ABSTRACT

Prenatal Stress in Rats and its Effects on Serum Corticosterone Levels

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Prenatal stress affects a variety of measures of stress in mature offspring, including corticosterone. The purpose of this experiment is to examine the effects of unpredictable, chronic prenatal stress on serum corticosterone levels, both at rest and in response to an acute stressor. Pregnant dams were stressed over the course of the final week of gestation using an unpredictable schedule of a variety of stressors. Offspring were exposed to foot shocks and trunk blood was collected, then analyzed by ELISA for corticosterone concentration. Prenatally stressed rats demonstrated both decreased baseline corticosterone and a slightly altered corticosterone release over time. We concluded that prenatal stress induces a general decrease in serum corticosterone, possibly due to increased negative feedback in the stress response system.

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PRENATAL STRESS IN RATS AND ITS EFFECTS ON SERUM CORTICOSTERONE LEVELS

A Thesis Submitted to the Faculty of

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By

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CHAPTER ONE

Introduction

Overview of Stress

When discussing a concept as broad and popularly used as stress, it is important to first define the terms of the discussion. Stress can be defined succinctly as a state of threatened homeostasis, and the stress response as an organism's physiological and behavioral responses to that threat (Chrousos, 2009). The forces threatening homeostasis, termed stressors, can comprise a variety of stimuli both physical and mental in nature. These stressors vary in both magnitude and duration, and any stressor exceeding a certain threshold in these two areas can activate the organism's adaptive stress response. This response compensates for the disruption caused by the stressor in order to restore homeostasis (Chrousos & Gold, 1992).

Physiological Effects of Stress Response

Since the general purpose of the stress response is to restore homeostasis, its primary effects on various organ systems tend to be short-term changes that can aid the organism in eliminating or otherwise handling the stressor. Some of the body systems affected by the stress response include the cardiovascular system, digestive system, immune system, and nervous system.

Acute physical stress tends to have predictable short-term effects on the aforementioned systems. In the cardiovascular system, blood pressure rises, heart rate increases, and the rate of sodium and potassium excretion increases after sustained

physical exertion (Herd, 1984). In addition to these excitatory changes, the functioning of the digestive system is aslo affected by the stress response. Delayed emptying of the stomach is a common response to stress; inhibitory signaling via the vagus nerve causes this delay. At the same time, motility in the colon is increased, while small intestine motility decreases (Tache & Bonaz, 2007). The immune system can also be affected in a variety of ways by acute stress. The activity and cytotoxicity of natural killer cells and cytotoxic T cells has been shown to increase in response to an acute stressor. Cytokine levels are also affected, with TNF-α levels increasing in response to short-term stress (Webster, Marketon & Glaser, 2008). Finally, the nervous system responds to stress by increasing levels of arousal, which manifests in a variety of physical changes including hyperattentiveness, pupil dilation, and other reactions commonly associated with the fight-or-flight response (Chrousos, 2009).

All of these physiologic changes in response to acute stressors work toward a common goal: to facilitate the organism's survival. In the short term, they increase the organism's ability to react to threatening stimuli, to defend itself or avoid the stressor altogether. However, stress can also have unforeseen long-term effects which are particularly potent in modern society. In place of the predators and temporary dangers of prehistoric humans, modern humans face less tangible and longer-term psychological stressors much more frequently. These chronic stressors can turn the body's stress responses against it, as the biological changes honed to counteract acute dangers can become self-destructive when applied over the long term.

The long-term effects of chronic stress impair the functioning of the systems the short-term stress response acts on, and additional compounding effects can be seen that

affect the entire body. In the cardiovascular system, blood pressure can increase and atherosclerosis can form or worsen; blood clotting can also be affected, increasing the risk of a serious clotting problem such as a thrombosis (Herd, 1984). Effects on the digestive system can manifest as a variety of chronic disorders such as irritable bowel syndrome, a disease whose hallmarks include irregular bowel movements and gut pain. A variety of early-life and chronic stressors have been tied to IBS incidence, including maternal neglect and fear conditioning (Tache & Bonaz, 2007).

