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CAN INDIVIDUAL DIFFERENCES IN THE RANGE OF STRESS HORMONE PRODUCTION PREDICT BEHAVIORAL COPING WITH CHALLENGE?

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ABSTRACT

Every animal handles unexpected stimuli and situations differently. An individual's ability to cope with challenge is dependent upon prior experience and intrinsic factors such as endocrine sensitivity. Our study assesses how the physiological range of stress hormone production, intrinsic to an animal, influences how it responds to chronic stress over time. In this experiment, we evaluate the hormone production of 30 male Sprague Dawley rats exposed to either chronic stress or control housing conditions. Normal, circulating, and challenged corticosterone levels were assessed before and after the stress treatment was applied. To assess the behavioral response to challenge, we conducted a successive negative contrast (SNC) test. This test assesses how the animal copes with a sudden decrease in reward value. Animals that are more sensitive to challenges show a greater sensitivity to this unexpected downshift in the reward value. After the SNC test took place, a third and final round of blood collections occurred. The corticosterone levels were evaluated using a radioimmunoassay kit and it was determined that the chronic stress period resulted in decreased peak corticosterone production [RMANOVA, $F_{(1, 28)} = 5.54$, $P < 0.03$], and decreased reactive scope of corticosterone production [RMANOVA, $F_{(1, 27)} = 5.120$, $P = 0.03$]. The SNC responses were not different between the stress and control rats [RMANOVA, $F_{(1, 28)} = 0.02$, $P = 0.90$]. Comparisons were then conducted on the hormone profiles of the animals with their behavioral response to the SNC challenge. A direct trend between reactive scope and SNC recovery score was found after the stress period at time point 2 and 3 [$R = 0.70$, $P < 0.01$] and [$R = 0.51$, $P < 0.01$] showing that stress does in fact cause individual variations in reactive scope and therefore coping ability.

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1. Introduction

Our bodies evolved to deal with our surroundings and unexpected stimuli using innate coping responses (Overli et. al., 2007). Without innate coping mechanisms we would not be able to survive the ever-changing world around us. Every individual is unique when it comes to dealing with unexpected stimuli and stress. How an individual copes with an unexpected obstacle is dependent upon prior experience and inherent factors such as endocrine sensitivity (Overli et. al., 2007). While the body's response to unexpected stressors is crucial to survival, overuse of stress-response systems can have adverse effects on behavior and physiology (McEwen, 1998). If an individual experiences a stressor, a stimulus that causes a stress response, repeatedly for an extended period of time this is considered a chronic stressor. Once a stressor becomes chronic, it may have pathological, disease causing, effects on the body. Continuous exposure to stress can lead to steroid diabetes, infertility, decreased growth, and impaired immunity to disease (Boonstra, 1998). An individual's ability to cope with chronic stress may also contribute to their likelihood of developing an addiction (Piazza & Moal, 1998).

Due to the deleterious consequences of chronic stress, it is important to understand what affects an individual's ability to cope with stress. One way to do this is to use animal models to gain insight into the coping response. By evaluating how individual animals respond to chronic stress, scientists can better understand the innate mechanisms that similarly operate in humans (Thanos et al., 2009). Such advances in understanding will promote easier identification of individuals that are uniquely vulnerable to stress and therefore have an increased risk of developing a disease or addiction.

Chronic stress has the ability to change the range of physiological and endocrine mediators of an animal, (McEwen, 1998) or the biological processes that function to maintain homeostasis. A common mediator used by an animal during the stress response is glucocorticoid hormone production (corticosterone in rats) from the hypothalamic-pituitary-adrenocortical (HPA) feedback system. The function of the HPA feedback system is crucial to responding to environmental challenges. This system, also known as fight-or-flight response, deals with a stressful situation and brings the animal back to homeostasis (Boonstra et al., 1998). Shortly after being stressed, an animal secretes corticosterone which mobilizes resources to fuel a behavioral escape or fight response (Boontra et al., 1998). Stress stimulates the pituitary to secrete adrenocorticotropic hormone (ACTH) which mediates the release of corticosterone from the adrenal cortex. Corticosterone then binds to receptors in the brain to regulate gene transcription and fine-tune the future stress response (McEwen, 1998). The HPA response is crucial to dealing with short-term stress but it is not designed to deal with chronic stress and is detrimental to long-term survival and fitness if sustained for a significant period of time (Boonstra et al., 1998).

