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Coupling of the HPA and HPG Axes

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Abstract

The Hypothalamic-Pituitary-Adrenal (HPA) and –Gonadal (HPG) axes have been considered mutually inhibitory; however, emerging evidence supports the proposition that this might not necessarily be the case. This idea is termed "coupling," in which the HPA-HPG axis are mutually activated or deactivated. Coupling is examined across three data sets with different time-courses of stress exposure, and results demonstrate HPA-HPG co-activation occurs. Furthermore, stress exposure influences this relationship. The discussion shows how it is physiologically possible to have positive coupling or co-activation between these axes according to complex regulatory feedback systems and overlapping neural structures. Findings are interpreted developmentally, because adolescence may be a critical time for this co-activation to occur. Finally, the discussion emphasizes an individual difference perspective because each individual differs in the duration and type of stress they experience, and these exerted individualized effects on HPA-HPG coupling.

KEYWORDS: Stress; HPA; HPG; Coupling; Cortisol; Testosterone

Introduction

The current project examines the manner in which Hypothalamic-Pituitary-Adrenal and –Gonadal axes fluctuate with one another. The interplay between adrenal and gonadal axes will be examined across three data sets, which include repeated hormone assessment of both axes. Historically, hormone axes have been examined in isolation, but an emerging perspective suggests that it is worthwhile to analyze them in conjunction with one another – to look at the manner in which axes are 'coupled' together. Coupling makes sense from the point of view of functional interconnections between the HPA and HPG axes, and from a developmental point of view. Furthermore, it is likely that stress exposure would influence the degree of coupling between the HPA in HPG axes, magnifying the individual differences in HPA and HPG coupling. The study examines stress reactivity, short-term stress, and long-term stress exposure, as stress is posited to influence the relationship between HPA and HPG axes.

Across development, a great deal of reciprocal communication goes on between the HPA and HPG axes, their end-products, and their up-stream regulatory structures (Viau, 2002). This high level of cross-axis communication, at multiple stages, is a possible mechanism by which coupling could occur. There is also a developmental reason to expect both the HPA and HPG axes to be mutually up-regulated during adolescence. This stage of development constitutes a time of great stress (and thus activation of the HPA axis) and also a time when the HPG axis goes from a dormant role to an activational role, and testosterone secretion from the gonads increases dramatically. Adolescence is a critical period during development: a period where (1) the body is re-

organizing itself and physiology is changing and adapting nearly as rapidly as intrauterine change; (2) the HPG axis takes on a vastly larger role than it does post-neonatally; and (3) environmental information is integrated for both short-term activation and long-reorganization of a wide variety of physiological processes. The malleable nature of the HPA and HPG axes during adolescence make this an excellent period in time to integrate environmental influences. It is reasonable to think that the body could allow the HPA and HPG axes to work together and be co-activated in order to adapt to environmental influences. The present study examines adolescent populations in two of the three studies to investigate this developmental view.

Stress exposure has been shown to influence both the HPA and HPG axes as they develop. Due to the highly interconnected nature of these axes (described below), it can be surmised that stress should also influence the way they interact with one another. Stress exposure, however, is a highly heterogeneous concept, with varying levels of chronicity and severity, and it is unlikely that stress exposure would exert a uniform or ubiquitous effect. Stress exposure likely impacts the way the HPA and HPG axis are coupled in different ways depending on the timecourse (i.e., chronicity) and severity of stress exposure. Three studies examined in this thesis vary systematically according to the timecourse of stress exposure.

In order to build a picture of the mechanism and context underlying coupling, we will look in depth at the interconnections between the HPA and HPG axes. Specifically we will consider the way that these interconnections are understood at present and the role specific hormones play physiologically, with the goal of building a cohesive picture of cross-axis communication. Three data sets with different indices of stress (short-term,

long-term, and reactivity) are analyzed. The core hypothesis of this thesis is that *positive coupling of the HPG and HPA axes occurs in certain contexts. A secondary hypothesis is that the strength of this association between hormones may be influenced by stress exposure.*

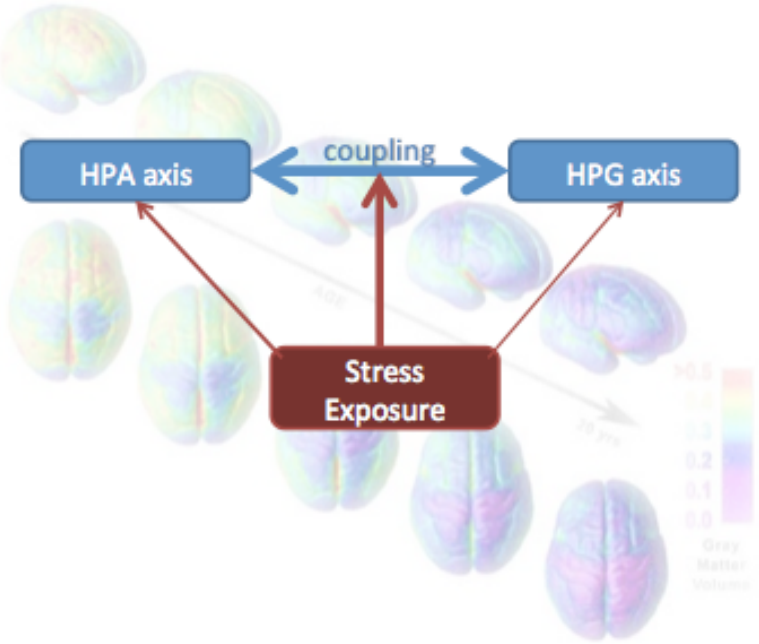

Figure 1: Stress exposure influences the HPA and HPG axes individually, as well as the communication between the two, possibly allowing coupling of the HPA and HPG axes. Adolescence is a period where this interaction could be especially tractable.

Stress, Hormones, and Development

Stress exposure is a possible moderator of HPA-HPG coupling which influences the degree of HPA and HPG axis activation and cooperation. Stress is a classic concept

which has evolved over time. In 1936, Hans Selye posited that stress had two components: its effects on the body, which he called General Adaptation Syndrome, and the long-term effects of those changes, which can result in pathology (Selye, 1936). Selye was building off the ideas of pioneers that had come before him – namely Claude Bernard (who pioneered the idea of homeostasis - the notion that the body has certain fixed points of optimum functioning it strives to maintain) and Walter Cannon (the first person to use the term fight or flight response). In 1988, the ideas underlying stress and physiological reactivity to the environment were refined further, when the concept of allostasis was first introduced. Allostasis expanded homeostasis theory by postulating that the body does not maintain fixed points, but instead continuously fluctuates in response to the world around it – that the body achieves 'stability through change'(Sterling & Eyer, 1988) . The ability of the body to adapt to the environment is a necessary prerequisite for coupling of the HPA and HPG axes, and is a core tenet of the way scientists view the stress response system.

Current conceptualizations of the stress response system (SRS) have progressed beyond allostatic theory. The adaptive calibration model (ACM) of stress responsivity integrates many of the ideas mentioned above into a theoretical framework which focuses on adaptation and the ability of the body to recalibrate itself in response to the changing world around it (Del Giudice, Ellis, & Shirtcliff, 2011). The ACM views the stress response system as having three overall functions: (1) to coordinate allostatic response to challenges, (2) to process information about the organism's environment, and in so doing, adjust how open to the environment that organism is, and (3) to regulate physiology and behavior such that optimal levels of functioning are achieved.

The ACM postulates that there are certain 'switch points' during development that optimize the trade-offs between the 'costs' of growth and maturation needs and the 'costs' associated with adapting to the environment. Gonadarche – the large increase in circulatory testosterone that signals the onset of puberty – is thought to operate as such a switch point, in part because the timing of this event is partly controlled by stress exposure. According to Del Giudice and colleagues, 'a switch-point is controlled by a condition-sensitive, quantitatively variable regulatory mechanism…' and as such both the timing and nature of this particular switch (and others) are subject to change under the influence of environmental stressors (Del Giudice, Ellis, & Shirtcliff, 2011). The activation of switch points has lasting consequences, and hormones often control these shifts (Del Giudice et al., 2011).

In adolescence, the HPG axis comes 'on-line' as part of the developmental switch-point of gonadarche. Furthermore, there is a great deal of cross-axis communication between the HPA and HPG axes. They both are modulated by stress exposure, and they modulate each other's activity (Viau, 2002). There is certainly a rapid change that takes place in the HPG axis during adolescence. In addition, developmental changes emerge in the HPA axis. It is reasonable to suspect that the timing and impact of this switch point on HPA and HPG development could be influenced be stress exposure, and, most importantly for this thesis, that the changes to these axes that occur through switch points in adolescence could be a mechanism that propagates 'coupling' of the HPA and HPG axis going forward in the lifespan. It is the goal of the current study to clarify some of the underlying mechanisms and pathways that inform the interaction between the HPA and the HPG axes during adolescence. An overlap in neural

architecture between the HPA and HPG axes can partially account for reactive and short-term coupling (evaluated in the skydiving and MRI/SPIT studies, respectively) but one must consider developmentally moderated adaptive recalibration to explain why coupling should be considered in a long-term context (the Mendota project).

The HPA and HPG Axes

Understanding the manner in which the HPA and HPG axes operate alone is a necessary prerequisite for understanding how these axes might interact during coupling. *Most importantly, there is a parallel structure that both the HPA and HPG axes conform to*. Within this parallel structure, there is a great deal of communication across, and within, common structures, and traditionally it has been suggested that intermediaries of one axis have roles as limiting agents on the other axis. There are possibly situations during which both the HPA and HPG axis are not necessarily negative feedback systems – where, in fact, both axes might be co-activated, or 'coupled'. Understanding the architecture of the HPA and HPG axes can provide insights into how this can happen, as can both the 'top-down' and 'bottom-up' regulatory mechanisms that influence these axes.

The HPA and HPG axes both begin and end in the brain. Tracking a hypothetical 'activation stimulus' can be a helpful way to make the ramifications of this point salient. A stimulus comes in, and is (sometimes, but not always, depending on the stimulus) processed by limbic neural structures, whereby both the HPA and HPG axis can be activated, and either (or both) of these axes undergo a complex biochemical cascade – this cascade *is* the axis. These parallel cascades can interact with one another as they are

promulgated through their respective axes, and the end products of these cascades have diverse effects throughout the body, but also, critically, on receptors in the brain; in point of fact, on those same limbic structures responsible for initiation of the cascade. Thus, salient stimuli begin and end in the brain. The combination of cross-axis communication, top-down effects, and bottom-up effects form the crux of the following section describing how the HPA and HPG axes work, are regulated, and possibly co-activated.

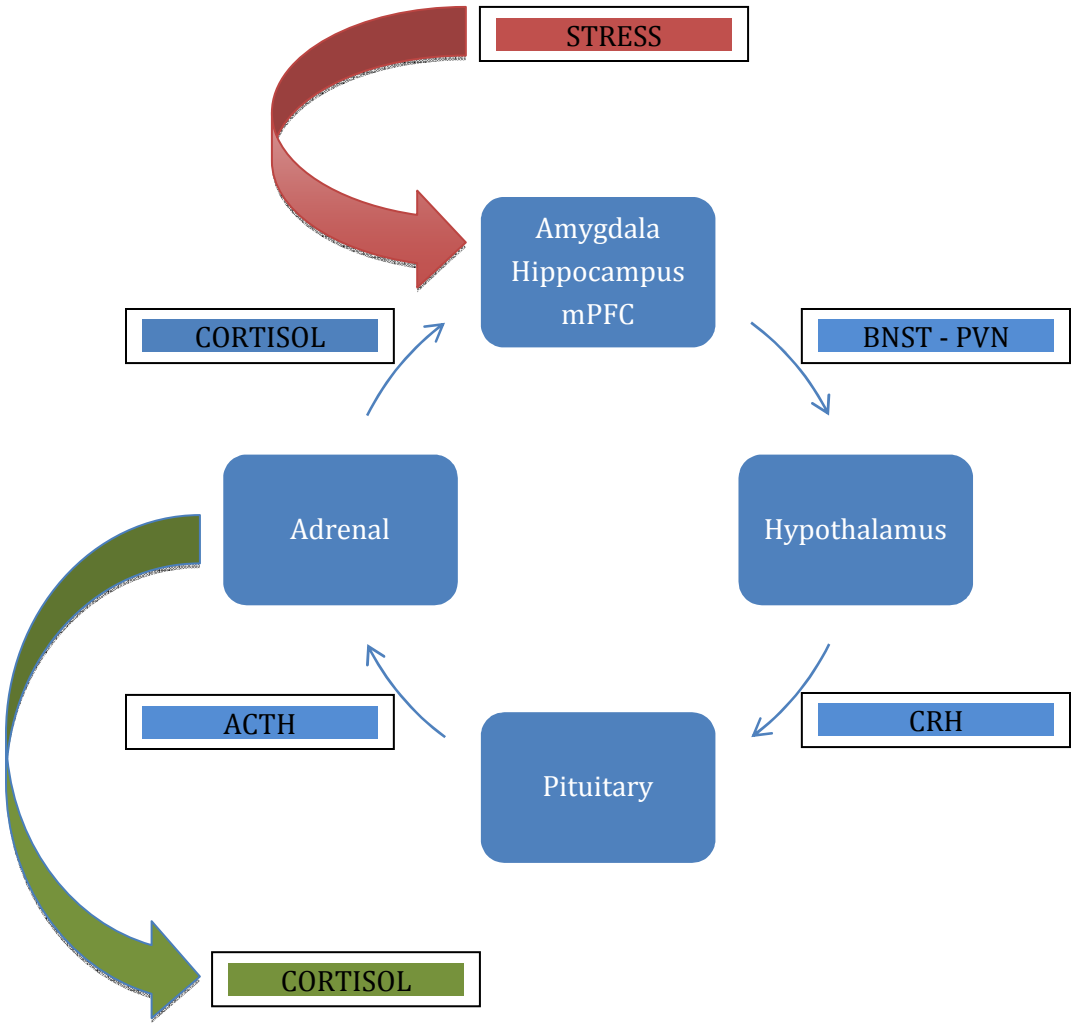

Figure 2: The Limbic-HPA Loop: Stress is (usually) processed by limbic structures,

which activates a biochemical cascade through the HPA axis, and results in the production of cortisol.