Chronic stress has a variety of detrimental effects on the immune system. Natural killer cell activity and T lymphocyte activity decrease after chronic psychological stress such as marital problems. Long-term stress can also weaken lymphocyte proliferation following infections, leading to an increased rate of infection in chronically stressed individuals (Webster, Marketon & Glaser, 2008). Adding to these detriments are the effects of chronic stress on nervous and cognitive processes. Psychiatric disorders such as anxiety and depression may also be triggered by the continual release of stress mediators, as can eating disorders like anorexia and hyperphagia. Sleep can also be severely disrupted, as chronic problems such as insomnia and daytime sleepiness can manifest after prolonged periods of stress (Chrousos, 2009). Given the prominence of these chronic conditions in modern society, it is safe to say that chronic stress plays a significant role in the overall health of the population.

In addition to these broad effects, post-traumatic stress disorder is a more specific stress-based ailment that merits discussion. Post-traumatic stress disorder (PTSD) is a disorder whose onset is precipitated by a traumatic event such as assault, combat exposure, divorce, or other significant acute stressors. Its symptoms affect up to 6.8% of

Americans at some point in their lives, and are characterized by a chronic hyperexcitability of the stress response system (Wright & Robinson, 2013). Symptoms include flashbacks or nightmares, irritability, insomnia, and exaggerated startle responses, symptoms that echo the short-term arousal effects of acute stress.

Overview of the Hypothalamic-Pituitary-Adrenal Axis

The neural and endocrine systems that govern or interact with the stress response are numerous, but the primary system responsible for this response is the hypothalamic-pituitary-adrenal (HPA) axis. This axis consists of a series of feedback loops involving the hypothalamus of the brain, the pituitary gland, and the adrenal glands, which work in tandem to regulate the release of chemical mediators of stress (Tsigos & Chrousos, 2002).

The HPA axis begins at the hypothalamus, which responds to a perceived stressor by releasing corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) (Tarullo & Gunnar, 2006). The area of the hypothalamus responsible for releasing these hormones, the paraventricular nucleus, contains a variety of subspecialized neurons for the purpose of said hormone production. Magnocellular neurosecretory cells contain a single long axon, which projects toward the posterior pituitary gland. They also have between one and three dendrites, each of which contains thousands of neurosecretory granules. The neurosecretory granules contain both vasopressin and oxytocin, another important neurochemical mediator, and release them by exocytosis in response to stimuli (Leng, Brown, & Russell, 1999). Upon exocytosis, vasopressin is carried down the axon of the neuron through the infundibulum and transported via Herring bodies directly to the posterior pituitary for storage and later release (Sawchenko, Swanson, & Vale, 1984).

Corticotropin releasing hormone, on the other hand, is a hormone primarily geared toward effecting an immediate response from the pituitary gland. CRH is stored in the parvocellular neurosecretory cells of the paraventricular nucleus, the axons of which also project to the median eminence (Sawchenko et al., 1984). This path brings CRH, like vasopressin, to the hypothalamic-pituitary portal system, through which it travels to the anterior pituitary. There it acts on corticotropes, basophilic cells that produce several pituitary hormones, to induce the release of adrenocorticotropic hormone, or ACTH (Tse, Lee, & Tse, 2012).

Once CRH reaches the corticotropes of the anterior pituitary, the pituitary portion of the HPA axis begins. Unlike the hypothalamus, the pituitary gland's function in the HPA axis is limited to the release of a single hormone: ACTH. ACTH is a peptide hormone cleaved from pro-opiomelanocortin, a longer peptide precursor whose cleavage by endopeptidases also results in the creation of other peptide hormones such as melanocyte-stimulating hormone (Seidah, Rochemont, Hamelin, Benjannet, & Chrétien, 1981). After secretion from corticotropes, ACTH is released into systemic circulation, which allows it to travel to the cortex of the adrenal glands. Here, it binds to cell-surface ACTH receptors in the adrenal cortex, and in particular the zona fasciculata of the cortex. The cells of the zona fasciculate respond in turn by releasing the last major link in the HPA chain, cortisol (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010).

Cortisol is the primary glucocorticoid stress hormone in humans, and its functional analog corticosterone fulfills similar functions in rats and other animals. These glucocorticoids fulfill a variety of functions, and are often associated primarily with stress. Produced in the zona fasciculata of the adrenal gland, cortisol enters

systemic circulation to exert a wide variety of effects on tissues throughout the body that are influenced by the stress response system. Cortisol is typically protein-bound in plasma, so cortisol measurements must measure the small percentage of cortisol that exists in an unbound state in plasma (Hamrahian, Oseni, & Arafah, 2004). It stimulates gluconeogenesis and facilitates glycogenolysis to regulate blood sugar (Coderre, Srivastava, & Chiasson, 1991), and is the final mediator for many of the stress response reactions detailed previously.