The reactive scope model, a model developed by Romero, Dickens, and Cyr (2009) that evaluates how animals respond to unpredictable stimuli, can be used to model corticosterone production during the stress response. There are four key ranges in the reactive scope model which can be used to describe corticosterone production: predictive homeostasis, reactive homeostasis, homeostatic overload, and homeostatic failure. Predictive homeostasis is the range of corticosterone production caused by predictable stimuli such as circadian and seasonal variations in the environment. Reactive homeostasis is the range of corticosterone production caused by unpredictable stimuli such as predators in the environment. Both the predictive and reactive homeostasis ranges define the normal reactive scope of corticosterone production for an animal. An animal's normal reactive scope is specific to them and shapes their ability to respond

to unpredictable stimuli. While within their normal reactive scope for corticosterone production, an animal experiences a healthy physiological coping response (Romero et. al., 2009).

When conditions reach extreme levels such as during periods of chronic stress, corticosterone production may be forced to function outside of this normal reactive scope. Homeostatic failure occurs when corticosterone production is below the predictive homeostasis range. When the normal processes of the HPA axis cannot be maintained during a stress response, the animal will fail to cope. Homeostatic overload occurs when corticosterone production is above the reactive homeostasis range. Corticosterone production can enter homeostatic overload but cannot be maintained without detrimental long-term effects. In these situations, the production of corticosterone becomes pathological and causes problems in the animal that could eventually lead to death. The threshold between an animal's reactive homeostasis and homeostatic overload ranges is dynamic and changes seasonally due to increased environmental stressors during certain times of the year (Romero et. al., 2009). Figure 1 shows a basic reactive scope model of corticosterone production.

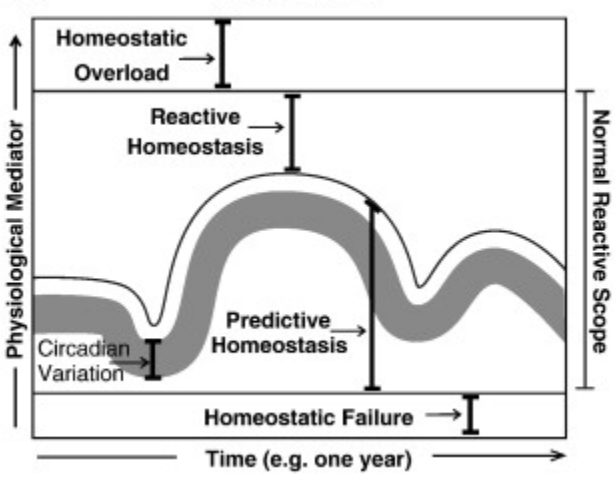

Figure 1: Basic Reactive Scope Model (Romero et. al., 2009)

Stress can change the threshold between reactive homeostasis and homeostatic overload due to "wear and tear". Wear and tear occurs when physiological systems are maintained for extended periods of time in the reactive homeostasis range causing systems to lose the ability to counteract threats and unpredictable stress. The decrease in threshold between the reactive homeostasis and homeostatic overload causes a decrease in reactive scope (Romero et al., 2008). According to the Romero model, exposure to prolonged unpredictable stimuli can temporarily decrease an animal's normal reactive scope. This results in more opportunities for the animal to experience homeostatic overload, which can result in a permanent decrease in the reactive scope Range. Therefore the individual reactive scope of an animal and their unique response to environmental stimuli can differ based on prior life experiences and stressors (Romero et. al., 2008). In Figure 2, Romero's reactive scope model is illustrated and highlights what can occur to an individual's reactive scope range due to chronic HPA axis stimulation and corticosterone production.

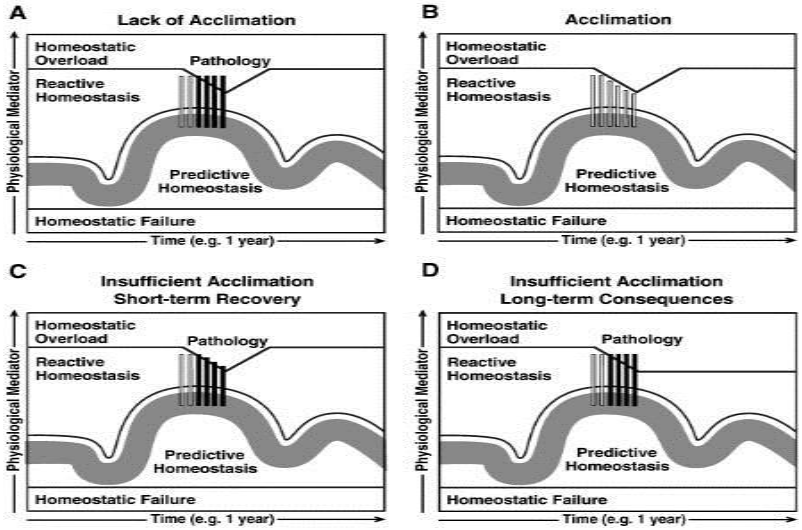

Figure 2: Effects of chronic stress on the Basic Reactive Scope Model (Romero et al., 2009)