Parallel Structure and Architecture

The HPA and HPG axes are highly interconnected and operate in a parallel fashion. The HPA and HPG axes are presented simultaneously in this thesis, in order to more easily convey the parallel structure and highly interconnected nature of these axes. After the hypothalamus has been activated, the hypothalamus projects to, and activates, the pituitary in both the HPA and HPG axes. For both axes, the hypothalamus and pituitary communicate with one another via a closed portal system (Popa & Fielding, 1930). The hypothalamus can be divided into anterior, posterior, and tuberal regions, with the anterior hypothalamus further segregated into medial and lateral divisions (Goodman, 2009). Two specific sites within the anterior medial hypothalamus are of specific relevance here. The paraventricular nucleus (PVN) of the medial anterior lobe of the hypothalamus is primarily responsible for the secretion of corticotropin-releasing hormone (CRH) to the pituitary gland via the closed portal system (Weinstock, 2005) between the hypothalamus and the pituitary, serving to communicate excitation from the hypothalamus to the pituitary in the HPA axis cascade. The PVN also receives many incoming signals from upstream neural regulatory structures, marking this as an important site of integration and regulation in the stress response system. In the case of the HPG axis, the preoptic nucleus of the hypothalamus releases Gonadotropin Releasing Hormone (GnRH) into the hypophyseal stalk, and thence to the anterior lobe of the pituitary (MacLusky, Naftolin, & Leranth, 1988). To summarize: both CRH (the HPA axis intermediary) and GnRH (the HPG axis intermediary) are secreted from the medial anterior hypothalamus (though from different nuclei within this highly specific region)

into a hypophyseal portal system connecting the hypothalamus and the pituitary, where they effect target cells within the anterior pituitary.

The anterior pituitary serves as the main target for CRH and GnRH secreted through the hypophyseal stalk. In the case of the HPA axis, CRH stimulates release of adrenocorticotropic hormone (ACTH) from corticotrophic cells. ACTH acts on the middle cortical layer of the adrenal cortex along the perimeter of the adrenal gland to stimulate the release of glucocorticoids. The initial HPA response to environmental threat occurs on the order of seconds (Sapolsky, Romero, & Munck, 2000). This three-step cascade represents an axis of stress responsivity, which culminates in the release of glucocorticoids.

In the case of the HPG axis, gonadotrope cells within the anterior pituitary are stimulated by GnRH to secrete Luteinizing Hormone (LH) and Follicle-stimulating Hormone (FSH) into the bloodstream, where they interact with Leydig cells in the testis to produce testosterone in males, and the ovaries in females. GnRH controls LH and FSH through pulsatile secretions: the size and frequency of pulses of GnRH that bind to the GnRH receptor in gonadotrope cells determine whether or not LH or FSH will be secreted, with higher frequency, more concentrated pulses leading to LH release, and lower frequency pulses leading to FSH release. This represents an important internal mechanism for regulation of the HPG axis.

Top-down Processes: Neural regulation of the HPA and HPG axis

The human stress response leads to the production of glucocorticoids via a biochemical cascade propagated through the HPA axis, but before this can occur, the

hypothalamus must be activated. There are two proposed pathways through which this happens: immediate (systemic) stressors are relayed directly to the paraventricular nucleus (PVN) of the hypothalamus via brainstem catecholaminergic projections, and stressors requiring mediation by higher brain structures are routed through limbic forebrain structures (J.P. Herman & W.E. Cullinan, 1997). Hindbrain activation of the PVN potentially occurs via neurons in the solitary tract of the medulla, which also receives inputs from efferent nerve fibers and chemoreceptors, marking this as an important integrative pathway (Zhang et al., 2010). Alpha-adrenoreceptors in the PVN of the hypothalamus appear to modulate the excitatory effects of catecholamines on the hypothalamus (Plotsky, Cunningham, & Widmaeir, 1989). Catecholaminergic projections from the brainstem into the hypothalamus represent a reflexive activation of the HPA axis, but there are higher-order structures that play a role in the regulation of the stress response. The type of stimulus received by the organism is critical for determining what physiological 'route' is taken. Reflexive threats (e.g., hypoxia) directly activate the hypothalamus without mediation by limbic structures, but these are not as common as 'processive' stressors, which are characterized by (1) input from multiple sensory modalities, and (2) the lack of a direct threat to the inner physiological milieu (such as oxygen deprivation), but instead represent input that is stressful only in context (J. P. Herman & W. E. Cullinan, 1997; James P. Herman, Ostrander, Mueller, & Figueiredo, 2005). It is the second, non-reflexive stressor paradigm that we are most concerned with, especially in the context of the particular structures this pathway employs, as these structures could possibly play a regulatory role on the manner in which the HPA and HPG axis interact with each other. Three main limbic structures involved in regulatory

control of processive stimuli are important to consider: the hippocampus, amygdala, and medial prefrontal cortex (Herman 2005). The majority of the effects of these structures are not accomplished through direct innervation, but instead relay with neurons in the bed nucleus of the stria terminalis (Herman, 2005).

The role of the hippocampus is largely inhibitory on the HPA axis (Jacobson and Sapolsky, 1991; Herman and Cullinan, 1997). Lesions to the hippocampus increase HPA axis activity (Sapolsky et al., 1984; Herman 2005). For the most part, the hippocampus is considered to inhibit the HPA axis, but this is not always the case: the dorsal hippocampus has been shown to stimulate the HPA axis (Feldman and Weidenfeld, 1993).

The amygdala plays a stimulatory role as a limbic regulator of the HPA axis (Herman 2005). Lesions to the amygdala reduce HPA axis activity (Allen and Allen, 1974; Feldman et al., 1994). Stimulation of the amygdala increases HPA axis activity (Matheson et al., 1971). This is consistent with other roles of the amygdala such as activation of autonomic responses (Gray, 1993) and involvement with fear and anxiety responses (Davis, 1992; Herman 2005).

The role of the medial prefrontal cortex in regulation of the HPA axis is complex. Different regions of the medial prefrontal cortex do different things, and this area contains an, 'intricate topographical organization' that is poorly understood with regards to processive stimuli. Some of this confusion can be attributed to differential output patterns of the mPFC (Herman 2005). The infralimbic cortex projects mainly through excitatory circuitry (Hurley et al., 1981; Vertes 2004), whereas the pre-limbic cortex

projects to areas implicated in stress inhibition and HPA down-regulation (Hurley 1981, Sesack, 1989). All in all, the role of the mPFC is differential and contingent upon subnuclei with regards to regulation of the HPA axis. It can both activate and inhibit the HPA axis and responds differentially both in terms of efferent projections and contextual transduction of incoming stimuli.

In summary, there are two main pathways by which the HPA axis is activated. The first of these involves brainstem projections straight into the PVN, and is reserved for reflexive responses to stressors. Other stressors, which we call processive (i.e. stressors that have an emotionally salient component), are processed through a Limbic–HPA circuitry. Three main limbic structures come into play when discussing processive stressors. These are: the amygdala, the hippocampus, and the medial prefrontal cortex. The amygdala is mostly involved with activation of the HPA axis, whereas the hippocampus is mostly inhibitory. The medial prefrontal cortex can occupy dual roles – it can be both an activating agent, and an inhibitory one.

The overarching reason for discussing this is to delineate the mechanisms underlying one of the core tenets in a discussion of coupling: that, in a complex way, the brain regulates the manner in which the HPA axis is activated. This is very important, because this regulation is not 'hard-wired,' but subject to change. Furthermore, as we will see in a later section, every limbic structure mentioned in a regulatory capacity for the HPA axis also contains glucocorticoid receptors. This means that the actions of the HPA axis (through the production of glucocorticoids) are able to exert influences on their own regulatory structures. This will be implicated more directly below when discussing bottom-up influences on the HPA axis. But, before we can move on to a discussion of

bottom-up effects, it is useful to understand the way that the HPG axis is regulated in the brain.

An extensive literature exists to delineate neural regulatory "top-down" mechanisms for the HPA axis. Less, however, is understood about the way the brain influences the HPG axis. Firstly, much of the literature that exists has a developmental focus, delineating the organizational – activational affects of testosterone, and will be discussed in the context of adolescence. Secondly, another body of literature exists that discusses the impact of the HPG axis on neural structure and functioning (as opposed to the impact of neural structure on the HPG axis, which we are presently interested in); this will be reviewed as "bottom-up" processes. It makes sense that so much of the literature concerning the interplay between testosterone and brain regions has to do with the effects of testosterone on neural circuitry, and not vice versa, as testosterone plays a significant role in the early organization of neural structures. Thirdly, many studies look at neural regulation in the context of behavioral outcomes. A number of studies look at the influence of the amygdala and prefrontal cortex, for example, in relation to outcomes such as aggression and violence (Davidson, 2000), but not in relation testosterone levels as a primary outcome of interest. Testosterone and the HPG axis are often a mechanistic intermediary between neural regulatory structures and aggression, but testosterone is rarely considered as an outcome, in and of itself, in these studies. Lastly, much of the literature describing neural structures that regulate the HPG axis is that many of them are genetically based, at present, and focus more on protein expression and receptors than on structural physiology and functional connectivity. These four reasons combine to make 'top-down' regulation of the HPG axis a bountiful area for future investigation, but limit

what we are able to state definitively at this point. What is known about neural regulatory processes on the HPG axis is largely through animal models.

Kisspeptin (encoded for by the KISS-1 gene) has been suggested as a principal activating agent for the hypothalamus, specifically in the context of stimulating GnRH release (Greives 2007). Expression of this protein responds to seasonal influences in animals. Kisspeptin has emerged as a linchpin in the neural regulation of GnRH, though this is a new finding (Popa 2008).

Proteins and neurotransmitters can impact the activity of the hypothalamus; however, when it comes to brain regions that regulate these proteins, very little is known. We can guess that some of the regions which play important roles in the HPA axis also have effects on the HPG axis. Lesions to the amygdala cause a decrease in aggressive behavior in rats (Vochteloo & Koolhaas, 1987). Because the HPG axis is often an intermediary in this behavioral phenotype, it is reasonable to think that amygdala lesions could cause down regulation of the HPG axis, and thus less aggressive behavior. This is not a direct link between the amygdala and the HPG axis. However, it does suggest that there is a possible connection, and that the amygdala could possibly play a regulatory role. In a similar vein, numerous studies have implicated the prefrontal cortex as a regulatory structure for aggressive behavior (Giancola, 2006). This marks the prefrontal cortex as another possible regulatory body.

In sum, there is limited information at this point about brain areas that directly regulate GnRH secretion. At best, we can speculate using behavioral phenotypes with which testosterone is commonly associated. Doing so leads one to believe that it is

largely limbic structures that regulate the HPG axis, and, most importantly, it is likely that the same structures which regulate the HPA axis are also involved with regulation of the HPG axis.

Bottom-up Processes: HPA & HPG

Stimuli that activate the stress response system are initially processed in the brain, and then propagated through an axis, whose end products subsequently impact those same brain regions that initially began the biochemical response cascade. Secretory products of both the HPA and HPG axis interact with the neural structures that regulate these axes. One of the central mechanisms by which both the HPA and HPG axes change is through feedback from endproducts of the HPA and HPG, respectively, back to the brain. A review of the effects of HPA and HPG axis hormones on certain brain regions aids in understanding how these axes change in response to the environment.

We have seen, when considering top-down regulation, that the HPA axis is regulated by series of limbic structures, especially the amygdala, hippocampus, and mPFC. The hippocampus plays a role in inhibition of the HPA axis. Furthermore, a special feature of the hippocampus is that it contains a number of glucocorticoid receptors (Aronsson et al., 1988). This means that cortisol can interact with the hippocampus to influence this limbic moderator of HPA axis activity. Decreased hippocampal glucocorticoid signaling has been associated with HPA axis inhibition (Issa, Rowe, Gauthier, & Meaney, 1990; Sapolsky, 1986). A similar situation exists with the amygdala. Central and medial amygdaloid nuclei contain glucocorticoid receptors, and are able to process both stress level and basal level expression of glucocorticoids (Ahima

& Harlan, 1990). Interestingly, the main effects of glucocorticoids on amygdaloid GR receptors is to stimulate CRH production (Herman, 2005). Just like the amygdala and the hippocampus, the medial prefrontal cortex also contains a large number of GR receptors (Ahima & Harlan, 1990). It has been suggested that stressful stimuli tend to promote feedback to this region (J. P. Herman et al., 2003).