Cortisol (or corticosterone) also plays an important part in the regulation process of the HPA axis and stress response. Cortisol provides negative feedback to the hypothalamus in its regulation of CRH, with high cortisol concentrations inhibiting CRH secretion. It simultaneously inhibits ACTH secretion from the pituitary, creating a two-pronged feedback regulation system that prevents the oversecretion of cortisol (Yehuda, Yang, Buchsbaum, & Golier, 2006). Failures of this feedback system can have important behavioral and psychological consequences; for instance, studies of patients with depression have revealed a lack of proper feedback inhibition in this system (Checkley, Sayal, Papadopoulos, Gibson, & Reynolds, 1996). Similar dysregulation occurs in PTSD patients, supporting the clinical relevance of negative feedback mechanisms in the HPA axis.

Limbic System Interaction with the HPA Axis

Although the HPA axis is a cohesive circuit that can respond to external sensory input, the limbic system also has an important role to play in the activation and regulation of the stress response. Some of the most prominent structures in this inter-system

dialogue include the hippocampus, the medial prefrontal cortex, and the amygdala (Herman, Ostrander, Mueller, & Figueiredo, 2005).

The hippocampus has been shown in previous studies to decrease glucocorticoid secretion and inhibit the HPA axis (Rubin, Mandell, & Crandall, 1966). This regulation of the HPA axis has been shown to be specific to both a particular region of the hippocampus and particular types of stressors. Restraint stress, open field, and elevated plus maze stressors were shown to trigger hippocampal interaction with stress-induced HPA activation, while hypoxia did not trigger this response (Herman, Cullinan, Morano, Akil, & Watson, 1995). While the hippocampus's role in the HPA axis is primarily inhibitory, it may also contribute to stimulation of corticosterone release, particularly if the dorsal hippocampus is activated (Feldman & Weidenfeld, 1993).

The medial prefrontal cortex is also implicated in regulation of the HPA axis. Lesion experiments have demonstrated a stressor-specific HPA inhibition by the medial prefrontal cortex (Figueiredo, Bodie, Tauchi, Dolgas, & Herman, 2003). Further studies have also concluded the topographic specificity of such lesions' effects on stress response, with lateral asymmetry of results demonstrated (Sullivan & Gratton, 1999).

The amygdala, another external source of mediation relevant to the HPA axis, is of particular interest to our laboratory. This region of the brain performs crucial functions in memory, emotional responses, and decision-making processes (Amunts et al., 2005). Anatomically, the amygdala is divided into multiple nuclei, which can be distinguished based on histological measures of tissue density, cell configuration, and fiber arrangement. These regions also differ based on evolutionary age, with the cortico-

medial region being older and the basolateral region being more evolutionarily recent (LeDoux, 2007). In addition, the types of nuclei differ in function.

The basolateral nuclei of the amygdala consist of the basal nucleus, lateral nucleus, and accessory basal nucleus. These nuclei have discrete, specific functional roles in the activation of the stress response system through fear conditioning. The basal nucleus and accessory basal nucleus are involved with contextual fear conditioning via projections from the hippocampus to both of these nuclei (Yaniv, Desmedt, Jaffard, & Richter-Levin, 2004). In contrast, the lateral nucleus of the amygdala has been pinpointed as a center of convergence for auditory conditioned stimulus inputs. This centralization of auditory stimulus processing is accomplished through projections from the thalamus and auditory cortex which stimulate the lateral amygdala to respond (Yaniv et al., 2004). This differentiation between amygdala nuclei based on stimulus modality has only been studied in recent years, and the implications of this stimulus specificity are still under investigation.

In addition to the basolateral complex, the corticomedial and central regions of the amygdala are also important in fear and anxiety. The central nucleus of the amygdala has direct projections to the lateral hypothalamus, and these projections can play a role in the activation of the sympathetic nervous system's response to fear-inducing stressors (Davis, 1992). The central nucleus also projects to the vagus nerve, through which it can further affect autonomic responses via the vagus nerve's dorsal motor cortex (Hopkins & Holstege, 1978). Projections to the parabrachial nucleus increase respiration in response to stress, while projections to the trigeminal nerve increase facial expressions of fear. Perhaps most relevant to the stress response system are the central nucleus's projections

to the paraventricular nucleus of the hypothalamus. (Sawchenko et al., 1984)These projections induce the hypothalamic release of ACTH, which continues the stress response cascade resulting in glucocorticoid release and subsequent systemic effects.