Figure 2 (A) represents the repeated production of corticosterone due to chronic stress. Prolonged exposure to stress can decrease the reactive scope range causing corticosterone release to become pathogenic until the threshold is adjusted back after the stressor is removed. Figure 2 (B) shows the HPA axis acclimating to the stress and decreasing corticosterone production in order to avoid reaching pathologic levels. In figure 2 (C) an acclimation response is shown by the HPA axis but not well enough to avoid the pathologic results of chronic corticosterone production. And finally, figure 2 (D) shows a permanent decrease in reactive scope. A permanent change in reactive scope will increase the likelihood of pathologic corticosterone production in the future. This can result in even more opportunities for detrimental long-term effects (Romero et al., 2009).

It is important to identify which survival and fitness mechanisms are affected by this permanent decrease of an animal's reactive scope of corticosterone production do to chronic stress. How an animal is reacting to stress can be determined by their coping response. By identifying individual coping capacity, an animal's unique ability to deal with stress can be determined (Koolhaas et al., 1999). Successive Negative Contrast (SNC) tests can be used to assess an animal's coping response as mediated by glucocorticoid production (Mitchell & Flaherty, 1998; Gomez et al., 2009). This test can be used to determine how an individual behaviorally copes with challenge. When an animal experiences a reward reduction or loss, they may identify and evaluate the change, search for a more rewarding substrate, become conflicted when it can't be found, and eventually recover from the change (Flaherty, 1999). Animals are more sensitive to reward losses than gains, especially those in a negative affective state (Harding et al., 2004; Burman et al., 2009). For example rats in unenriched housing show increased sensitivity to reward loss compared to rats in an enriched housing environment (Burman et al., 2008). Additionally, rats that experienced chronic stress during adolescence are more sensitive to the SNC test than rats raised in control settings (Chaby et al., 2013). The success of SNC testing

in showing how animals differ in coping ability makes it useful in evaluating the efficacy of the reactive scope model in determining the long-term effects of chronic stress.

The SNC test gives a unique insight into how well an animal's stress response is dealing with unexpected stimuli. The research in this thesis attempts to link the coping ability of rats to their range of corticosterone production. If a rat's range of corticosterone production could be shown to determine their ability to cope with stress, this would provide us with an early indicator for those individuals at risk of developing stress related diseases and addictions. It is hypothesized that rats that experience the greatest decrease in reactive scope range of corticosterone release will act the most abnormally (in comparison with control animals) during the SNC coping test.

2. Materials and Methods

2.1. Subjects and Housing

This protocol was IACUC approved before beginning (IACUC #44459). Thirty adult male Sprague Dawley rats (*Rattus norvegicus*) were used in this study. These rats were housed at The Pennsylvania State University (Chandlee Building, University Park campus). They were housed in pairs and were kept on a 12L: 12D reversed light-dark schedule (lights on at 21:00h). This allowed for behavioral testing to be done during the dark when rats are most active. The room was kept at 70°F and 30% humidity and the cages were changed at least once a week. Food and water were available to the rats at all times except when they had to be briefly food deprived for behavioral trials. The rats were handled at least once a week to help habituate them to human contact and to decrease their stress due to handling during tests. The rats were divided into two experimental groups. The first group, made up of 16 rats, was exposed to stressful social, physical, and predation events over the course of 9 days. The second group, the control group consisted of 14 rats, and was not subjected to stress but otherwise received the same treatment as the stressed group (i.e. housing conditions, handling, etc.). As the rats were pair housed we could not split the animals into two groups of 15, so we decided to have two more treatment animals compared to the controls. Figure 3 shows a timeline of the procedures the rats experienced.