The amygdala, hippocampus, and medial prefrontal cortex are all influenced by the activity of the axis they control. Feedback and interaction take place during the processing, transduction, and execution of a response to a stimulus. In addition, many of the structures responsible for control of the HPA axis are also likely relevant for control of the HPG axis. The synchronicity in parallel structure of those regulatory mechanisms, the axes themselves, and feedback mechanisms provide a wealth of mechanistic possibilities that could allow coupling. We've discussed bottom-up mechanisms for control of the HPA axis, now it is time to turn our attention to the HPG axis.

In animal models, testosterone has been shown to act on the amygdala to mediate dominance behavior (Delville, Mansour, & Ferris, 1996; Stanton, Beehner, Saini, Kuhn, & LaBar, 2009). This finding has also been extended to the VmPFC, where testosterone acts to lower the threshold for engaging in dominance and behavioral aggression (Ambar & Chiavegatto, 2009). There are also a number of functional interconnections between the amygdala and the VmPFC. Global grey matter has been shown to be positively correlated with Testosterone levels in boys, though in one study at least, regional brain volume differences were not able to be explained by testosterone (Peper et al., 2009). Testosterone has also been shown to increase functional connectivity between the left prefrontal and the right parietal cortex. Most importantly, overall, there are androgen

receptors on these limbic structures, just as there are glucocorticoid receptors (Meethal & Atwood, 2005). This allows androgens to feed back on the (potential) limbic structures that regulate the HPG axis in a parallel fashion to glucocorticoids.

The architecture of the HPA and HPG axes have been historically discussed in terms of a parallel structure, from the hypothalamus, to the pituitary, and lastly to the adrenals and gonads, respectively. This does not clearly delineate the role of the brain in both initiating these axes and as a primary target organ for the end-products, however. We have seen that there are both top-down and bottom-up structures that regulate these axes, and it is likely that many of these structures are conserved between axes; therefore the same neural structures that are involved in top-down control are also implicated in bottom-up feedback. Looking at this bigger picture of the neuroendocrine axes emphasizes the potential interconnected nature of these axes. It opens the possibility that the HPA and HPG have cross-axis communication (which may be largely inhibitory as the classical model suggests), but also that the communication of these axes encompasses neural structures that both initiate and are influenced by the HPA and HPG axes. Very little research has been carried out to examine the relationship between neural structure, feedback, regulation of individual axes, and coupling. This represents another exciting possibility which is indirectly suggested by positive associations between HPA and HPG biomarkers.

Further Notes: DHEA

The review above emphasized testosterone and cortisol as measures of the HPG and HPA axes, respectively. However these are not these are not the only endproducts of

these axes. DHEA is the most widely circulating endogenous hormone in the human body. In the bloodstream, DHEA is largely found in its sulfated form, DHEA-S. DHEA partially binds to androgen receptors in the human body (Chen et al., 2005), as well as some estrogen receptors (Webb, Geoghegan, Prough, & Michael Miller, 2006). Like cortisol and testosterone, DHEA exhibits a diurnal rhythm, and tends toward a linear decline across the day. Concentrations of DHEA also change across development, and tend to increase through puberty, beginning to increase around age 7 and continuing to increase through early adulthood, at which point levels begin to decline (Matchock, Dorn, & Susman, 2007; Susman & Rogol, 2004). DHEA is associated with onset of adrenarche through increased production in the zona reticularis of the adrenal cortex, and results in effects associated with progression from Tanner stage 2 to Tanner stage 3, such as onset of acne, new body hair growth, body odor, and oiliness of the skin (Susman & Rogol, 2004).

Interestingly, DHEA biosynthesis is not restricted to the adrenal cortex, however. DHEA is also produced in the brain of many animals (including humans) in a poorly understood process. It has only been recently discovered that astrocytes and neurons in the brain contain P450c17, a key enzyme responsible for synthesis of DHEA in the 4 step conversion from cholesterol to DHEA (Zwain & Yen, 1999). Furthermore, DHEA is also produced in the gonads, marking this as an important hormone from a regulatory point of view, as it is produced in both the adrenal cortex and the gonads. Little is understood about regulation of DHEA production, nor are physiological control mechanisms (beyond direct biosynthetic pathways) well elucidated (Auchus & Rainey, 2004). However, when considering a dual axis perspective, DHEA is impossible to ignore, as it is produced at

the end-point of both axes, as well as by one structure implicated in the regulation of both the HPA and HPG axes – the hippocampus (Baulieu & Robel, 1998; Kimoto et al., 2001; Mukai et al., 2006). There is little available information to guide how DHEA should be considered in the model of cross-axis communication. What DHEA illustrates by being an end-product of both axes, however, is that simple cross-axis inhibition would be difficult to accomplish physiologically for this molecule.

Coupling: An overview of cross-axis communication

Coupling is a complicated phenomenon, and there are multiple levels of organization that can influence the way that hormone axes interact – from top-down regulatory processes, to bottom-up feedback, to the actual manner in which intermediaries in hormone axes interact with one another. It is the latter component that can be most illustrative in the current analysis, as this is the area where research has shed the most light thus far.

There is burgeoning literature on the field of cross-axis coupling. In this section, HPA-HPG coupling will be approached in two ways: first, the role of the actual, physiological interplay between hormone axes and their intermediaries will be reviewed. The relevant literature that has examined coupling thus far will then be laid out.

Mechanistic Concerns: How do the HPA and HPG Axis talk to one another?

The HPA and HPG axes, through both top-down and bottom-up processes, form biochemical cascades associated with each individual axis in parallel using many of the same structures, but these axes interact as well. This is critical to coupling, as regulatory structures can inform the way that the HPA and HPG axes interact, and bottom up

regulation can inform the degree to which each axis is expressed. Coupling has another dimension as well – the immediate manner in which intermediaries of each axis communicate with each other. To date, the amount of research that has been carried out on this subject is limited; in large part this is due to the complexity of modeling multiple hormone system simultaneously, and the complex nature of the interactions that occur as these axes communicate with one another. To understand how these systems interact, it is important to know the details of their feedback mechanisms.

Traditionally, the actions of the HPA axis have been considered down-regulatory on the HPG axis; this is largely due to the fact that glucocorticoids suppress reproductive facilities during the second wave of GC activity (the 'modulating' phase) (Rivier & Rivest, 1991; Tilbrook, Turner, & Clarke, 2000). However, recent advances and new theoretical perspectives have suggested that this picture could be incomplete, The interaction between the HPA and HPG axis occurs via feedback of the secretory products of these axes (i.e. cortisol and testosterone) and the regulatory actions of the intermediaries involved (CRH, ACTH, GnRH, LH, FSH) as well as other molecules (inhibin, activin) recruited into this complex chain of activation and suppression (Viau, 2002).

In rodent models, CRH has been shown to limit synthesis of LH (Porter, Lincoln, & Naylor, 1990). CRH also has a similar effect on FSH, though exogenous CRH administration does not seem to influence GnRH levels (Barbarino et al., 1989). There is some evidence in animal models that the effects of CRH are dependent upon the rest of the HPA pathway through the adrenal glands; in adrenalectomized animals the full effects of CRH on gonadotropin release are attenuated by background glucocorticoids and not

achieved unless background cortisol is high (XIAO, LUCKHAUS, NIEMANN, & FERIN, 1989). Interestingly, CRH has two receptor subtypes, coded for independently, designated CRH-R1 and CRH-R2. CRH-R1 receptors are mostly found in the pituitary and in the brain, whereas CRH-R2 receptors are mostly found in the periphery (Tsigos & Chrousos, 2002). This possibly suggests a differential central regulatory pathway and peripheral allostatic response pathway for CRH. In animal models, ACTH seems to retard LH secretion, but this is again dependent on intact adrenals; however, in one study at least, ACTH does not appear to *directly* down-regulate gonadotropins (Mann, Evans, Edoimioya, Kamel, & Butterstein, 1985). Several studies have investigated the effects of glucocorticoids on HPG axis activity, and have indicated that GC's tend to down regulate the HPG axis at all levels, and act primarily on the intermediaries (GnRH, LH, FSH) (Rivier & Rivest, 1991; Tilbrook et al., 2000). Regarding the HPG axis, less is known about the effects of intermediaries on inter-axis communication. Similarly to glucocorticoids, testosterone has been shown to down-regulate the HPA axis (Viau, 2002), but this is possibly mediated by androgen receptors, especially in the context of stressful stimuli (Viau, 2002). Furthermore, testosterone is also converted between aromatized substrates whose affinity for receptor sites is different than that of the parent compound (Viau, 2002). These represent two areas where the effects of testosterone could be moderated, and perhaps allow for HPA and HPG axes to be stimulated simultaneously. This is very important as the concept of permissive coupling of HPA and HPG axis intermediaries and end-products moves forward; modulation of androgen receptor-site sensitivity in the PVN and possible aromatization of testosterone represent possible mechanisms by which positive coupling can be achieved.

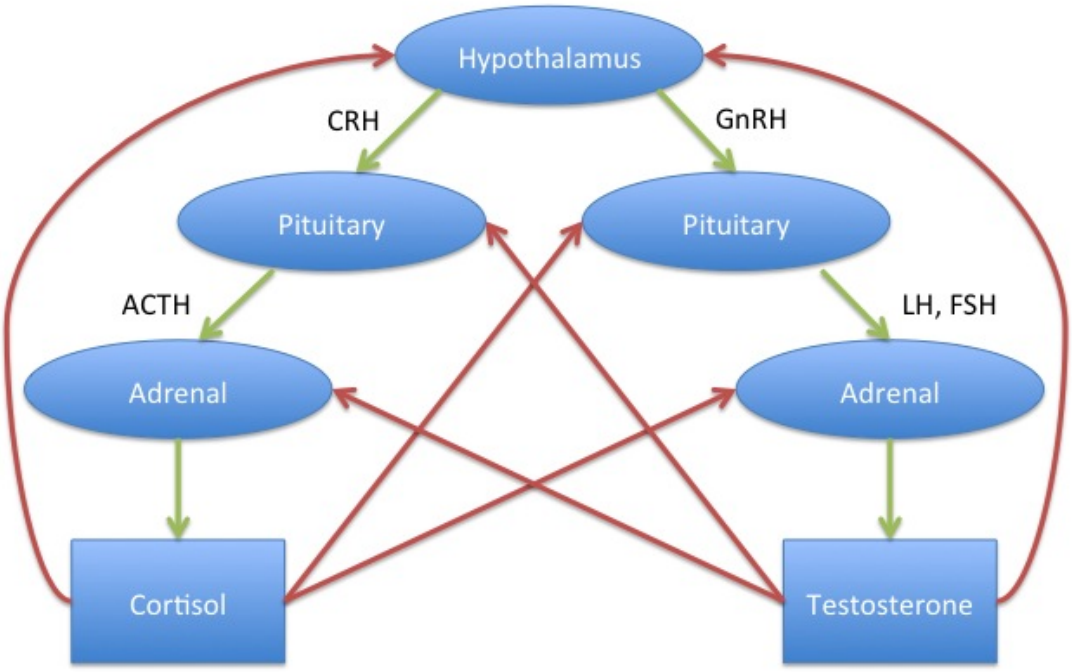

Figure 3: Traditional model of suppressive cross-axis communication

Standing on the shoulders of giants: What does the existing literature say about coupling?

A limited body of research has been conducted looking at HPA and HPG axes simultaneously, and, for the most part, has shown that it is not the case that these axes are mutually suppressive in humans. Adolescence is a period of great change and re-organization, and diverse (and even paradoxical) patterns of behavior and physiological activity are possible (Buchanan, Eccles, & Becker, 1992). Viau (2002) delineated the highly interconnected nature of the HPA and HPG axes, and posited that they could

interact in unexpected ways. Glenn and colleagues (2011) have suggested that the ratio of baseline testosterone to cortisol responsivity to a stressor acts as a significant predictor of psychopathy, when baseline cortisol, cortisol responsivity, and baseline testosterone as individual predictors do not, and goes on to suggest that the utility of the ratio score is likely due to the highly interconnected nature of the HPA and HPG axes(Glenn, Raine, Schug, Gao, & Granger, 2011). Mehta and colleagues (2008) found that, after losing a competition, men who were high in testosterone and lost a competition tended to have a drop in cortisol, and men who were high in testosterone and succeeded in a competition tended to have a rise in cortisol (Mehta, Jones, & Josephs, 2008). Interestingly, in both Glenn's work and Mehta's research, it was only individuals high in testosterone who had differential patterns of cortisol reactivity. In two different projects published in 2010, Mehta and colleagues suggested that the HPA and HPG axes interacted to regulate dominant behavior (Mehta & Josephs, 2010). Mastorakos has discussed the HPA and HPG axes in the context of pathology and shown that normative functioning, and interactions between axes are disturbed in a number of syndromes (Mastorakos, Pavlatou, & Mizamtsidi, 2006). Bateup (2002) demontstrated a pre-game rise of both cortisol and testosterone in female rugby players. In a study of 630 Filipino males, Gettler and colleagues found that waking and evening samples of cortisol and testosterone were co-elevated, and that this relationship was strengthened by actively seeking a romantic partner (Gettler, McDade, & Kuzawa, 2011). Furthermore, Marceau (2013) has shown that, in a sample of 213 adolescents, cortisol and DHEA were positively coupled throughout the day, as well as cortisol and testosterone, within persons. Ruttle (2013) found tightly coupled HPA and HPG axis activity in a longitudinal study at age 11,

though this diminished at ages 13 and 15, suggesting adolescence as a critical period during which to consider this phenomenon. Interestingly, in both of these studies, the effects are more pronounced in females.