Through its many projections to other areas of the brain, the amygdala has the potential to interact significantly with stress and fear responses. The primary functional lens through which it effects fear-based and stress-based changes is through memory consolidation, a key component of fear learning. The basolateral complex is of particular importance in the process of stress memory modulation and consolidation, which is mediated by both catecholamines and glucocorticoids.

Norepinephrine enhances memory consolidation in fear learning when injected into the basolateral complex. This effect is specific to the basolateral complex, as norepinephrine has no effect when applied to the central nucleus of the amygdala (Roozendaal, McEwen, & Chattarji, 2009). The effect of norepinephrine is pronounced when administered immediately after fear learning exercises, which implies its particular importance in the consolidation phase of fear-associated memory. In addition to this positive correlation, the inverse effect that lowering norepinephrine levels has on fear learning (i.e. impairment of the process) has also been indicated via the use of β-adrenoceptor antagonists (Liang, McGaugh, & Yao, 1990). The activity of noradrenergic neurons in the BLA is regulated by GABAergic receptors, as antagonists for these receptors enhance memory consolidation, while antagonists for β-adrenoceptors negate this memory enhancement (Brioni, Nagahara, & McGaugh, 1989).

The direct effects of norepinephrine on the amygdala can also be attenuated by epinephrine and glucocorticoid hormones. Epinephrine cannot cross into the brain from

systemic circulation due to its inability to bridge the blood-brain barrier, but systemic epinephrine can activate β-adrenoceptors in afferent pathways leading to the amygdala (McGaugh, Cahill, & Roozendaal, 1996). Glucocorticoids, on the other hand, can easily enter the brain to directly affect the basolateral amygdala (Roozendaal et al., 2009). Glucocorticoid receptor activity in the BLA has been shown to improve memory consolidation, while antagonists to these receptors diminish post-fear-conditioning memory consolidation (Roozendaal & McGaugh, 1997).

Along with these broad hormonal effects, acute and chronic stressors can cause notable changes to neuronal activity and transmission, as well as to neuron growth and structure. Regarding the changes incurred on neuron activity, several specific experimental results are relevant to the discussion. A study of feline BLA activity after foot shocks demonstrated a marked increase in BLA neuron firing rate that climbed until 30-50 minutes post-shock, two hours after which it declined to normal levels (Pelletier, 2005). This finding could indicate the role of stress and emotional arousal on memory consolidation, and suggests a possible time frame during which this consolidation enhancement would be most potent. Another series of experiments by Stutzmann, McEwen, and LeDoux (1998) found a link between corticosterone levels and serotonin inhibition of glutaminergic lateral nucleus activity. This finding was of particular note because corticosterone's effects were found to be dosage-dependent: high doses of corticosterone facilitated this inhibitory action, but low doses did not. This provides another clear picture of the link between stress and the functioning of BLA neurons.

Stress can also directly affect the structure of amygdala neurons, making changes important to long-lasting neural plasticity. One study that has been supported by further

literature is that of Vyas et al., who found that chronic stress can augment arborization of dendrites in the amygdala (Vyas, Mitra, Rao, & Chattarji, 2002). This increased branching was linked to increased emotional responses and possible mood disorders brought about by chronic stress. Morphologic changes can additionally come about after acute stress, even a one-time stress event. The changes, in the form of heightened dendritic spine formation, accompany similarly heightened anxiety behavior; both of these effects were found to be delayed by 10 days after the acute stress (Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005). Chronic repetition of the acute stressor was then shown to amplify spine formation and anxiety increases over time. Thus the amygdala, and in particular the basolateral complex, can undergo noticeable structural changes over time in response to a variety of acute or chronic stressors. Underlying structural changes can then contribute to changes in higher-order cognition and behavior that characterize long-term anxiety.

Effects of Prenatal Stress

Stress has a wide range of effects on the body, and those effects can be particularly potent to a developing body and brain. While the prenatal environment is relatively free of direct stressors to the developing fetus, the child's intimate physiologic connections to the mother's body make stress on the mother into stress on the child. Prenatal stress, through maternal stress response and the transmission of its neurochemical mediators to the child, can significantly alter the development of the fetal brain (E. P. Davis, Glynn, Waffarn, & Sandman, 2011).