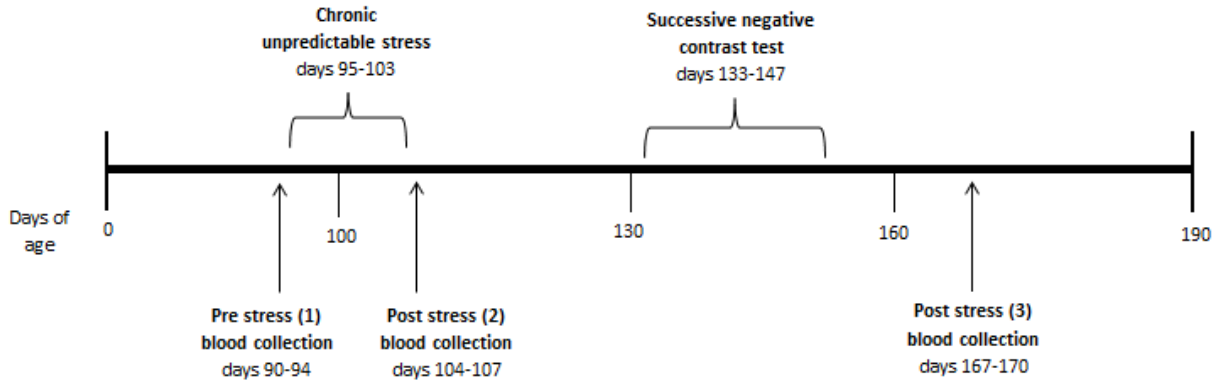

Figure 3: Timeline of procedures

2.2. Hormone Levels

Plasma hormone levels were analyzed by collecting blood through the saphenous vein. Three periods of blood collections occurred in this study: a pre stress (1) collection at 90 to 94 days of age, a post stress (2) collection at 104 to 107 days of age, and a post stress (3) collection at 167 to 170 days of age. During each collection, 4 different blood samples were taken from the rats: basal corticosterone level, dexamethasone suppression level, ACTH peak level, and ACTH recovery level. The dexamethasone suppression level was taken to evaluate the efficacy of the negative-feedback system of the brain, which detects corticosterone produced and reduces ACTH and corticosterone production. This dexamethasone collection occurred 2 hours after the rat was injected intramuscularly with dexamethasone, an artificial glucocorticoid. The ACTH peak level evaluated how well the rat's adrenals were producing corticosterone due to the increased stimulation from the ACTH. The ACTH peak collection occurred 30 minutes after the rat was injected intramuscularly with ACTH. The ACTH recovery collection occurred 1 hour after the rat was injected with ACTH and evaluated whether the adrenals recognized the overstimulation from the ACTH and diminished the production of corticosterone. These blood samples were

taken in order to get the range of corticosterone production for each rat at 3 different time points. Figure 4 shows how the bloods collections align with the reactive scope of corticosterone production.

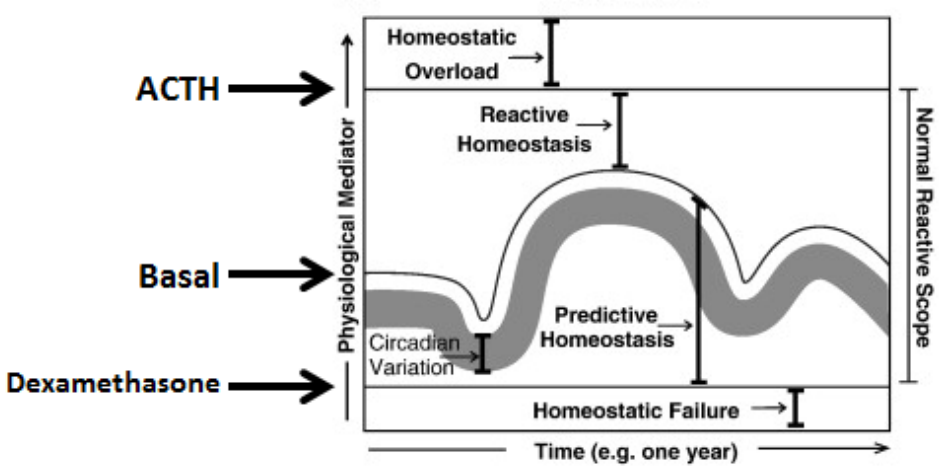

Figure 4: Reactive scope representation of manipulated corticosterone production

For the collections, a clean handler randomly brought each rat to a surgery room. The blood samples were collected in labeled micro centrifuge tubes and stored on ice. After the collections, the blood was spun with a centrifuge and the plasma containing the corticosterone was removed from the top of the sample and collected into a separate micro centrifuge tube. The plasma samples were stored in a -80 °C freezer until the radioimmunoassay. The pre stress (1) collection occurred over the course of 4 days. On the first day the basal and dexamethasone levels were collected. The ACTH (peak and recover) collections occurred three days later over the course of two days. Both of the post stress collection periods occurred over a 3 day period with basal and dexamethasone levels collected on the first day and all of ACTH (peak and recovery) levels collected 3 days later. Each of these blood collections occurred at the same time of day (11 am to 5pm) in order to control for daily circadian rhythms and variations in hormone production.