In sum, there is a limited body of research that has thus far looked at co-activation of both the HPA and HPG axes, but this perspective is gaining momentum. It is becoming more and more apparent to simply say that these axes are mutually inhibitory is insufficient, and that the complex interplay between axes allowing co-activation makes sense from physiological, empirical, evolutionary, developmental, and theoretical standpoints. This is especially true in adolescence, a developmental period in which both the HPA and HPG axes undergo rapid maturation.

Adolescence: A period when the HPA and HPG axes are both excited.

Adolescence is a particularly important time in which to capture data about the coupling of hormone axes. Since 1904, adolescence has been considered a period of heightened 'storm and stress' (Hall, 1904), for a number of reasons; these include (1) identity formation, (2) conflict with parents, and (3) high levels of risk-taking behavior. During adolescence, both adrenal and gonadal hormone axes undergo significant changes, and there are times at which it makes sense for both testosterone and cortisol to be co-elevated. Indeed, pre-natally, post-natally, during juvenility and during adolescence, cortisol and testosterone exhibit similar activity (Ruttle, 2010), and it does not make sense for these axes to be unable to co-activate (Shirtcliff & Ruttle, 2010). Adolescence is also likely a point during which the body can recalibrate these axes, for long-term potentiation of these effects (Del Giudice et al., 2011).

A great deal of neural architecture is controlled by testosterone, in a very complex way, according to principles laid out in the 'organizational-activational' hypothesis first put forward by Phoenix and colleagues (Phoenix, Goy, Gerall, & Young, 1959). This theory suggests that testosterone operates in different roles at different time points in development, such that, perinatally, testosterone serves to organize neural structures and physiology, is dormant during childhood, and activates the previously laid out architecture when the HPG axis comes back 'on-line' during adolescence. In males, testosterone is produced during gestation, and there is a peak in testosterone concentration about one month after birth. Following this peak, testosterone levels decline for approximately the next six months (WINTER, HUGHES, REYES, & FAIMAN, 1976) to basal levels, where they remain until puberty. In the perinatal time frame, testosterone is responsible for many of the masculinizing effects that occur in males, and it is considered a general rule that more testosterone in this early, vulnerable period equates to more masculinizing characteristics during development (Mazur & Booth, 1998). In the time since Phoenix and colleagues put forward the organizational-activational hypothesis, a number of experiments have been carried out to delineate the timing of the critical periods in development(Arnold & Breedlove, 1985; Wallen & Baum, 2002). Adolescence has come to be more and more regarded as a critical period in the intervening years and the original findings of Phoenix and colleagues have been somewhat modified, such that adolescence is now regarded as both an organizational and an activational period (Romeo, 2003). The organizational-activational hypothesis predicts that the HPG axis will come on-line during adolescence, and that this is a period

of critical integration of information. However, the HPG axis is not alone in being predicted to activate during adolescence.

The HPA axis is likely to be highly active in adolescence as well. Adolescence, more than any other developmental period, has been characterized as a period of tremendous interpersonal, and intrapersonal, stress. Problems with emotion and behavior regulation lead to increased risk of morbidity and mortality in this period (Dahl, 2004; M. R. Gunnar, Wewerka, Frenn, Long, & Griggs, 2009) During adolescence, the HPA is characterized by prolonged activation, more-so than during adulthood (McCormick & Mathews, 2007). Indeed, changes in HPA axis activity during puberty have been suggested by some as possibly accounting for gender differences in psychiatric disorders such as depression (Spear, 2000). In animal models, adolescence is implicated as a sensitive period for programming effects of stressors on the central nervous system, and the HPA axis is suggested as a pathway by which programming might occur (McCormick & Mathews, 2007).

Adolescence is a critical period during which many unique developmental events take place. Both the HPA and HPG axis appear to become activated, but it is unknown how these axes would interact together during this developmental stage. However, a mutually antagonistic architecture may be detrimental to the developing organism. The body evolves adaptive response systems, and it is logical that mechanisms allowing for co-activation of the HPA and HPG axis would emerge during the critical period of adolescence. If this idea is supported, it will lend dramatic support to emerging theoretical models for adolescent neuroendocrine development.

Stress Revisited: Context for the Current Proposal

Stress exposure is a very broad term. In the case of this particular proposal, there are three types of stress to examine. Each of these stressors is addressed in a different data set. A version of the same overarching analytic strategy will be applied to each data set, using Hierarchical Linear Modeling. First, data collected from participants before and after going skydiving, as well as on a basal day, is assessed to look at stress responsivity. Short term stressors will be assessed in the MRI/SPIT data set, a project carried out at the University of Wisconsin where subjects experience a stressful 'lab day' environment, and provide several hormone samples across both the lab day and basal days. Third, Long term stress will be evaluated using data collected from participants incarcerated at the Mendota Juvenile Detention Center in Madison, Wisconsin. Prison itself is stressful, as well as the environment many of these children grew up in. There tends to be a great deal of abuse reported in this sample; this represents a high stress exposure participant pool. The next section frames the forms of stress exposure as concepts to examine, connecting them with the study that will be used to operationalize them. These studies will be described in later sections.

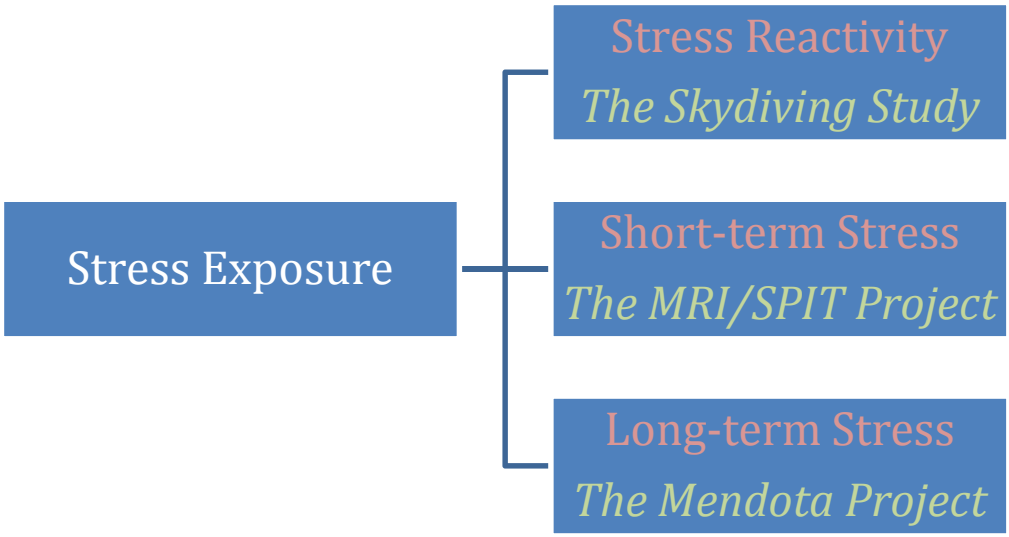

Figure 4: Organization of the study, and types of stressors to be evaluated

Stress Reactivity: The Skydiving Study

Stress reactivity is the manner in which the stress response is mobilized in response to a stressor. There is a wealth of literature that delineates the manner in which HPA axis reactivity changes in response to stress exposure. Some of this literature will be discussed in this review, but it is important to remember that we are considering the HPA axis, the HPG axis, and the way that they work together in response to stress exposure as well. It might seem that the HPG axis is a somewhat neglected in this section; that is not through oversight, but instead due to the fact that very little is known about HPG reactivity, and especially HPA-HPG coupled reactivity.

Stress reactivity constitutes the manner in which the body mobilizes resources in response to a threat (W.T. Boyce & B.J. Ellis, 2005; G. P. Chrousos & Gold, 1992;

Meaney, 2001). One of the main (though not only) actions that the body undertakes as a part of the stress response is activation of the HPA axis. Glucocorticoids are produced as a function of this activation, and exhibit diverse effects throughout the body, including immune, reproductive, and metabolic changes. In addition to the HPA axis, the locus coeruleus-norepinephrine system is activated; together, these represent a set of biobehavioral changes throughout the body meant to orient and respond to stimuli (W. T. Boyce & B. J. Ellis, 2005; G. Chrousos, 2000). The HPG axis is reactive as well, and testosterone levels are known to increase in many contexts, though this is not as well researched as HPA axis reactivity. The HPG axis is mostly discussed in the context of competitive tasks or sexual encounters, and reactive increases in testosterone are observed in both (Mazur, Susman, & Edelbrock, 1997; McIntyre et al., 2006).

Short-term Stress Exposure: The MRI/SPIT Project

Short term stress exposure is the set of physiological changes to the body that occur on the order of hours or days. This forms the middle part of a three pronged analysis designed to assess the way that stressors influence the interaction between the HPA and HPG axes, and is the first of our research paradigms for which the diurnal rhythm becomes important. The diurnal rhythm is the natural, rhythmic pattern that hormones express over the course of a day. For cortisol, the glucocorticoid that is produced when the HPA axis is activated, there is a natural ebb and flow that occurs throughout the day and is fairly well understood at this point. Cortisol levels tend to rise gradually prior to waking, and experience a significant increase immediately after waking up in the morning. This is known as the cortisol awakening response, and happens about 45 minutes after an individual wakes up. This morning spike in cortisol level is anteceded

by a significant decline. After this early spike and return to baseline, cortisol exhibits fairly linear decline throughout the rest of the day.

The diurnal rhythm is relevant for short-term stress exposure because it is changes to this natural rhythm that we are concerned with, on the timescale of lab-based stressor days.

Long-term Stress Exposure: The Mendota Project

The effects of chronic stress exposure are numerous and profuse. There are a number of adverse health outcomes associated with child abuse and maltreatment, including increased risk for cardiovascular disease, increased hospitalizations, increased prevalence of obesity and drug abuse, and overall greater risk for poor physical health (Batten, Aslan, Maciejewski, & Mazure, 2004; Flaherty et al., 2006; Hussey, Chang, & Kotch, 2006). In addition to physical manifestations, abuse is highly associated with myriad mental health outcomes. Both depression and anxiety disorders are linked to early life adversity, as well as attachment disorders and problems with emotional regulation, and memory disorders in later life (Bos et al., 2011; Bremner, 2003; Bremner & Narayan, 1998; Heim & Nemeroff, 2001). The economic impact of child abuse and neglect is startling, totaling roughly 103.8 billion dollars in 2007 (Wang & Holton, 2007). Abuse represents a potent risk factor for a variety of psychiatric and health outcomes, but scientific understanding of the mechanisms underlying these associations are complex and continually evolving (Bremner, 1999; Kaufman & Charney, 2001). It is becoming apparent that examinations of early life adversity must incorporate context, developmental stages, genetic predisposition and individual differences into models to

gain a more robust understanding of the impact such experiences have through the lifespan (Belsky, 1993; Bremner, 2003; Caspi et al., 2002; Kaufman & Charney, 2001). The reactive, contextually dependent nature of endocrine axes suggest that these might play a role in the transmission of early adversity effects on through life (Shirtcliff & Ruttle, 2010).

Methods

Three different projects are employed in this analysis, with the idea that each study captures a different aspect of stress exposure. Stress Reactivity will be examined using the Skydiving study, short-term stress will be examined in the MRI/SPIT data set, and long-term stress will be looked at in a data set collected at Mendota Treatment Center. Each of these data sets have unique aspects that make them germane for a comprehensive analysis of stress exposure. It is worthwhile at this point to take a moment to reflect on each of these projects, as the data that is collected is only as good as the experimental protocol that underlies it. Each project will be briefly described in the following sections.

Stress Reactivity: The Skydiving Study

In the skydiving study, 44 participants were recruited from Gold Coast skydiving company in Lumberton, Mississippi. Only individuals who expressed their own desire to go skydiving participated in the study. All participants took part in a mandatory training course held by the skydiving company. In addition, the Institutional Review Board of the University of New Orleans approved all aspects of the study.