In neurochemical terms, glucocorticoids are crucial modifiers of fetal brain development, with cortisol (in humans) or corticosterone (in rats and other animals)

playing a particularly important role. The maternal HPA axis undergoes dramatic changes during pregnancy, and maternal cortisol levels have been shown to double over the course of human pregnancy (Sandman et al., 2006). In humans, the increased cortisol levels are regulated by 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), a fetal enzyme that inactivates cortisol via oxidation to cortisone. Since cortisone is not a human neurochemical mediator, it has no effect in this oxidized state (Brown et al., 1996). 11β-HSD2 provides moderate protection from increased maternal corticosterone levels, but its protection is incomplete and thus the fetus can receive some effects of placental corticosterone. Subsequent studies have shown a significant correlation between maternal and fetal glucocorticoid levels, mothers under considerable stress will show upregulation of the stress response system, increased systemic cortisol or corticosterone, and a resultant increase in fetal glucocorticoids (Gitau, Fisk, Teixeira, Cameron, & Glover, 2001).

Prenatal stress also has important developmental consequences for the amygdala and HPA axis. Prenatally stressed rats have decreased size in multiple amygdala nuclei, including the basolateral and central nuclei. Kraszpulski et al. hypothesized that these findings could indicate a fundamental lack of development in the amygdala components that control fear behavior. Such undeveloped elements could include key GABAergic interneurons in several amygdala nuclei (Kraszpulski, Dickerson, & Salm, 2006). In contrast to these results, Salm et al. have reported an increase in the size of the lateral nucleus of the amygdala as a result of prenatal stress (Salm et al., 2004). This increase in size was associated with an upregulation of CRFergic neurons, an important indicator of HPA axis regulation. It is clear that prenatal stress makes a significant impact on the

neuroanatomy of the amygdala, even if the precise nature of the change is not fully known.

A more broad analysis of the possible physiologic effects of prenatal stress reveals a large pool of far-reaching consequences. Early on, prenatal stress can affect the developmental trajectory of the offspring by decreasing the weight of offspring, which can correlate with a host of developmental delays (Schneider, Roughton, Koehler, & Lubach, 1999). Maternal stress in rats also specifically decreases adrenal and pancreas weight, as well as plasma glucose levels (Lesage et al., 2004). These changes could potentially predispose prenatally stressed offspring to type two diabetes mellitus later in life. Changes in sexual physiology have also been observed as a result of prenatal stress, with prenatally stressed males demonstrating reduced copulatory behavior caused by increased adrenal cortex androstenedione production and decreased testosterone production (Ward, 1972). These broad changes to fundamental neural and endocrine physiology underscore the potential potency of prenatal stress on the developing animal.

Experimental Goals and Hypothesis

Given the existing literature regarding prenatal stress and the host of questions that remain regarding its effects, we set out to conduct a small-scale study of the effects of prenatal stress on serum corticosterone of mature offspring. We believe this to be an important subject of inquiry because the regulatory imbalances of stress response that prenatal stress can potentially induce (which would be indicated by altered corticosterone levels) have such long-lasting and pervasive effects on the offspring. Implications for such imbalances extend well beyond rat physiology and into human behavior and disease,

making this a topic relevant not only for pure scientific inquiry but also for inquiries related to epidemiology and medicine.

CHAPTER TWO

Materials and Methods

Experimental Design

Adult female Sprague-Dawley rats were bred, either in-house or by the supplier (Harlan Laboratories). Dams were randomly assigned to one of two stress conditions, either unstressed control (USC, n=4 dams) or prenatal stress (PNS, n=4 dams). Rats in both groups were housed in the university animal care facility for the duration of their pregnancy. Mothers in the PNS group were subjected to mild, unpredictable chronic stress beginning on gestational day 14. This stress schedule is detailed in Table 1. Unstressed control dams remained undisturbed in their home cages, except for normal animal husbandry procedures.

