dinucleotide sites, in sperm DNA has been characterized as a potential substrate of transgenerational transmission, with reports associating changes in specific or global methylation patterns in sperm with offspring phenotypes following early life stress (10,44) or adult glucocorticoid administration (51). Paternal exposure to cocaine self-administration or olfactory fear conditioning has been similarly associated with changes in sperm DNA methylation patterns (53,55). These environmental cues may reprogram DNA methylation in sperm through the direct interaction with germ cell membrane receptors, as has been proposed for cocaine (76,77), or through activation of complex signaling cascades, perhaps involving the metabolic regulation of spermatogenesis by supportive Sertoli cells (78).

**Sperm RNAs**

The third class of epigenetic mark implicated in transgenerational transmission, sperm RNA populations, was first proposed in C. elegans, where induction of RNA interference induced gene silencing through four to five generations in the paternal lineage (83). More recently, messenger RNA (mRNA) and small noncoding RNA populations have been identified in mammalian sperm (84–86), where changes in RNA populations have been implicated in the programming of offspring stress-related phenotypes following paternal stress exposure, including HPA axis hyporeactivity (11,13). In fact, total RNA isolated from the sperm of stressed sires and injected into zygotes was shown to recapitulate aspects of the offspring phenotype (13). Sperm mRNA populations may mediate transgenerational transmission to some extent (87); however, due to their key
role in regulating embryogenesis, small noncoding RNA populations have emerged as a primary mediator of transgenerational reprogramming (88).

Populations of small ～22 base pair RNA molecules known as microRNA are essential to early embryogenesis, where they regulate the selective degradation of stores of maternal mRNA in the zygote through the activity of the RNA-induced silencing complex (RISC) (88,89). Loss of Dicer, the protein that preprocesses microRNA and loads them into the RISC, or of Argonaute 2, the functional component of the RISC with endonuclease activity, in mouse oocytes results in early embryonic lethality (90,91). As each microRNA directly targets hundreds of different mRNAs, disruption of specific microRNA may elicit profound programmatic and developmental effects (92,93). Recently, the targeted silencing of miR-34, a microRNA present in sperm but not oocytes, was reported to significantly restrict early embryogenesis, supporting a critical role of paternal microRNA in offspring development (94). We have also proposed more subtle, long-term functions of sperm microRNA, associating a significant increase in nine specific sperm microRNA in a model of paternal stress with an altered HPA stress axis in their adult offspring (11).

Emphasizing the interdependence of epigenetic machinery, the increase in specific microRNA in our model of paternal stress represented a larger transcriptional shift, such as one mediated by underlying histone modification or DNA methylation patterns (11). This crosstalk between microRNA and other epigenetic marks may be crucial for the transgenerational transmission of paternal stress. The genomic loci of sperm microRNA are associated with regions of retained histone proteins, hypomethylation, and the histone mark H3K4me3 (86), suggesting that the expression of sperm microRNA may be controlled by these upstream epigenetic marks. Additionally, postfertilization, microRNA may program lasting effects by inducing transcriptional activation or repression within the nucleus through other epigenetic mechanisms (95). In fact, microRNA can regulate de novo methylation in embryonic stem cells (96).

Far less is known about the potential role of PIWI-associated RNAs (piRNAs) as a potential mediator of epigenetic inheritance. piRNAs are small ～30 base pair noncoding RNAs expressed mostly in spermatids at much higher levels than microRNA and primarily function to silence transposable elements during spermatogenesis (97,98). piRNAs have been associated with transcriptional silencing in C. elegans through multiple generations (99), but their role in mammalian epigenetic inheritance requires further study (100).

### INFLUENCE OF GERM CELL MARKS ON OFFSPRING NEURODEVELOPMENT

The mechanisms by which sperm epigenetic marks program lasting consequences on offspring stress reactivity and shape disease susceptibility or resilience remain unclear. In this section, we explore potential pathways by which these molecular signatures may facilitate brain reprogramming, specifically how germ cell marks may influence maturation of the HPA axis and behavioral stress responses. Activation of the HPA axis is critical for the body’s physiological response to homeostatic challenge, and as described earlier, stress axis dysregulation is a predominant feature of neuropsychiatric diseases, including PTSD (7). Importantly, transgenerational effects of parental lifetime stress experience on offspring stress reactivity may be adaptive or maladaptive depending on the environmental context (101).