For participants in the skydiving project, both hormone and autonomic measures were assessed. Researchers were present at the skydiving site in the afternoon, and recruited participants from the site itself. No one was excluded from the study based on skydiving ability or experience – both novice and experienced jumpers were allowed to participate. Once a researcher had discussed the project with a participant, and the participant had indicated that they were willing, informed consent was given, and the first hormone sample was collected. At this point, participants also filled out a daily diary – a small questionnaire which collects data on exercise, eating, sleep habits, mood and emotion at the time of each sample. Skydivers then completed a small training task run by the facility, and put on the appropriate attire for the jump. Immediately before boarding the plane to jump, participants provided a second saliva sample, and fill out the corresponding second daily diary. Immediately after landing a third saliva sample was collected, along with a daily diary, and 15 minutes after that a fourth samples collected. A fifth, and final, sample was collected one hour after the jump was completed.

On a separate day, participants would provide time-matched samples to each of the saliva samples they provided on the original day. This allows for a comparison day, and a baseline to be established in terms of normal diurnal fluctuations in the afternoon. Skydiving provides an excellent vehicle for examining stress reactivity. There has been concern in the past over the ecological validity of laboratory-based stressors for being sufficiently stressful, but skydiving is less subject to these limitations. Skydiving represents an acute, intense stress on participants. Five samples taken before and after the jump allow researchers to model the reactivity profile from skydiving. Samples matched on a basal day allow for differences in reactivity and basal profiles to be modeled.

Participants also complete a daily diary at each point of hormone collection, which indexes mood, medicine usage, attitude, food intake, and other factors that could potentially influence hormone profiles.

Short-Term Stress Exposure: The MRI/SPIT Project

The MRI/SPIT project is a 5-day study with 32 discrete points of hormone sampling. Participants undergo a physical exam, an MRI, and a series of interviews and questionnaires on a lab day. Eight hormone samples are collected on the lab day: these are sampled: (1) upon arrival, (2) after the puberty assessment, (3) before the MRI, (4) after the MRI, (5) after lunch, (6) after the interviews, (7) before dinner, and (8) at bedtime. A daily diary (described above) is also collected at each of the sampling points. One each of two home days and school days, six hormone samples are collected as well, these are sampled: (1) upon awakening, (2) mid-morning at least an hour after breakfast, (3) prior to lunch, (4) mid-afternoon after school, (5) before dinner, and (6) before bedtime. In sum, the MRI/SPIT study has five days of hormone sampling – 1 lab day, 2 home days, and 2 school days, there are 8 samples taken on the lab day, and there are 6 samples taken on each of the 2 school and home days. This allows for a data-rich mapping of the diurnal rhythm.

The lab day experience is a stressful one. Firstly, arriving at an unknown, laboratory-type setting is naturally disturbing. Secondly, the MRI, in and of itself, is a highly salient stressor (Eatough, Shirtcliff, Hanson, & Pollak, 2009; Muehlhan, Lueken, Wittchen, & Kirschbaum, 2011). Both sex (testosterone) and Stress (cortisol) hormones have been shown to be responsive to participation in an MRI. In addition, participants go through a physical exam and a puberty assessment, as well as a completing the Life

Stress Interview (described below) and the CTS (described below). All in all, this adds up to a highly stressful day that would likely cause disruption to the normative functioning of the diurnal rhythm of cortisol and the baseline levels of testosterone, and provides a great data set for understanding how these two hormones might change together in the context of a day long stressful event.

Long-Term Stress Exposure: The Mendota Project

Participants included 50 male incarcerated adolescents (M age=16.08, SD=1.06, range 14-18 yrs) from Mendota Juvenile Treatment Center (MJTC), a detention facility located in Madison, Wisconsin. Incarceration provides a stable environment where saliva can be collected rigorously, and the context is relatively stable over time. MJTC receives youth referred by general corrections facilities for extreme behavior problems throughout the state of Wisconsin.

Informed assent was obtained from each participant before testing. Saliva collections were conducted 1-2 weeks after admission to preclude treatment effects and to allow the individual to acclimate to the incarceration context. While incarceration is likely to be stressful, its impact would not occur through a mechanism of novelty or unpredictability; most stressors require novelty in order to stimulate a hormone response. Testing occurred over 3 days, including two days for collecting saliva samples and one for conducting the Life Stress Interview and administering self-report measures of abuse and maltreatment and demographic information.

Researchers collected saliva samples over two consecutive days. Five samples were collected each day. Samples were collected (a) upon waking (*M*=7:07am, *SD*=11min, range=6:10-7:53am); (b) 45 minutes later to capture the response to

awakening (*M*=7:46am, *SD*=15min, 7:10-8:25am) (Wust, Federenko, Hellhammer, & Kirschbaum, 2000); (c) before lunch to minimize the influences of mealtimes (*M*=11:31am, *SD*=5min, 11:19-11:47am); (d) before dinner (*M*=5:33pm, *SD*=9min, 5:10-6:38pm); and (e) immediately before bedtime to capture the entire rhythm (*M*=10:00pm, *SD*=16min, 9:30-11:55pm). Saliva was collected following published protocols (Schwartz, Granger, Susman, Gunnar, & Laird, 1998) and frozen immediately (-80^{o}C).

Maltreatment was measured through a combination of self-report measures and interviews. The Life Stress Interview (LSI) is a semi-structured interview administered one on one with each participant, and is highly sensitive to stress in adolescent environments. The LSI measures stress across familial, academic, behavioral, cross-gender and peer domains. The interviewer asks for specific episodes in each of these domains, and participants rate the impact and describe the context of each event to the interviewer. Chronic stressors are assessed over the same domains as well. Once this data has been gathered, an independent rating team blinded to the child rates the stressfulness or severity of the events, with higher ratings corresponding to more stressful events. The rating team incorporates the participants rating of severity into their own independent evaluation and a total score emerges across each domain. The Life Stress Interview (LSI) represents a valid and reliable (κ>.80) measure of both acute and chronic stress over the past year (Adrian & Hammen, 1993).

In addition to the LSI, self-report measures of abuse were collected from all participants, in the form of the Childhood trauma questionnaire (CTQ) and the conflict tactics scale (CTSPC). The CTQ yields scores in five domains: emotional abuse, emotional neglect, sexual abuse, physical abuse, and physical neglect, and is well

validated as a sensitive and specific measure in adolescent populations (Bernstein, Ahluvalia, Pogge, & Handelsman, 1997). The CTSPC assesses frequency, prevalence and severity of physiological or psychological aggression and discipline instances in the child-parent relationship, and has been widely utilized in research involving violent or potentially violent relationships (Straus, 1998). Abuse and adversity are quantified in each of these measures, providing an operational definition for the construct.

Analytic Strategy

This thesis hypothesizes that the coupling of the HPA and the HPG axis is increased in the context of stress. In order to determine if this occurs, a statistical model that has the capacity to assess coupling has to been set up: this was accomplished using hierarchical linear modeling (HLM). Hormones have a reactive component as well as a diurnal profile. In order to assess the diurnal rhythm of each participant, multiple samples have to be taken, which means that there are multiple samples nested within each participant in each study. Using HLM allows researchers to account for the inherent nesting of samples within individuals. For this reason, HLM is employed as the analytic tool in each analysis set.

On the day of assay, each saliva sample was thawed and assayed within 24 hours for cortisol, testosterone and DHEA in duplicate by Madison Biodiagnostics (Madison, WI), using well-established enzyme-immunoassay kits (www.salimetrics.com) . All samples from an individual were assayed on the same kit to minimize measurement error. All samples were assayed in duplicate. Duplicates that varied by more than 15% were repeat tested.

Each data set (short-term, long-term and reactivity) had a unique hierarchical model associated with it because there were unique variables in each data set, and models were changed to reflect this. Level-1 equations were modified to represent the best fit of the data in each set.

Each base model was similar, and each model had at least one conserved component – the coupling parameter. The coupling parameter is the beta weight associated with one hormone in the prediction of another hormone. For initial analyses, cortisol was used as the outcome of interest at level-1, and testosterone (the coupling parameter) was conserved as part of the level-1 equation in all three data sets. Additional level-1 predictors (such as time since waking for diurnal analyses or time since stressor for reactivity analyses) were loaded on to the model to create an accurate level-1 equation that represents the data well. In addition, DHEA was run as a separate analysis in all three data sets, in a parallel fashion to testosterone. These level-1 predictors subsequently became outcomes-of-interest when additional levels were brought in to the model, such that, for example, a measure of stress exposure was brought in on level-2, in order to see if this level-2 variable changed the manner in which testosterone was coupled with cortisol.

.Furthermore, in each data set, a trivariate model was run. The trivariate model contained both DHEA and testosterone terms loaded onto the level-1 model, in the prediction of cortisol as the outcome of interest. Examining the manner in which the beta weights associated with each hormone change when another hormone is allowed to account for variance in the same model allows one to assess the degree to which competing hormones are accounting for variance – to tell, in effect, which hormone is

really 'driving' the relationship underlying coupling. If, for example, testosterone is a significant predictor of cortisol in a bivariate model, but is not in a trivariate model, it can be surmised that DHEA is responsible for much of the variance that was originally attributed to testosterone in the bivariate model.

Lastly, variables representing long-term stress were loaded onto the MRI/SPIT base model, in order to see if the presence of long-term stress changes the short-term reactivity pattern found in the base model. This is possible because the MRI/SPIT project contains many parallel measures to the Mendota project and allows for indexing long-term exposure to stress. A summary of all analyses corresponding with each project is detailed in figure 5.

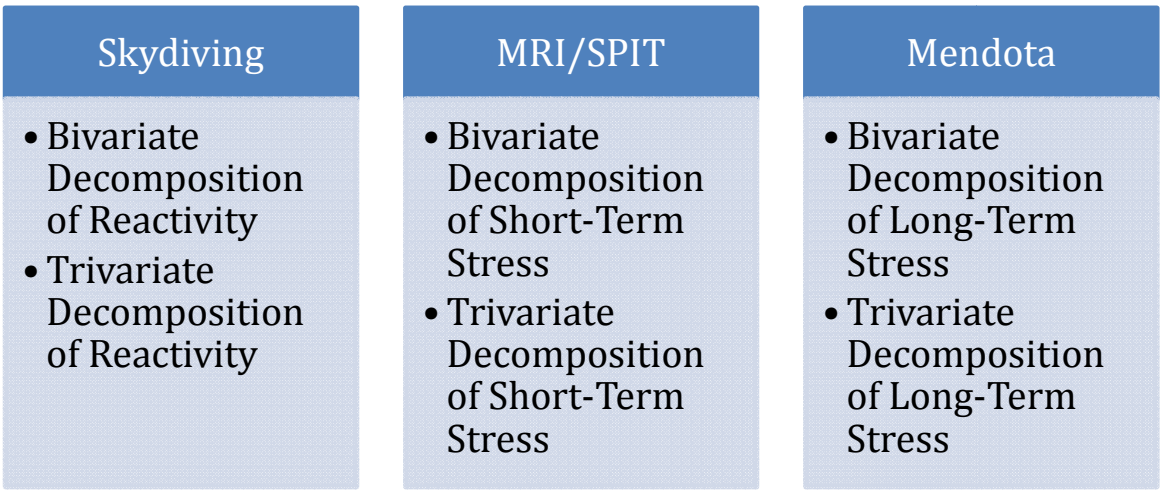

Figure 5: A summary of each analysis associated with each project.

Results

Stress Reactivity: The Skydiving Study

In the skydiving study, saliva samples from participants were collected on both jump and basal days, allowing a disambiguation between contexts. Participants were on average 29.6 years old (SD 9.60), with 40 caucasian participants, and 4 other participants, and a male:female ratio of 32:12.

A number of variables went into the base model. These include time to jump (TBJ), time after jump (TAJ) a variable to represent the jump day versus the basal day (JB) and an interaction term between the jump/basal variable and the time to jump/time after jump variable. The TBJ variable allows participants to have different slopes leading up to the jump, and the TAJ variable allowed participants to have different slopes after completing the jump, while the JB variable allowed participants to have different slopes on the jump and basal days. The interaction term allows for the slopes of the TAJ and TBJ terms, respectively, to differ according to whether the duration of time was on the jump day or not. These interaction terms essentially allow for the reactivity and recovery slopes to be captured on the jump day but not the basal day.

In addition, a variable for sex hormone concentration (either testosterone or DHEA) at the same time of day was loaded onto the level 1 model. The full model is as follows:

Level 1:

$$Y_{CORT} = B_0 + B_1*JB + B_2*HORMONE + B_3*TBJ + B_4*TAJ + B_5*Interactionterm + B_6*Interactionterm$$

No predictor variables were loaded onto level 2, as the point of the exercise was to assess the impact of a stressor – skydiving. Controls including SES, medication, age, race and gender were all assessed and statistically controlled for. Body Mass Index was the only control variable to have any impact on the model: BMI loaded onto the intercept term (B=-.090, p<.001). In the base model, JB was significant, indicating that those participants on the jump day had different slopes than those on the basal day (B = .943, p<.001). TBJ, the time before the jump, was significant and positive (B=.348, P<.05), as was TAJ, the slope after the jump (B = .210, P<.05). The results of the analysis confirm that testosterone is indeed positively coupled with cortisol (B=.652, p<.001) in this reactivity paradigm. In addition, in a separate model, DHEA reliably predicts cortisol as well (B=.633, p<.001). The full results of this analysis can be seen in figure 6 and table 1.