Gestational Day	14	15	16	17	18	19	20	21
AM (9-11)	11:00 AM Restraint (30 min)		9:00 AM Swim (15 min)		10:00 AM Injection	9:00 AM Swim (15 min)	Cold Stress 10:00 AM - 4:00 PM	11:00 AM Swim (15 min)
Noon (12-3)	2:00 PM Swim (15 min)	Cold Stress 12:30-6:30 PM	12:00 PM Restraint (30 min)	1:00 PM Injection	2:00 PM Swim (15 min)	2:00 PM Injection		2:00 PM Restraint (30 min)
PM (4-7)	6:00 PM Injection	Overnight Fasting	4:00 PM Injection	5:00 PM Restraint (30 min)	4:00 PM Restraint (30 min)	Lights on overnight		5:00 PM Swim (15 min)

Table 1: Prenatal Stress Schedule

For restraint stress, each rat was placed in a plexiglass tube, closed off at both ends, with minimal space for movement. The rats were returned to their cages after 30 minutes in the tube. In the swim stress procedure, rats were placed in a large plexiglass

cylinder filled with water, where they swam for 15 minutes under experimenter supervision. After swimming, the rats were towel dried and returned to their cages. Injection stress was accomplished by injecting each rat intraperitoneally with a sterile 0.9% saline solution. In the cold stress procedure, rats were moved to a cold room set at a temperature of 4 degrees C, and remained there in their cages for 6 hours before being returned to their standard housing room.

Prenatal stress procedures were terminated either as scheduled or when the mothers gave birth, whichever came first. Offspring were allowed to mature under normal conditions in the animal care facility. Experiments began when the pups attained a postnatal age of 30 days. The effect of prenatal stress on the stress response of adult pups was investigated using a two factor design. Factor one is the prenatal stress condition (USC or PNS). The second factor is time after acute stress (0, 2, 10, 30 or 60 min). One pup from each litter of the unstressed control (USC) and prenatal stress (PNS) groups were randomly assigned to one of the levels of time after acute stress. The acute stressor consisted of a 1 mA foot shock for a duration of 1 second. At the designated time, animals were sacrificed by decapitation. Immediately after decapitation, trunk blood was collected in test tubes containing anticoagulant (EDTA) and immediately placed on ice. Samples were then centrifuged at 1300G for 10 minutes. Supernatant was pipetted from each sample and stored in individual vials and stored at -80 degrees C until used.

Analysis of Data

Corticosterone levels were determined by ELISA using a kit according to the manufacturer's instructions. Baseline corticosterone concentrations were assessed, and

the standard curve below was used to calculate corticosterone concentrations from measured optical density levels.