#### Epigenetic Marks Persist from Sperm to Brain

Transgenerational models of parental lifetime exposures first suggested that an initial epigenetic mark in germ cells may persist throughout developmental stages and into the adult brain, where it was directly or indirectly responsible for changes in behavior or physiology (Figure 1). For example, in a mouse model of early life stress, lasting changes in DNA methylation patterns were reported, with the methylation status of two specific genes, Chvr2 and Mecp2, altered in both the sperm of the stressed dads and in the brains of their female offspring (10). The persistence of microRNA changes has also been observed, where an increase in miR-339 initially identified in the sperm from stressed sires was similarly detected in their offspring’s hippocampus and associated with offspring behavioral changes (13).

Evidence from models of diverse paternal exposures corroborates the potential of epigenetic marks to persist from the mature sperm into the adult brain first observed following early life stress (44,53–55,57), and the specificity and permanence of the proposed epigenetic signatures are remarkable. The precise activation of sperm epigenetic machinery at genetic loci involved in stress axis regulation suggests a very specific targeting mechanism that stands in contrast to the more commonly characterized role of epigenetic marks as a broad transcriptional control mechanism (69). However, such limited epigenetic changes reported may actually be representative of more global modifications that were not examined in these studies. Second, as gametes fuse to form the zygote, which is a pluripotent cell that gives rise to all differentiating cell lineages, an epigenetic modification that persists from sperm into neurons may be expected to propagate universally (63). Studies have reported correlations in epigenetic signatures between peripheral tissues such as white blood cells and postmortem brain tissues of psychiatric patients (102,103), supporting the persistence of germ cell changes and offering a potential biomarker of brain epigenetic status or parental experience. However, others have described significant between-tissue variability (104,105), perhaps because various cell types may not uniformly maintain inherited epigenetic marks across development (106,107). Such differential maintenance of epigenetic marks may underlie the tissue or brain region-specific outcomes observed in offspring following parental stress (13,44).

#### Sperm Epigenome Alters the Trajectory of Offspring Development

Alternatively, a germ cell epigenetic mark programmed by parental environmental exposure may initiate a cascade of molecular events in the early zygote, eventually altering offspring development and affecting long-term changes in offspring behavior or physiology (Figure 1). Evidence of dissimilar epigenetic signatures in sperm and offspring brain tissue...
following paternal perturbation supports the notion of a cascading signal (9,52,58,108). For example, our model of paternal stress elicited an increase in microRNA populations in sperm but did not observe parallel changes in these miRs in key stress regulatory brain regions examined, the paraventricular nucleus and bed nucleus of stria terminalis (11). We instead found a broad reduction in gene expression in these regions, reflecting an as yet unknown upstream epigenetic mechanism in the adult brain that was likely responsible for the reported alterations in offspring HPA axis reactivity (11). Similarly, in a recent study examining the specific role of stress hormones in programming these epigenetic signatures in both sperm and offspring tissues, different methylation patterns were observed in the sire sperm and the offspring hippocampus or kidney following glucocorticoid administration (51).

Mechanistically, it is likely that epigenetic alterations in the zygote subtly shift developmental processes, where slight changes in cell proliferation, migration, or differentiation are the next step in the molecular cascade from the sperm epigenome to offspring brain function (65). The links between a germ cell epigenetic mark and eventual changes in offspring stress responsivity are no doubt complex and currently unknown, though recent evidence of brain microRNA regulating behavioral responses to chronic social defeat has emphasized the continual importance of epigenetic mechanisms in offspring stress susceptibility or resilience (109). A careful stepwise approach can forward our understanding of this cascade of molecular events and offer insight into transgenerational consequences of parental stress experience on offspring disease risk. Evidence of altered HPA axis regulation following maternal or paternal exposure to such diverse challenges as alcohol, lead, chronic stress, and social defeat suggests that the developing hypothalamus, the brain region ultimately responsible for stress axis reactivity, may be particularly susceptible to parental experience (42,110–115).

CONCLUSION

It has been estimated that nearly 75% of the population experience a severe traumatic event in their lifetime, yet only a small percentage of these individuals will subsequently present with PTSD (116). Parental lifetime stress exposures, likely communicated to offspring through epigenetic marks in germ cells, contribute to the programming of subsequent generations’ stress axis development and reactivity, factors critical in individual disease vulnerability to PTSD, as highlighted in this special issue but equally relevant to a wider array of neuropsychiatric diseases. In this light, the role of experience in conferring disease risk or resilience spans wider than the lifetime of an individual to encompass the lifetime of stress experienced by his or hers parents or grandparents and perhaps generations before that. A growing appreciation for epigenetic inheritance as a source of individual variability will influence the future of clinical diagnosis and treatment of PTSD, affording the identification of at-risk individuals and allowing preventative or early intervention following trauma exposure.

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Parental Stress and PTSD Risk