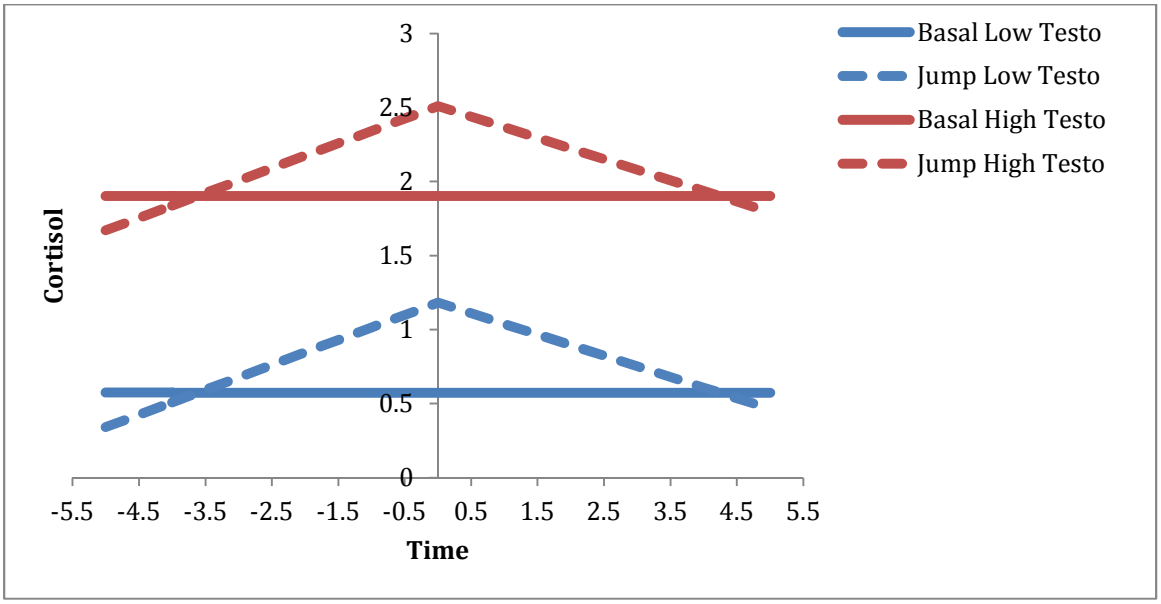

Figure 6: Jump days show reactivity, whereas basal days do not, and those with higher testosterone have higher predicted cortisol values.

Table 1: Bivariate coupling of cortisol with Testosterone and DHEA, respectively

Cortisol as Outcome					
Variable	***Intercept***	***JB***	***Testosterone***	***TBJ***	***TAJ***
Beta Weight	-.319	.943***	.652***	.348***	.210*
Variable	***Intercept***	***JB***	***DHEA***	***TBJ***	***TAJ***
Beta Weight	-.548	.700***	.633***	.204**	.198*

**p<.05 **p<.01 ***p<.001*

In a subsequent analysis, a trivariate model was computed where both DHEA and testosterone predicted cortisol in the same model. In this trivariate analysis, DHEA remained a reliable predictor of cortisol level (B=.548, P<.001), but testosterone did not (B=.138, p=.197), suggesting cortisol is more tightly coupled with the adrenal sex hormone DHEA than the gonadal hormone testosterone. Full results can be seen in table 2.

Table 2: Tri-variate coupling of Cortisol, Testosterone and DHEA in the same model

Cortisol as Outcome						
Variable	***Intercept***	***JB***	***Testosterone***	***DHEA***	***TBJ***	***TAJ***
Beta Weight	-.746	.775***	.138	.548***	.170	.252***

**p<.05 **p<.01 ***p<.001*

Short-Term Stress Exposure: The MRI/SPIT Project

The MRI/spit project represents an index of short-term stress, and it is necessary

to employ a 3-level hierarchical linear model to accurately assess hormonal coupling. There are two main reasons for a 3-level model. First, there are five days of hormone assays; consequently hormones are nested within days, which are nested within individuals, giving rise to three levels of disambiguation. Second, the 'day' level (level 2) is systematically different across days (i.e., school, lab, home) rather than being two similar days of collection. Third, the relevant measure of stress is specific to the entire rhythm of cortisol on the lab day, as it is short-term stress of interest, so it is necessary to capture the day-level in order to examine the impact of the lab-day stress on the entire diurnal rhythm. HLM is an optimal analytic tool for analyses such as these because it allows for modeling of the diurnal rhythm,. Three level modeling of this type is a fairly straightforward extension of the analytic method we have already discussed. Age, SES, race, gender, body mass index and medication usage were all controlled for.

The three TSW variables allow the curve to contain linear components, curved components, and quadratic components, allowing the curve to best fit cortisol as the diurnal rhythm changes over the course of the day. The school and home variables allow for the curve to be different on these days, and the interaction term says that coupling is not fixed and immutable, but can change over the course of a day. The presence of the variable lab day on level two constrains the model to those samples collected on the day in which the participant is in the lab. In the base model, with no level three predictors added, this establishes a short-term stress coupling parameter. A summary of the model is provided below:

Level 1:

$$Y_{CORT} = \pi_0 + \pi 1*TSW + \pi 2*TSW2 + \pi_3*TSW^3 + \pi_4*TSNOON +$$

π_5*HORMONE + π_6*SCHOOL + π_7*HOME + π_8*HORMONEINTERACTION

+R

Level 2:

$\pi_0 = \beta_{00} + \beta_{01}$*(LABDAY)

$\pi_1 = \beta_{10} + \beta_{11}$*(LABDAY)

$\pi_2 = \beta_{20} + \beta_{21}$*(LABDAY)

$\pi_3 = \beta_{30}$

$\pi_4 = \beta_{40}$

$\pi_5 = \beta_{50} + \beta_{51}$*(LABDAY)

$\pi_6 = \beta_{60}$

$\pi_7 = \beta_{70}$

$\pi_8 = \beta_{80} + \beta_{51}$*(LABDAY)

Level 3:

$\beta_{00} = \gamma_{000}$

$\beta_{01} = \gamma_{010}$

$\beta_{10} = \gamma_{100}$

$\beta_{11} = \gamma_{110}$

$\beta_{20} = \gamma_{200}$

$\beta_{21} = \gamma_{210}$

$\beta_{30} = \gamma_{300}$

$\beta_{40} = \gamma_{400} + \gamma_{401}$*Stressvariable

$\beta_{50} = \gamma_{500} + \gamma_{501}$*Stressvariable

$\beta_{60} = \gamma_{600} + \gamma_{601}$*Stressvariable

$\beta_{70} = \gamma_{700}$

$\beta_{80} = \gamma_{800} + \gamma_{801}$*Stressvariable

In our base model, there is a significant lab day effect on the intercept, such that participants have lower cortisol at the point of first sampling on the lab day (B = -.634, p<.001) than the basal day, but this is to be expected as the stressor has not begun yet, and participants arrive at the lab approximately one hour into their lab day. The effect on the slope is significant for lab day as well (B = .021, P<.001), such that cortisol does not decline as quickly on the lab day as it does on basal days.

For testosterone, there is a significant effect (B = .417, p<.05) on Cortisol, which indicating that cortisol and testosterone are coupled on waking, such that those participants who have higher testosterone have significantly higher cortisol as well. The effect of labday seems to indicate de-coupling (B = -.090, p<.05), but this predictor is included for statistical purposes only; this is likely capturing de-coupling across a typical day as it occurs at a point in time on the intercept when the stressor hasn't happened yet (see below). Instead, in order to further examine the relationship between testosterone and cortisol over the course of a stressful day, we focus on an interaction term between testosterone and time since waking, which captures hormone effects across the lab day duration. This interaction terms is negative on basal day (B=-.019, p<.05) indicating that, on average, as the day goes by, cortisol and testosterone seem to de-couple. Most interestingly, this interaction term was significant and positive on the lab day (B = .010, p=.05), suggesting that testosterone remained coupled with cortisol across challenges unfolding on the lab day, whereas it does not in the context of the basal day. The interaction term becomes the real coupling parameter in this case, as it indexes the co-

elevation of cortisol and testosterone in response to a continual stressor, whereas the labday effect on level is indexing a point in time when the participants have just arrived at the lab and have not yet undergone the MRI. This effect can be seen in figure 7 below. When diurnal rhythms are accounted for, the interaction between testosterone, cortisol, and time of day is significant and positive. For this reason, it is necessary to expand our analyses to include the full diurnal rhythm. Doing so leads to the conclusion that participants in the lab day setting maintain a more robust coupling of testosterone and cortisol throughout the day (as indicated by the interaction term) than otherwise would have been the case. Results can be seen in table 3 below.

Table 3: Bivariate Coupling in the MRI/SPIT Data set+

Coupling Parameter		***Interaction Term (TestoxTSW)***	
Intercept	*Labday*	*Intercept*	*Labday*
.417041*	-.090037*	-.019182*	.010383*

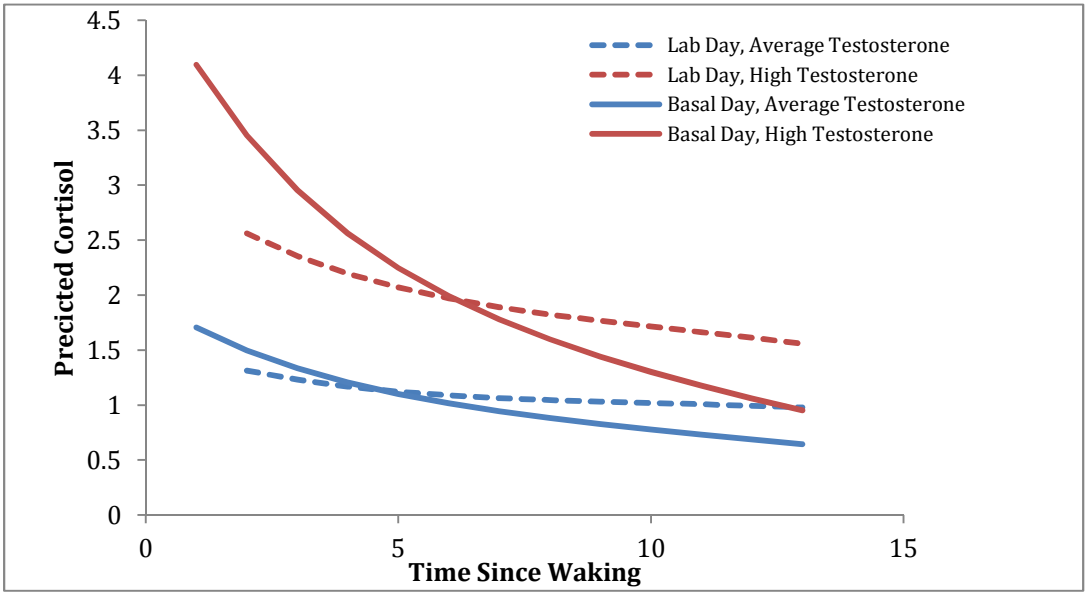

Figure 7: Coupling of testosterone and cortisol across the day in the context of a short-term stressor. Participants on the lab day tend to have a relatively flat coupling pattern across the day, such that cortisol and testosterone tend to remain coupled as the day

progresses, whereas on the basal day participants tend to de-couple.

In the tri-variate model, with testosterone, DHEA and cortisol all loaded onto the same model, neither testosterone nor DHEA were significant predictors of cortisol on the lab day. This indicates that neither testosterone nor DHEA are recruited preferentially to account for the challenges associated with a stressful lab day, and we cannot gain insight into which axis is primarily accounting for the variance in the model. As such, it is unclear whether it is DHEA or testosterone that is driving coupling in this model.

Long-Term Stress Exposure: The Mendota Project

In the Mendota Project, fifty participants provided a total of 443 saliva samples. In the first set of analyses run, cortisol was the outcome of interest, and the base model was established to index the coupling parameter. In order to best capture the diurnal rhythm of cortisol across the day, four additional variables were loaded on to the level 1 model. A dummy coded variable to capture the cortisol awakening response (CAR) was added, a winsorised time since waking (TSWW) variable was added to capture the diurnal slope, and two dummy coded time point variables to capture the before lunch and before bedtime samples were introduced due to the non-linear slope of the diurnal rhythm later in the day. Because we are interested in modeling coupling, a sex hormone variable was added as the final predictor on the level 1 model.