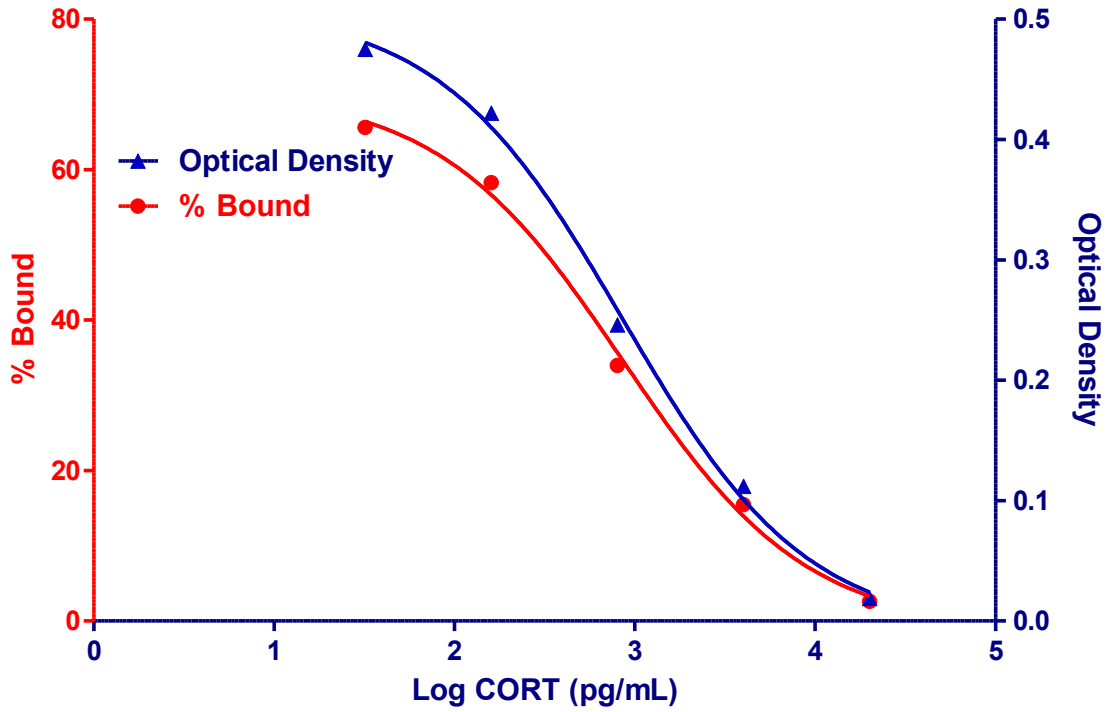

Figure 1: Corticosterone ELISA Standard Curve

The effect of prenatal stress condition on baseline corticosterone level was first analyzed by Student's unpaired t-test. Corticosterone levels over time after the acute shock stress were analyzed by a two-way ANOVA (prenatal stress x time after shock). Both factors were manipulated as between groups factors. Following a significant ANOVA, specific time points were compared between stress conditions by post-tests corrected for multiple comparisons. Data are expressed as mean plus/minus standard error. Statistical significance was defined as the probability of type I error less than 5% ($P < 0.05$)

CHAPTER THREE

Results

Baseline Corticosterone Concentrations

Baseline corticosterone concentrations were measured for each litter and mean concentrations were compared between the prenatally stressed and unstressed groups, as shown in Figure 2. Prenatal stress was associated with a decrease in corticosterone concentration that did not reach statistical significance [T(5) = 1.491, $p > .05$]. Unstressed control rats had a baseline corticosterone concentration of 120 ± 61 pg/mL (N=4), while corticosterone concentration in prenatal stressed rats was 11 ± 10 (pg/mL N=3).

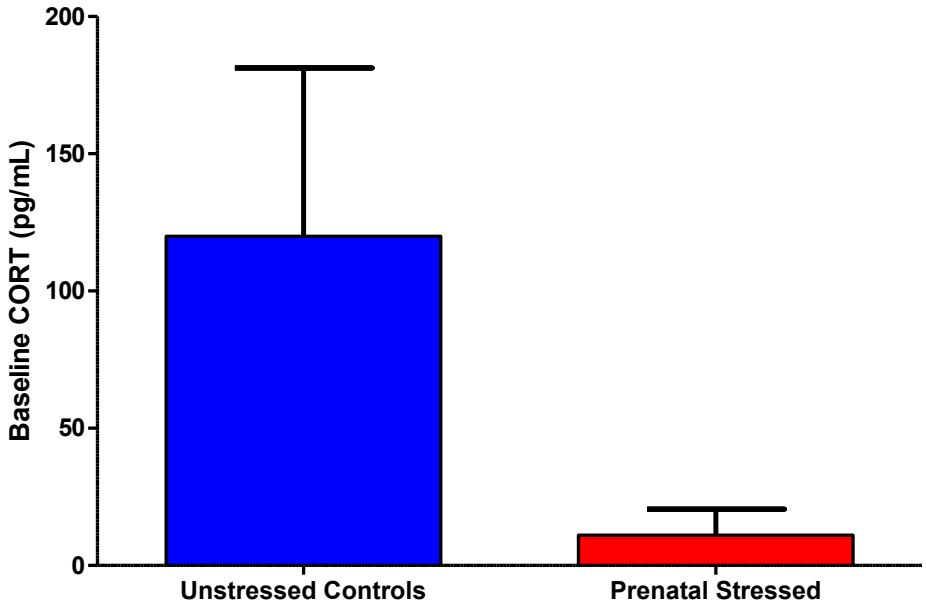

Figure 2: Baseline serum corticosterone concentration (mean ± SEM) for unstressed controls and prenatal stressed rats

Serum Corticosterone in Response to Acute Stress

Serum corticosterone concentration was measured over time following an acute footshock stress (see Methods). the results are shown in Figure 3 below. Unstressed control rats showed a progressive increase in corticosterone levels over time, reaching a peak of 2868 ± 350 pg/mL 30 minutes after the acute stressor. At 60 minutes, corticosterone levels decreased. In contrast, corticosterone levels in prenatal stressed rats showed a more constant level of corticosterone between 10 to 60 minutes, with a mean at or near 1000 pg/mL. Corticosterone levels over time were analyzed by two-way ANOVA, examining the effects of time and stress on corticosterone concentration. Analysis revealed a significant main effect of time on corticosterone concentration [$F(4,17) = 8.20$; $p < .05$]. However, there was no significant main effect of prenatal stress condition [$F(1, 17) = 1.62$; $p > .05$], and no significant stress x time [$F(4, 17) = 2.52$; $p > .05$]. Post-hoc comparisons between prenatal stress condition at each time point revealed that corticosterone level 30 post shock stress was increased [$T(17) = 2.791$; $p > 0.05$