2.3. Chronic Unpredictable Stress

The stress group rats experienced chronic unpredictable stress from 95 to 103 days of age beginning on the day following the pre stress (1) blood collections. A combination of social, physical, and predation stressors were used on the designated 16 stress treatment group rats. The predation stressors included introducing the rats to cat hair or fox urine, wheeling around a taxidermied bobcat, and playing feline vocalizations. The social stressors included isolating the rats from their cage-mate, and placing the rats into a cage with foreign rat bedding. The physical stressors included tilting the rat's cage at a 30° angle, placing them in damp bedding, and decreasing the size of their cage. Full descriptions of the stressors are listed in Table 1. These stressors were not introduced to the rats prior to this chronic stress period. This allowed each stress to be completely unpredictable and therefore elicit the desired stress response. The stress period occurred over the course of 9 days with 2 different stressors per day. Predation, social, and physical stressors were randomly assigned over the time. The exact timeline of stressors is found in Table 2.

Table 1: Description of Stressors (Adapted from Chaby et al., 2013)

Stressor	Description
Predation	
Cat Hair	A mesh bag of cat (*Felis catus)* hair was introduced into the rat cages for 30 minutes (Kendig, et al., 2011)
Bobcat	An adult taxidermied bobcat was wheeled around in front of the rat cages for 30 minutes
Fox Urine	Cotton balls soaked with Tink's Red Fox-P were introduced into the rat cages for 30 minutes (Fendt, 2006)
Feline Vocalization	A feline (*Felis catus)* vocalization track was played for the rats continuously for 30 minutes
Social	
Isolation	Each rat was separated from its housing partner for 30 minutes (McCormick et al., 2012; Zurita et al., 2000)
Foreign Bedding	Rat pairs were placed into another pair's empty home cage for 12 hours (Harding et al., 2004)
Physical	
Cage Tilt	The rat cages were tilted at 30° degrees for 4 hours (Harding et al., 2004)
Damp Bedding	200 mL of water was added and mixed into 2/3 of the bedding in each rat cage for 6 hours (Harding et al., 2004; Zurita et al., 2004)
Smaller Cage	Rat pairs were housed in a cage with a 25% reduction in volume for 4 hours (Doyle et al., 2011)

Table 2: Timing of Chronic Unpredictable Stress Application

Stressor	Time (hrs)
Cat Hair	07:00
Foreign Bedding	21:00
Isolation	08:00
Bobcat + Cat Hair	17:00
Cage Tilt	13:00
Damp Bedding	19:00
Isolation	11:00
Fox Urine	19:00
Bobcat + Feline Vocalization	08:00
Damp Bedding	14:00
Smaller Cage	06:00
Foreign Bedding	21:00
Cage Tilt	13:00
Damp Bedding	17:00
Feline Vocalizations + Cat Hair	09:00
Smaller Cage	15:00
Bobcat + Feline Vocalization	10:00
Fox Urine + Isolation	16:00

2.4. Successive Negative Contrast Test

The coping response of each rat was measured using a Successive Negative Contrast (SNC) test from 133 to 147 days of age. The SNC test evaluated each rat's response to a downshifting reward. For the SNC test the rats were individually placed in an opaque plastic square container for 5 minutes every day (Flaherty, 1999). A plastic bottle filled with a sucrose solution was present on one of the walls of the container for the rats to drink from. An electronic device was used to record every lick of the sucrose solution. A conductive metal plate was placed on the bottom of the container for the rat to stand on. Metal clips attached the metal plate to the metal spout of the sucrose solution. With each lick the circuit between the metal plate and the metal spout was closed and the number was tallied. The lick-o-meter device was able to count the licks of 4 rats at a time. The order was randomized every day. For 12 days a 32% sucrose solution (high value reward) was used (Chaby et al., 2013). The solution concentration was then decreased to a 4% sucrose solution (low value reward) for 5 days (Mitchell & Flaherty, 1998). The number of licks each day was recorded for each rat over the course of the 17 day coping test. Each rat's response to this downshifting reward determined how well they were coping with the previous chronic stress period (Mitchell & Flaherty, 1998; Gomez et al., 2009). Typically, a poor coping response leads to a decreased frequency of licking towards the devalued reward.

2.5. Radioimmunoassay Analysis

A commercial radioimmunoassay [^{125}I] kit (MP Biomedicals, Solon, OH) was used to evaluate the corticosterone levels in the various plasma samples. In order to standardize for the various amounts of plasma collected, 5 µL of plasma was used from each rat. The samples were

diluted to 1:200 using steroid diluent. An ^{125}I artificial corticosterone tracer was added to each diluted sample followed by the primary antibody specific to corticosterone binding. The samples were incubated for 2 hours while competitive antibody binding took place between the endogenous and artificial corticosterone. After the incubation period, the second antibody was added which allowed for further binding. The samples were centrifuged at 3200xg for 15 minutes in order to create a density gradient with the bound antibody-corticosterone conjugate at the bottom of each tube in a pellet. The solution from each tube was dumped out after centrifuging leaving the pellet behind. Each sample was run twice in the gamma counter to determine the amount of bound tracer. Due to the competitive binding process, samples with more bound artificial tracer contained less endogenous corticosterone.