With the level 1 model established, HLM allows level 2 equations to be set up with the slope coefficients of the level 1 predictors as the outcome of interest for the level 2 predictors. The present study was focused on life adveristy as a between-subjects

variable that could influence the coupling parameter between sex and stress hormones. The level 1 model is represented by the equation:

$$Y_{CORT} = \beta_0 + \beta_{1CAR} + \beta_{2TSW} + \beta_{3testosterone} + \beta_{4after\text{-}lunch} + \beta_{5pre\text{-}dinner} + R$$

And the level 2 predictors were as follows:

$$\beta_0 = \gamma_{00} + \gamma_{01(Abuse\ measure)} + U_0$$

$$\beta_{1CAR} = \gamma_{10} + \gamma_{11(Abuse\ measure)}$$

$$\beta_{2TSW} = \gamma_{20} + \gamma_{21(Abuse\ measure)} + U_1$$

$$\beta_{2Testosterone} = \gamma_{30} + \gamma_{31(Abuse\ measure)} + U_2$$

$$\beta_{3after\text{-}lunch} = \gamma_{40} + \gamma_{41(Abuse\ measure)}$$

$$\beta_{4pre\text{-}dinner} = \gamma_{50} + \gamma_{51(Abuse\ measure)}$$

The level 1 model with no level 2 predictors was run to establish the coupling parameter and to model the diurnal rhythm for the sample. In the morning, Cortisol rose sharply according to the CAR (B=.59, p<.001). After this initial spike, cortisol exhibited a steady drop over the course of the day (B=-.09, p<.001). The lunch time measurement of cortisol was not significantly different than that predicted by the diurnal rhythm (B=-.05, p=.51), but the pre dinner sample was higher than would be expected (B=.70, p<.001). Testosterone was positively coupled to cortisol rhythm (B=.61, p<.001) in this sample, such that, in moments when individuals had higher testosterone, they also had higher cortisol release. This relationship can be seen in Graph 3.

Table 4: Descriptive Statistics for the Mendota Stress Variables

MEASURE	MEAN	SD
ICU CALLOUS	10.24	5.17
ICU UNCARING	10.97	5.05
ICU UNEMOTIONAL	8.08	3.26
ICU TOTAL	29.27	9.98
IRI TOTAL	55.6	15.11
IRI FANTASY	14.32	5.14
IRI PERSPECTIVE	14.08	4.29
IRI EMPATHIC	16.04	4.45
IRI PERSONAL	11.34	4.87
LSI ACADEMIC	3.2	.939
BEHAVIORAL	4.22	.913
PEER	3.72	.650
XGENDER	2.76	.839
ROMANTIC	3.15	.851
FAMILY	3.30	1.09
MARRIAGE	2.94	.968

Next, a principal component analysis of the centered LSI, CTS, and CTQ subscales was conducted to provide an aggregate vantage point for the heterogeneous construct of abuse. The first principal component to emerge was able to account for 49% of the variance in the abuse measures, and was coded as an overall 'abuse factor'. Since the Mendota Project is designed to assess the effects of long-term abuse, this represents a homogenous factor designed to encompass the overall effects of abuse, as a starting point

in the analysis. When abuse factor was loaded as a level 2 predictor, the cortisol level upon awakening was overall lower (B=-.81, p<.05), the CAR was significantly steeper (B=.16, p<.01) in males with higher abuse factor scores, and the slope was relatively stable. The coupling parameter between testosterone and cortisol was also positive and significant (B=.16, p<.05), indicating that, in adolescents who have experienced higher levels of abuse, the coupling between testosterone and cortisol (and, by extension, the HPA and HPG axes) is greater than in those with less life adversity (see figure 3). This same pattern held true for DHEA. In a similar base model, DHEA was strongly positively coupled to testosterone (B = .531, p<.001).

To understand which specific type of adversity was driving this effect, individual subscales were loaded on to the model as level 2 predictors. Consistently across subscales, positive (tighter) coupling was observed between cortisol and testosterone, as indicated by the coupling coefficient (β_3) in individuals who had greater life adversity (see table 4). The findings were not as robust for DHEA, though still present.

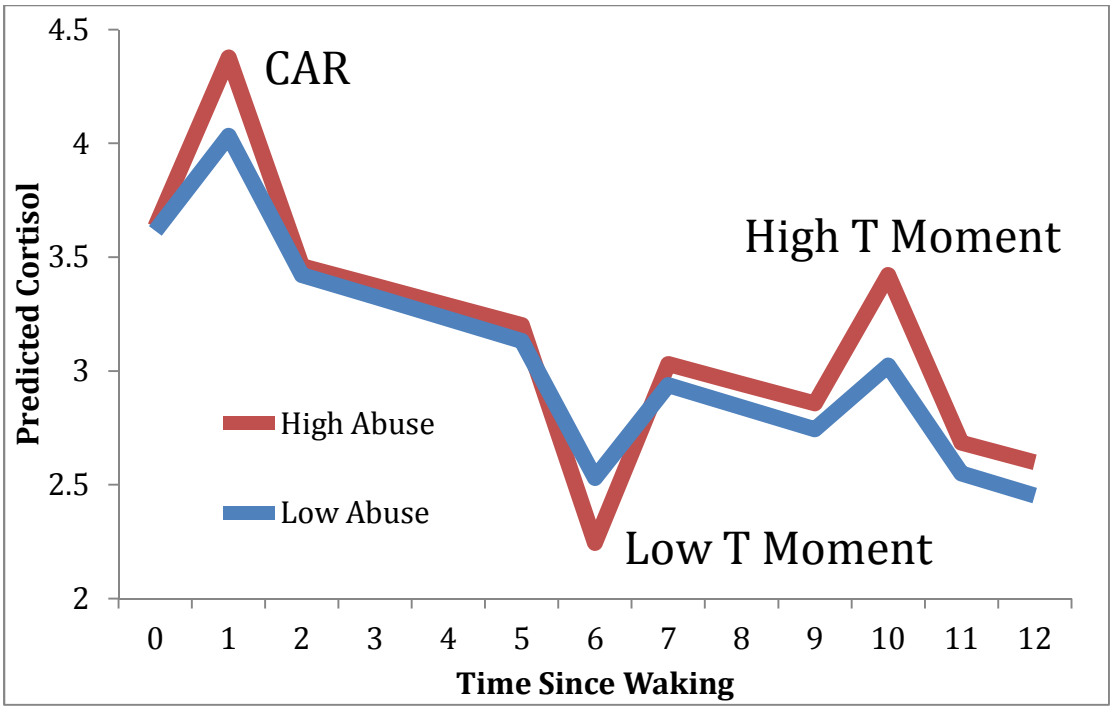

Figure 8: Abuse and predicted cortisol levels.

Table 5: Coupling in the context of long-term stress

	Cortisol as Outcome				***Cortisol as Outcome***			
Variable	*Intercept*	*CAR*	*Slope*	*DHEA*	*Intercept*	*CAR*	*Slope*	*Testo*
Abuse Factor	---	.118**	---	.531***	-.816*	.158**	.005	.160*
LSI - Life	-.290	---	.003	.040+	---	---	---	---
LSI -Chronic	---	.204*	-.018*	.016	---	.253*	-.021*	.039+
LSI -Family	---	---	---	---	---	---	-.007	.024*
LSI - Peer	---	.231*	---	-.019	-.822*	.219*	---	.161*
CTQ-PA	---	.025+	---	-.003	-.201	.036+	.002	.035
CTQ-PN	---	---	.002	.001	-.272*	.008	.006*	.052*

Aside from the bivariate model loading testosterone or DHEA onto cortisol functioning in the base model, a separate set of analyses were run loading both DHEA and testosterone onto cortisol functioning as a dependent variable. The purpose of these analyses was to assess whether the influence of prior life stress is related to DHEA operating more androgenergically than adrenally, given that DHEA can have effects on both the HPA and HPG axes. The trivariate model partials out the degree to which DHEA is acting adrenally (coefficient with cortisol) or androgenergically (coefficient with testosterone). For abuse factor, an omnibus variable, testosterone stayed significant (B=.252, p<.001)and positive in this analysis, whereas DHEA switched from positive to de-coupled (B=-.151, p<.05). This pattern held true for all other stress variables loaded on to the trivariate model. See Table 5 for full results.

Table 6: Trivariate Coupling of Cortisol, Testosterone and DHEA in the Mendota Data Set

	Cortisol as Outcome				
Variable	*level*	*CAR*	*Slope*	*DHEA*	*Testo*
Abuse Factor	-.418*	.149**	---	-.151*	.252***
CTQ - Physical Abuse	---	.034*	---	-.048+	.051
CTQ - Physical Neglect	-.201+	.009	.005*	-.009	.050+
LSI – Peer	-.997***	.219*	---	-.036	.225+
LSI – Family	---	---	---	-.029	.045
LSI – Life Rank	-.234*	.040+	.001	-.072*	.126***
LSI – Chronic	-.465	.271**	-.021*	.023	.001

Discussion

The present study found that cortisol, DHEA, and testosterone all work together in response to environmental influences in a more cooperative manner than has previously been predicted. This was validated across three stress domains – reactivity, short-term, and long-term, suggesting that positive coupling may be a robust phenomenon although it runs counter to the extant literature. These findings will be looked at individually, in terms of how they fit with our specific hypotheses and the existing literature, and then overall findings across data sets will be discussed.

Stress Reactivity: The Skydiving Project

The skydiving study is designed to assess environmentally reactive capabilities of the HPA and HPG axes. In accordance with our hypothesis, coupling was observed between the HPA and HPG axes, as indexed by the positive coupling parameter. This is a unique finding that is not in accord with the prevailing literature, but consistent with the hypothesis of cross-axis communication.

Skydiving is inherently stressful, and induces cortisol responsivity. Knowing that cortisol is stress reactive makes it easy to predict a change in level in response to skydiving. Testosterone is also reactive to challenge. Testosterone reactivity is observed in a number of competitive paradigms, including athletics, intellectual challenges, economics, and laboratory-induced challenges, such that testosterone tends to rise before competitions, and in winners, tends to stay high, but in losers, tends to drop (Booth, Shelley, Mazur, Tharp, & Kittok, 1989; Coates & Herbert, 2008; Edwards, Wetzel, & Wyner, 2006; Mazur, Booth, & Dabbs Jr, 1992; Suay et al., 1999). Insofar as skydiving represents a challenge, it could be expected that testosterone levels would rise to meet

this challenge. In one previous study, cortisol and testosterone were both looked at in response to skydiving; in this study, both cortisol and testosterone were acutely lower on a skydiving day than on a non-reactivity day, and cortisol reversed this pattern after the jump, whereas testosterone did not (Chatterton, Vogelsong, Lu, & Hudgens, 1997). There is a precedent in the literature that supports the point of view that cortisol would definitely react to skydiving, and that this reactivity would, in turn, shut off the HPG axis and testosterone would remain down-regulated. At a group level, Chatterton's findings are consistent with this overall profile of HPA reactivity and HPG suppression. This is only one study which failed to replicate with our larger sample size; moreover, we hypothesized a different interaction would occur between the HPA and HPG axes in a skydiving context. This is largely based on a more comprehensive approach to the HPA and HPG axis, and a different approach to the assessment of cortisol and testosterone using hierarchical linear modeling. The analysis of Chattterton et al. relies on a between-subject design, which does not yield a significant elevation in testosterone after the jump event. However, we used HLM, which employs a within-subject approach, making it non-susceptible to the ecological fallacy. There is a possibility that some, but not all, of the subjects in Chatterton et al. did in fact show positive coupling, but this did not show up in the overall group (for a statistical review see Marceau et al., 2013). That testosterone did not react overall also is a divergence between these two studies. However, applying group-level characteristics to the individual could have obscured the coupling that might have occurred.

We have discussed the neural regulation of both the HPA and HPG axes, and argued that these systems must be expanded upon to incorporate both top-down and

bottom-up regulatory structures, as well as functional interconnections between axes at all levels, to adequately capture the full scope of hormone production. An extensive regulation and communication system could allow for interactions that go beyond negative feedback, and could, in certain contexts, promote co-activation. It is this systems-and-structure level interaction that informs our hypothesis, and this is exactly what was found to occur in the skydiving paradigm: functional integration of the HPA and HPG axes allowed permissive co-activation, and in our HLM analysis, testosterone levels increased with cortisol levels in the skydiving event. Coupling was observed in a reactive paradigm.

This interaction was decomposed further still, however, and a model with both testosterone and DHEA predicting cortisol was run – the trivariate model. This analysis was compared to that of just testosterone predicting cortisol, in an attempt to parcel out the variance accounted for by each unique hormone. In so doing - when DHEA is included in the base model with testosterone - the variance accounted for by testosterone in the initial model is subsumed by DHEA, which remains a strong, positive predictor of cortisol level, while testosterone did not. DHEA is thus a more powerful predictor in *this* reactive context than testosterone. The implications of this await replication to fully decompose, but may imply that DHEA recruits testosterone to act with cortisol in this highly reactive context. It appears that DHEA is the 'driving force' in the coupling phenomenon in an acute event. Functionally, the stress axis may be recruiting additional hormones to cope with the challenge of skydiving. At a neural level, structures that stimulate the HPA axis to manage the stressor may be activated to such a degree that multiple stress-responsive axes are active in this context. This may not be true for

challenges of different (i.e., longer) durations, however, as indicated below. Duration of effects might have a significant impact when it comes to understanding which axis is driving the coupling phenomenon, if indeed there is a driver and the axes are not equally recruited.

