This video series was made possible through a generous grant from the National Institute of General Medicines (Grant Number: 5 R25 GM133017-03), awarded to Cohen Veterans Bioscience (Principal Investigator: Chantelle Ferland-Beckham, PhD)

Module 1, Video 5: Sex differences in stress reactivity and its implications for neuropsychiatric diseases

Similar to humans, laboratory animals are constantly surveying their environment and determining whether changes introduce a potential threat—real or perceived—to bodily processes. This process is fairly conserved among mammals. Information from the environment is judged as stressful based on sensory input such as what we see and what we hear, AND on what we remember from previous similar encounters. If the situation is determined to be threatening, the body responds by activating several systems, including central systems to increase arousal and attention, the autonomic nervous system, which initiates the fight or flight response, and the endocrine system, which includes the structures that make up the Hypothalamic—Pituitary—Adrenal axis, or HPA axis. The reactions of these various systems, including the HPA axis, form the basis for how our brains and bodies perceive, react to and behave in response to a stressor. But emerging evidence from both humans and animals suggests that the response to different types of stressors is different in males and females. In this video, we will introduce sex differences in behavioral and neurological responses to different types of stressors, with a focus on the role of the HPA axis. We will also discuss how sex differences in stress reactivity may mediate observed differences in the prevalence of various neuropsychiatric diseases.

Activation of the HPA axis in response to real or perceived stress initially involves the release of CRH and vasopressin by the hypothalamus. Rises in CRH and vasopressin trigger ACTH release from the pituitary gland, which then acts on the adrenal medulla, culminating in the release of glucocorticoids—mainly corticosterone in rodents and cortisol in humans. Glucocorticoids in turn feedback onto the hypothalamus and pituitary gland by acting on two types of glucocorticoid receptors—the glucocorticoid receptor and mineralcorticoid receptor. This feedback modulates activity of the HPA axis in what is termed a negative feedback loop.

Glucocorticoids act on virtually all tissues of the body, including the brain, inducing physiological and behavioral changes necessary for survival. In rodents, there are prominent sex differences in how the HPA axis responds to stress: For example, females typically have a more robust HPA response following exposure to a number of different types of acute stressors, as evidenced by their increased corticosterone and ACTH levels [1-5].

Sex differences at each level of the HPA axis, as well as in other limbic structures that regulate activation of the HPA axis such as the amygdala, likely underlie these

differences between males and females. The expression of HPA axis-related genes also differs between males and females. Female rats show greater mRNA expression of vasopressin and CRH in the paraventricular nucleus of the hypothalamus, and greater mRNA expression of the ACTH precursor, POMC, in the anterior pituitary following acute stress compared with males [1, 3, 4, 6].

As important as activation of the HPA axis is to survival, being able to shut off the response of the HPA axis after cessation of the stressor is equally important. Shut off of the HPA axis occurs through negative feedback of glucocorticoids on brain regions critical to HPA axis initiation. In addition to a more robust HPA response to stress, females also show a delayed return to baseline after exposure to an acute stressor, indicating sex differences in glucocorticoid-mediated HPA negative feedback [1-4]. These differences may be due to underlying differences in glucocorticoid receptor activation of the limbic structures known to inhibit the HPA axis, including the frontal cortex, cingulate cortex, piriform cortex, and hippocampus [8]. Females show both lower glucocorticoid binding in the hypothalamus [9] as well as a lower density of both types of glucocorticoid receptors in the pituitary compared to males [10]. Furthermore, glucocorticoid receptor mRNA regulation in response to acute stressors varies between males and females in brain structures that regulate HPA activity, with higher glucocorticoid receptor mRNA levels in the hypothalamus of male rats compared to female rats [11, 12].

Sex differences in HPA regulation are further highlighted by brain region-specific glucocorticoid receptor knockout studies. For example, the loss of glucocorticoid receptors in the forebrain of male mice leads to HPA axis dysregulation in males but not females, as evidenced by increased morning corticosterone levels at baseline and changes in the time-dependent corticosterone release pattern in response to an acute stress challenge [13]. Selectively deleting glucocorticoid receptors in the paraventricular nucleus of the hypothalamus also increases ACTH and corticosterone levels in knockout male mice versus wild-type mice, but no such change is observed in females [14]. Taken together, sex differences in stress reactivity and inhibitory control may be brain region specific.

Sex differences in the neuroendocrine response to acute stress during adulthood are mediated in part by interactions between the HPA axis and the neuroendocrine system that controls reproduction. This system is known as the hypothalamic–pituitary–gonadal (or HPG) axis [15]. The HPG axis is responsible for the production and secretion of testosterone and estrogens from the testes and ovaries, respectively. Both testosterone and estradiol can in turn modulate the HPA axis in adulthood and contribute to sex-specific differences in HPA function.