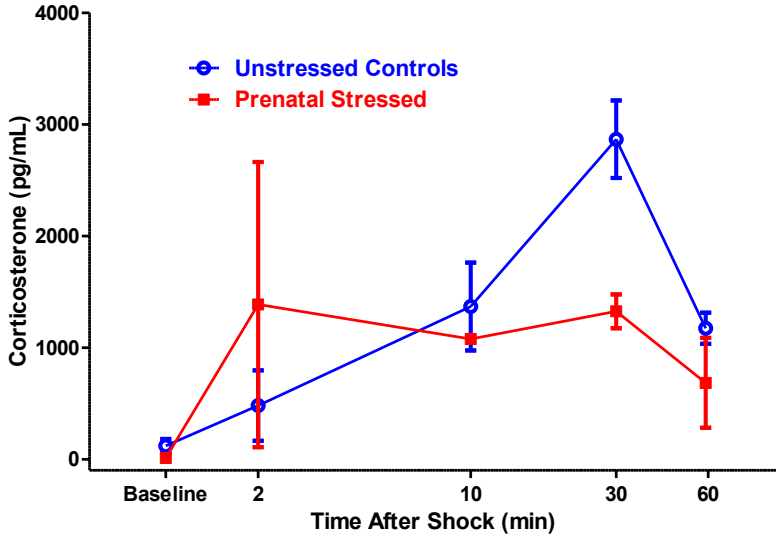

Figure 3: Serum corticosterone concentration over time after foot shock in prenatal stressed and unstressed control rats.

CHAPTER FOUR

Discussion

Summary

We measured blood corticosterone concentration in stressed and unstressed animals, analyzing their concentrations by ELISA. We found that resting corticosterone concentration was higher on average in unstressed control rats than in rats exposed to prenatal stress. When measured over time after a foot shock, corticosterone concentration increased significantly over time. Unstressed control rats reached a higher average corticosterone concentration by 30 minutes after foot shock, followed by a decline. Prenatal stressed rats' average corticosterone concentration jumped higher than the controls' value at 2 minutes, but maintained a consistent level below that of the unstressed controls for the remainder of the measured period. Ultimately, prenatal stress conditions did not display a statistically significant effect on corticosterone concentration.

Comparisons to Existing Literature

To put the results of this experiment in perspective, comparisons can be drawn to the findings of prior studies. For instance, we did not expect the results of our baseline corticosterone measurements to turn out the way they did, believing instead that prenatally stressed rats would show a significant increase in baseline corticosterone concentration over controls. This expectation was due to results from the literature such as those of Weinstock et al. that indicated consistently higher baseline corticosterone in

prenatally stressed rats than in controls (Weinstock, Poltyrev, Schorer-Apelbaum, Men, & McCarty, 1998). Takahashi, Turner, and Kalin found a similar trend, concluding that baseline cortisol levels increased significantly in stressed mothers and their prenatally stressed offspring compared to controls (K. Takahashi, G. Turner, & H. Kalin, 1998).

The results of the corticosterone over time measurement for unstressed control rats align very well with prior measurements found in the literature. A peak at 30 minutes followed by a decline is characteristic of existing corticosterone data, and thus this result seems to be supported by previous findings (cf. Weinstock et al., 1998). The data for prenatally stressed rats were less similar to what we expected, given the tendency of the mean to be below the mean for prenatally stressed rats. However, there have been prior studies that have demonstrated a decreased corticosterone response over time in prenatally stressed rats, so again there is some precedent for this finding (Burton et al., 2007).

Possible Future Studies

Overall, the prenatal stress effects demonstrated in this experiment were surprising but not unprecedented. The hypothesis that prenatal stress would increase serum corticosterone was refuted, and Future studies could benefit from increased sample size, as this experiment was designed as more of a pilot study and thus had a smaller sample size than many related experiments in the scientific literature. Further inquiry might also be directed toward relating behavioral abnormalities with the corticosterone changes detailed herein.

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