2.6. Data Analysis

Reward intake during the SNC test was evaluated using a Repeated Measures ANOVA (RMANOVA). The SNC coping response was evaluated by calculating a difference score for each rat that represented the decrease in reward consumption resulting from the downshift. The difference score was determined by subtracting the number of the licks on the first day of the 4% sucrose solution from the number of licks on the last day of the 32% sucrose. This represents the downshift in licks from the high reward to the low reward sucrose solution. The difference scores were analyzed using a t-test. SNC data met the assumptions for parametric analysis.

Plasma corticosterone determined from the radioimmunoassay was also analyzed using RMANOVAs to compare hormone production between the stress and control treatment groups at all three time points. Separate RMANOVAs were run on basal, dexamethasone, ACTH peak, and ACTH recovery hormone levels. If significant differences between the stressed and control

groups were observed in the RMANOVA, posthoc univariate ANOVAs were run on each time point separately to determine at which time point the differences occurred. All corticosterone data met the assumptions for parametric analysis, with the exception of dexamethasone levels which were log transformed to achieve normality. Normality was assessed using Levene's test for equality of variances.

Reactive scope and recovery variables were also analyzed using RMANOVAs to compare the groups across the 3 time points. The reactive scope variable was determined for each rat by subtracting the dexamethasone corticosterone level from the ACTH peak corticosterone level. The recovery variable was determined for each rat by subtracting the ACTH recovery corticosterone level from the ACTH peak corticosterone level. If treatment differences were observed, posthoc ANOVAs were run on each time point separately to identify the time points at which the groups differed. Values are reported as mean $\pm$ standard deviation.

3. Results

3.1. Successive Negative Contrast

The stress and control groups did not differ in reward consumption during the SNC test [RMANOVA, $F_{(1, 28)} = 0.02$, $P = 0.90$]. The response to the downshift in reward also did not differ between the stress and control group [$T_{(1, 28)} = 0.02$, $P = 0.90$, see Figure 5].

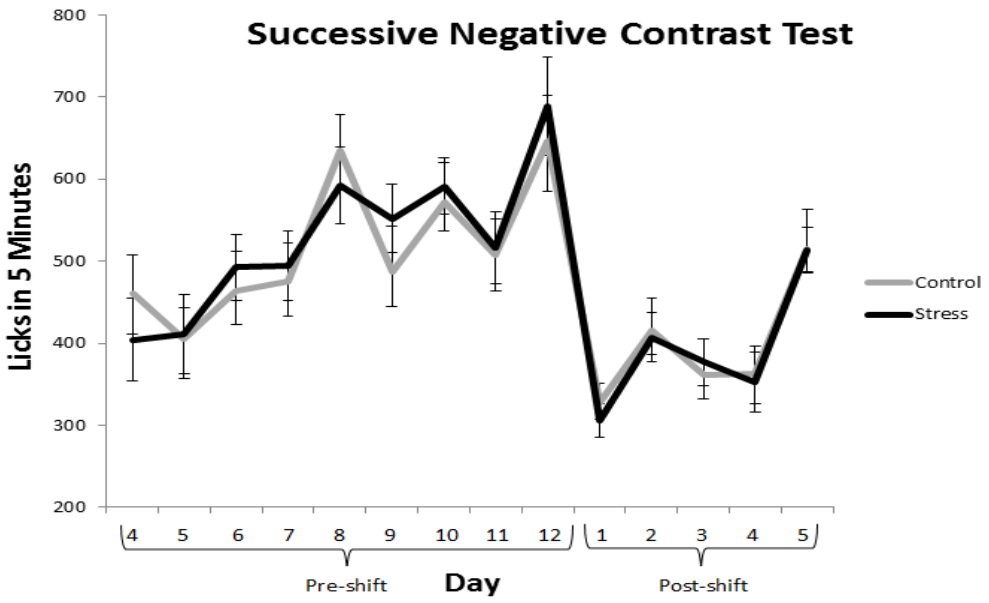

Figure 5: Results of the Successive Negative Contrast Test on Stress and Control Rats (values mean ± standard error)

3.2. Basal Corticosterone Production

Basal corticosterone levels in rats decreased due to a period of chronic stress [RMANOVA, $F_{(1, 28)} = 1.59$, $P = 0.01$]. There was no difference in control and stress basal corticosterone levels before stress, at time point 1 [RMANOVA, $F_{(1, 28)} = 1.35$, $P = 0.25$, Mean

control = 320.97 ± 141.99 ng, Mean stress = 270.27 ± 93.72 ng]. Immediately following stress, however, the stress group's basal corticosterone levels during time point 2 were lower [RMANOVA, $F_{(1, 28)} = 7.56$, P = 0.01 Mean control = 321.89 ± 77.81 ng, Mean stress = 241.70 ± 81.30 ng]. At time point three, there were trending differences in control and stress basal corticosterone levels [RMANOVA, $F_{(1, 28)} = 2.48$, P = 0.13 Mean control = 281.71 ± 67.62 ng, Mean stress = 241.29 ± 72.36 ng].