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As mentioned in Video 3, sex hormone receptors are distributed widely throughout the brain, including in key parts of the neural circuitry controlling the HPA axis. This allows gonadal steroids to modify the neuroendocrine response to stress in parallel to changes in reproductive function. Variations in sex hormone receptor expression across brain regions may ultimately explain why males and females show different stress responses. For example, neurons in the paraventricular nucleus of the hypothalamus, an area that directly regulates HPA axis activity, show robust expression of estrogen receptor beta and G-protein-coupled estrogen receptors, but limited androgen receptor expression. Estradiol has been shown to alter HPA axis function either directly via the paraventricular nucleus or indirectly through brain regions that project to the paraventricular nucleus; in contrast, androgens, predominantly modulate HPA function through indirect mechanisms [2]. Thus, sex hormones modulate HPA activity through distinct mechanisms [2, 16].

Changes in HPA axis function may also fluctuate across the estrous cycle. Studies show that as estradiol levels increase during proestrus in female rats, basal and stress-induced activity of the HPA axis also increases. For example, female rodents in diestrus—which is characterized by low estradiol levels—show similar resting glucocorticoid secretion and a similar HPA on-off response to stress as males [18, 19]. By proestrus, which is characterized by high estradiol and progesterone levels, and estrus, which occurs just following the estradiol peak, females exhibit higher basal and stress-induced ACTH and corticosterone levels [18-20]. The highest levels of HPA output are observed on proestrus morning, when estradiol levels are at their peak, but elevations in progesterone have not yet occurred. Female rats in proestrus and estrus also have a delayed return to baseline glucocorticoid secretion following stress [18, 19].

This may be due to less robust glucocorticoid negative feedback on the HPA axis and/or decreased input from limbic structures that are known to inhibit the HPA axis [8, 19]. Overall, changes in HPA axis function across the reproductive cycle highlight the need to consider variations in estradiol when examining mechanisms or behaviors with known or potentially unknown interactions with the HPA axis.

While outside of the scope of this video series, it should also be noted that the HPA axis is not immune to the effects of androgens. Additionally, there are also organizational effects of sex hormones during key developmental stages [21, 22], as well as effects of puberty, where rises in sex steroids in both scenarios can drive permanent changes in brain HPA function [23].

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How does all of this relate to stress- and trauma-associated diseases? Our ability to adapt to acute stressors provides an evolutionary advantage, allowing us to mobilize resources when necessary to survive. But chronic or repeated exposure to stress can drive constant activation of the HPA axis, which is detrimental and leads to an increased risk of disease, particularly psychiatric disorders [24, 25].

Females show a higher incidence of many psychiatric disorders known to be precipitated or worsened by stress, and they also show different responses to stressors in terms of their coping behavior. For example, consistent with mice, men exposed to acute social stress have impaired cognitive flexibility while women do not [26, 27]. Stress habituation, or the ability to adapt to a repeated homotypic stressor, is also lower in females compared to males; this process is known to be influenced by estrogens [28-31]. Finally, research suggests that corticosterone levels do not predict depressive-like behavior in female rodents, as they do in males [32].

But historically, females have been largely omitted from this line of research. The underlying mechanism of how chronic stress exposure leads to long-terms changes in HPA reactivity and the activation of other systems as well as the role of sex hormones is unknown. But better diagnoses and treatments for these diseases, especially in women, cannot be achieved until we know this information [33]. Animal models still offer the best hope for understanding these mechanistic processes but some challenges exist: Many of these challenges, including the need for more robust and better validated animal models, will be discussed in more detail in Video 17. But one of the most important challenges in modeling psychiatric diseases is the heterogeneity of these disorders—between AND within males and females—including the patterns of stress-induced neuronal activity in the brain. This heterogeneity is difficult to capture in animals, as most animal models use highly homogenous conditions characterized by genetic uniformity. Understanding the heterogeneity of how stress responses and coping behaviors differ across the full spectrum of males and females is important for discovering better therapeutics for mood and anxiety disorders. Therefore, better preclinical models designed to study and capture clinical heterogeneity are urgently needed [32, 34-36].

As we have shown in this video, males and females show vastly different responses to stress, mediated in part by underlying differences in neural circuitry related to the stress system as well as the interplay of sex hormones with critical brain regions. As psychiatric disorders affect nearly 500 million individuals worldwide and are nearly two to three times more prevalent in females [37], studying the effects of stress in both males and

females is critical to revealing the mechanism of and developing diagnostics and therapeutics for these conditions.

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