Short-Term Stress Exposure: The MRI/SPIT Project

In the MRI/SPIT study, a short-term index of stress exposure, coupling of HPA and HPG axis hormones was again observed overall, but in a different manner than in the other studies as expected by the unique nature of the data set involved, and the duration of stressor. Cortisol and testosterone were tightly coupled overall. Cortisol and testosterone naturally de-coupled over the course of a day. For the short-term stress day, however, this de-coupling occured less quickly. This subtle interplay between the HPA and HPG axes highlights the importance of a shift in thinking from reactivity to diurnal profiles. Duration of stressor was the entire day, and analyses were designed with this full-day duration in mind. Results illustrate HPA – HPG coupling is influenced by stress exposure across an entire days' length. Diurnal rhythms are often neglected when it comes to testosterone, though this aspect is more prevalent in the literature on cortisol. However, considering the full days' worth of effects helped discern short-term coupling in the MRI/SPIT data set which was magnified by a stressful day.

Just as in the other studies, diurnal coupling is consistent with our hypotheses, though not with the existing literature. At first blush, it may seem as though the lab-day was related to a de-coupled pattern because the cortisol intercept showed a negative association with testosterone on the lab-day. This interpretation is inaccurate, however, as

the intercept captures waking hormone levels, but on the lab-day participants did not arrive at the laboratory until at least an hour into their day. Furthermore, the first sample in the lab was collected immediately after informed consent, before any stressor had begun. Consequently, the intercept was not capturing short-term coupling in their waking samples, but rather the natural circadian de-coupling of HPA-HPG axis activity evident on the non-stressor days.

Instead, coupling was limited to the interaction term between testosterone and the diurnal rhythm on the stressful lab-day. The most stressful component of the lab day – the MRI (a known stress inducer; see Eatough et al., 2009) induced reactivity early on, but the effects (along with the remaining stressors of the participant day) were felt over the course of the entire day. Consequently, the diurnal slope captured the short-term stress exposure effect in its interaction with sex hormone levels. In this conceptualization, this coupling pattern is similar to that seen in the skydiving study regarding reactivity. Continuing stress then obscured the rebound to baseline, and both axes continued to work together to manage the stressors of the entire lab-day, as indicated by co-elevated cortisol and testosterone across the remainder of the lab day.

In the trivariate analyses, neither testosterone nor DHEA predicted cortisol level or diurnal rhythm. This suggests that DHEA and testosterone each behaved independently in their interaction with the HPA axis, and neither one nor the other was truly driving the relationship.

Long-Term Stress Exposure: The Mendota Project

In the Mendota project, positive coupling of the HPA and HPG axes was observed, indicating this within-individual association of hormone levels is robust. Moreover, positive coupling was larger in the context of long-term stress, as indicated by the influence of abuse factor on the coupling parameter; i.e. higher levels of abuse increased co-activation, again illustrating that this dual-axis relationship is consistent.

Incarceration is not a stress-free environment, but it is a stable one. The days are highly conserved, with schedules fairly stringently applied in adolescent settings. Predictability and novelty are two components that trigger HPA axis activity, but which are not present in an incarceration context. There are acute stresses associated with incarceration, but anecdotally, these may not compare to the unpredictability of home life. Thus, the effects of stress observed in incarcerated settings likely reflect the strong, unpredictable influences of home life. Rather than focus on incarceration as a stressor, the long-term stress of lifetime stressors and child abuse were the focus of the third study.

As with the prior literature review, the effects of abuse were consistent with the expectations of the guiding theory for the thesis, but did not necessarily frame easily within the extant literature. Nonetheless, the effects of long-term stress on the HPA axis are anything but ubiquitous, and can lead to both hypo-arousal and hyper-arousal (M. Gunnar & Vazquez, 2001). The main effects of abuse on the HPA axis remain to be fully disentangled. Adding in cross-axis effects makes the question even more complicated, and renders conceptualizations of the 'HPA up, HPG down' variety somewhat superficial. A lack of consensus in the hormone literature made it difficult to reconcile with our hypotheses. The findings are in line with our hypotheses, however. The behavior of the coupling parameter in the bivariate analysis of the Mendota project does provide evidence for long-term

coupling, as we predicted. This is encouraging because the findings appear consistent across multiple studies and with the conceptual life history theory.

Interestingly, while Table 5 reflects strong coupling between cortisol and DHEA in the bivariate analyses (suggesting that DHEA is acting as a stress hormone), Table 6 reflects that the majority of the variance found for the relationship between DHEA and cortisol gets partialed out when accounting for testosterone in the trivariate model. The switch in beta weight for DHEA (moving from positive to negative) in the bivariate compared to the trivariate model suggests that DHEA is operating more androgenerically than adrenally. The positive correlation between DHEA and cortisol found in the bivariate models likely reflects the degree to which stress and sex (DHEA in this case) hormones are coupled together. Testosterone seems to be driving the coupling relationship with cortisol, and recruiting DHEA to act androgenically. In the face of early life stress, sex hormones may become even more strongly coupled with stress hormones, and potentially cause DHEA to become even more androgeneric in function than it otherwise would be in adolescent males. This is in contrast to the acute trivariate model run in the skydiving study, and suggests that

The Big Picture

Positive coupling was observed across three stress domains: reactive, short-term and long-term. Across all three data sets, findings were not in accord with what a review of the literature would suggest; however, they were in accord with our hypotheses. The mechanisms that led us to believe positive coupling was a possibility differ across data sets. The idea that neural architecture could allow, and even promote, coupling is novel, but neural architecture is certainly acutely environmentally responsive, and multiple

levels of interaction and feedback could possibly allow for 'in the moment' changes in cort-testo-DHEA coupling. This idea (and the limited literature available on the subject) led us to suspect reactive coupling was a possibility. This extended through a short-term stress day as well, especially in our design, where participants were exposed to a very stressful stimuli early in the day (the MRI), and then continually to low-grade stress as the rest of the stress day progressed. The brain would continually integrate and adapt to this acute information, thus allowing reactive coupling to extend into a short-term stress response. However, this acute consideration of stress responsivity is insufficient to explain long-term coupling; for that, we must take into account developmental considerations.

The Adaptive Calibration Model of stress responsivity posits that there are certain points in human development where significant changes can occur in limited windows of time, that these points are variable, and are timed to occur adaptively (Del Giudice et al., 2011). In addition to the promotion of broad developmental goals (such as puberty), these switch-points serve to integrate environmental information and re-calibrate the organism to best adapt to its environment. These critical periods in human development possibly set a trajectory – a sort of 'flight-plan' for the individual. Long-term stress of this type is just the sort of environmental information that the Adaptive Calibration Model posits that the body integrates during switch points. Therefore, long-term stress exposure leading into inflection points in the developmental trajectory of an organism could possibly lead to hormone axes changing the way they communicate with one another during adolescence, and these changes would then be carried forward as an adaptive response to the environment. Each axis is uniquely implicated in integrating environmental

information in the ACM; it is possible, therefore, that they could do so together, as well as individually. It is for this reason that we predicted the HPA and HPG axis could work together beyond just a reactive setting. Whilst our predictions are not wholly in line with the prevailing hormone literature, they do extend well from the literature on life history theory and adaptive calibration. The ACM is useful, especially for long-term stress exposure. Future studies should consider how this theory applies to reactive or short-term stress.

The fact that different mechanisms underlie our conceptualizations of reactive, short-term and long-term stress is born out when considering the tri-variate models. For reactive stress, much of the variance that testosterone was responsible for in the bivariate analysis was overwhelmed by DHEA in the trivariate. In reactive settings, DHEA may take charge and recruit testosterone to act more like a stress hormone, and not vice-versa.

However, in the short-term stress paradigm, neither DHEA nor testosterone were significant predictors of cortisol. Lastly, in the context of long-term stress, all of the variance that DHEA was able to account for in its bivariate analysis was lost to testosterone in the trivariate model. DHEA interacts in a highly contextual way – with long-term stressors DHEA may be recruited to act synergistically with testosterone and help speed up sex hormone maturation. In sum, the coupling between testosterone and cortisol may be understood better by using the intermediary hormone DHEA (which is an end-product of both the HPA and HPG axes) as a third variable.

This shift across time courses from adrenal dominance in acute settings to gonadal dominance in long-term settings acts in accordance with differing mechanisms underlying reactive coupling, short-term coupling, and long-term coupling. The

mechanisms underlying coupling differ, and the hormones that drive the coupling relationship differ accordingly.

We have seen that a compelling argument for coupling can be made on neuro-regulatory grounds, and that this can be extended past reactivity to long-term patterns when developmental considerations are applied. Taken together, this can provide evidence that coupling is a real phenomenon that makes sense both neurobiologically and developmentally.

Limitations and Future Directions

Each particular study has a unique set of limitations, yet the advantage of looking at coupling across multiple data sets is that the disadvantageous characteristics of individual designs are bolstered by advantages in other projects. The skydiving study, for example, would benefit from more participants, and furthermore, is not limited to adolescence. This distinguishes it from the Mendota study, as well as from the MRI/SPIT study, which are both centered on adolescence. However, this becomes less of a disadvantage, and more of an aspect of broadened scope, when looked at in conjunction with the other projects in this thesis. The Mendota project possibly suffers from an effect of the short-term stress of incarceration; however, this becomes less likely to be the case, as we have other data sets that validate these findings. The findings of the MRI/SPIT project on the interaction term make more sense when looked at in the context of reactivity and long-term stress effects, and make it less counter-intuitive to consider the day-long effects as an altered diurnal profile in reaction to a short-term stressor. Without the context of the other projects this would not make sense, however. This does not mean that there are not improvements that could be made across the board, however. Certainly

more participants validating each of the stress contexts would be useful. Strong preliminary evidence for coupling of the HPA and HPG axes was found across three data sets, however. These findings await replication in larger data sets, yet a good point from which to embark on these investigations has been established across the three studies we have discussed thus far.

The ideas underlying coupling necessitate viewing hormone axes as functionally integrated across multiple levels, and capable of a greater degree of malleability and cooperation than has previously been acknowledged. This suggests a number of future directions for promising research. Firstly, the notion of top-down regulation needs to be further explored, especially in the case of testosterone. There is a paucity of research that looks at the manner in which the brain regulates the HPG axis, and this bears further clarification. This requires experimental paradigms shifting from exogenous administration studies, which are limited to assessing the impact testosterone has on participants, to environmental manipulations that influence endogenous levels. Furthermore, there is insufficient research to delineate the bottom-up effects of cortisol on relevant structure in the brain that regulate the HPA axis, and indeed on the HPA axis itself. This builds into a consistent picture of two parallel systems with gaps in our understanding both in top-down regulation by the brain, and bottom-up feedback. Our understanding of both the HPA and HPG need to be expanded to include the brain, and the gaps in the parallel pathways need to be filled in.

Secondly, the structural foundation and neuroanatomical interconnections that inform coupling, on the level of the axes themselves, need more investigation. We need to better understand the way the brain regulates each axis, and both axes jointly, and

furthermore, we need to understand the way each axis communicates with its counterpart as they progress through their respective biochemical cascades. There is a great deal of basic, translational research with animal models still to be done, coupled with fMRI, in order to really disentangle the full scope and scale of interconnections that inform cross-axis communication. This needs to be done on the level of the brain, but also on the level of the axes themselves.

Thirdly, it is becoming apparent that the diurnal rhythm has a great degree of variability, and that coupling might not be best considered using ratio scores, which do not accurately reflect the way hormones change with each other over the course of a day. The C/T ratio is interesting, but limited, and bringing into account the full diurnal rhythm of hormones individually (especially testosterone, whose diurnal rhythm is sadly neglected) and especially when considering hormones in conjunction with one another, would be a useful tool for future research to incorporate.

Fourthly, emerging research in this area suggests a more robust and malleable interpretation of the interconnections between the HPA and HPG axes is worthwhile, but this needs to be extended forward into utility. The information in this thesis is useful as basic science, but even more so when it comes to real world utility, and the avenues which can carry this science forward.

There are developmental ramifications for co-activated axes, especially when dealing with adolescents. We must remember that this is a critical period for alignment and re-calibration of endocrine axes. The fact that the HPA and HPG can change so fundamentally in response to environmental influences means we must carefully consider the environment that children are placed in when they have undergone significant stress

exposure, for example, children removed from the home due to abusive parents. The malleable nature of the HPA and HPG axes as these children enter into adolescence means we must consider not just mental health effects, but long-term physiological effects as well.

Conclusion

Bivariate coupling of the HPA and HPG axes have been shown to occur across three separate stress domains. This is a novel finding which demands a reconsideration of the way we view the sex and stress axes, and especially the manner in which they communicate with one another. While not fully in accord with the existing literature, our findings are not unexpected. Firstly, by expanding our conceptualization of the HPA and HPG axes to include top-down and bottom-up feedback from the brain, coupling begins to make sense. Furthermore, by extending this possibility through developmental considerations, and remembering that adolescence is a critical period in which environmental information such as stress exposure is integrated, we were able to hypothesize that the way in which the HPA and HPG axes communicate with one another during adolescence could change in not just a reactive, but a long-term manner. Altogether, this builds into a cohesive picture of a stress response system which is able to react in the short term (through top-down, bottom-up, and inter – axis channels) and in the long term (through long-term changes integrated in developmental switch points), through both the HPA and HPG axes, allowing coupling to occur.

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Vita

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