3.3. Dexamethasone Corticosterone Production

The efficacy of the negative feedback system for corticosterone (measured using dexamethasone suppression) was not altered by chronic stress [RMANOVA, $F_{(1, 25)} = 1.68$, P = 0.21]. The stress and control groups were not different during time point 1 [Mean control = 116.95 ± 58.13 ng, Mean stress = 122.18 ± 83.86 ng], time point 2 [Mean control = 23.74 ± 4.87 ng, Mean stress = 23.09 ± 4.41 ng], or time point 3 [Mean control = 62.26 ± 52.39 ng, Mean stress = 27.59 ± 11.56 ng].

3.4. ACTH Peak Corticosterone Production

The maximum amount of corticosterone a rat can produce (ACTH peak) increased due to a period of chronic stress [RMANOVA, $F_{(1, 27)} = 9.671$, P < 0.01]. There was no difference in maximum corticosterone productions between stress and control rats during time point 1 and time point 3 [RMANOVA, $F_{(1, 28)} = 1.93$, P = 0.18 Mean control = 577.92 ± 93.90 ng, Mean stress = 534.00 ± 94.85 ng] and [RMANOVA, $F_{(1, 28)} = 0.908$, P = 0.349 Mean control = 489.92 ± 150.46 ng, Mean stress = 441.48 ± 126.61 ng]. There was however an increase in maximum corticosterone production between stress and control rats at time point 2 [RMANOVA, $F_{(1, 28)} =$

5.54, P < 0.03 Mean control = 599.69 ± 129.59 ng, Mean stress = 495.75 ± 108.46 ng]. Over the course of the three time points, the reactive scopes of all the rats changed significantly [$F_{(1, 28)}$ = 4.40 and P < 0.02).

3.5. ACTH Recovery Corticosterone Production

The recovered amount of corticosterone a rat can produce (ACTH recovery) did not change due to a period of chronic stress though a difference is trending [RMANOVA, $F_{(1, 27)}$ = 3.96, P = 0.06]. There were no significant differences during time point 1 [Mean control = 594.95 ± 126.01 ng, Mean stress = 528.28 ± 131.53ng], time point 2 [Mean control = 545.10 ± 109.79 ng, Mean stress = 487.00 ± 85.62 ng], or time point 3 [Mean control = 499.70 ± 110.64 ng, Mean stress = 453.10 ± 127.71 ng].

3.6. Reactive Scope

The reactive scope of corticosterone production (ACTH peak – dexamethasone suppression) diminished due to a period of chronic stress [RMANOVA, $F_{(1, 24)}$ = 6.01, P = 0.02]. There was no difference in reactive scope range between stress and control rats during time point 1 and time point 3 [RMANOVA, $F_{(1, 27)}$ = 3.95, P = 0.06 Mean control = 493.30 ± 62.75 ng, Mean stress = 409.11 ± 126.03 ng] and [RMANOVA, $F_{(1, 28)}$ = 0.230, P = 0.635 Mean control = 419.94 ± 150.36 ng, Mean stress = 398.28 ± 165.19 ng]. The decrease in reactive scope occurred at time point 2 [RMANOVA, $F_{(1, 27)}$ = 5.120, P = 0.032 Mean control = 560.92 ± 94.64 ng, Mean stress = 476.17 ± 116.88 ng, see Figure 6].

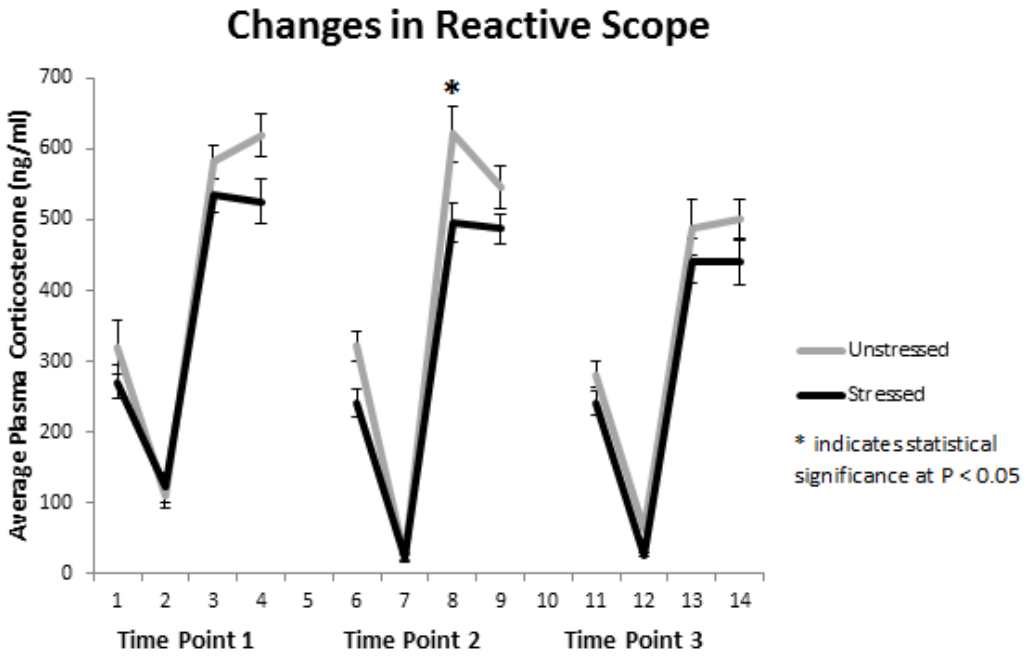

Figure 6: Changes in Reactive Scope for Stress and Control Rats (* indicates statistical significance at P < 0.05; values average ± standard error)

3.7. Recovery Score

The ability to recover from stimulation with exogenous ACTH (ACTH peak – ACTH recovery) did not change due to a period of chronic stress [RMANOVA, $F_{(1, 25)} = 0.05$, $P = 0.83$]. There were no differences during time point 1 [Mean control = 3.62 ± 83.24 ng, Mean stress = -4.02 ± 89.46 ng], time point 2 [Mean control = 33.17 ± 118.24 ng, Mean stress = 18.69 ± 82.29 ng], or time point 3 [Mean control = -2.7 ± 165.00 ng, Mean stress = 3.70 ± 105.56 ng].

3.8. Reactive Scope and Successive Negative Contrast Test Correlation

The size of the reactive scope, immediately after the stressors were applied, was strongly related to a rat's success in the coping test [R = 0.51, P = 0.04, see Figure 7]. In the stress group,

larger reactive scopes were related to higher licking scores on the second day of the downward sucrose shift.

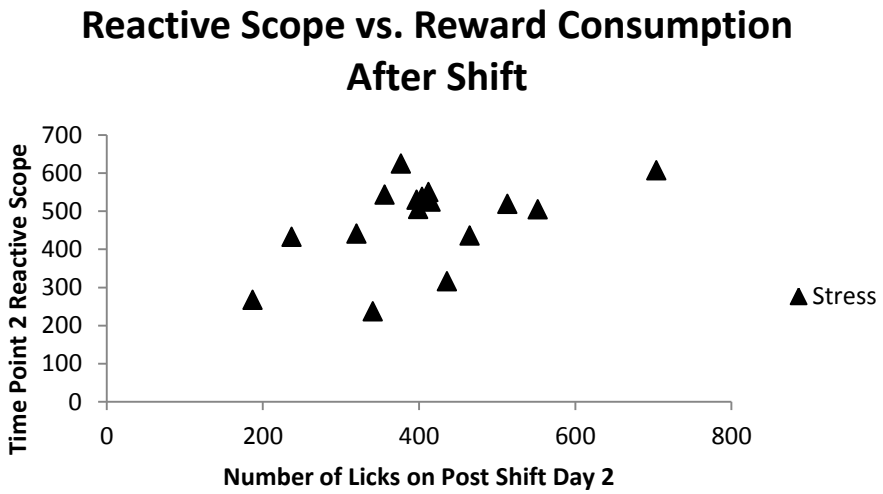

Figure 7: Correlation of Time Point 2 Reactive Scope and the Number of Licks on the 2nd Day of the Downshifted Sucrose Reward in Stress Rats

The size of the reactive scope in control group rats was strongly related to a rat's success in the coping test [R = -0.57, P = 0.03]. In the control group, larger reactive scopes were related to lower licking scores on the first day after the downward sucrose shift.

3.9. Recovery Score and SNC Difference Score Correlation

The time point 2 recovery score (ACTH peak – ACTH recovery) was strongly related to the SNC difference scores of the rats (day 12 of high reward - day 1 of low reward) [R = 0.43, P = 0.02] and [R = 0.40, P = 0.03, see Figure 8]. Rats with larger recovery scores had larger SNC difference scores. This trend was present in control rats [R = 0.70, P < 0.01] but not in stress rats [R = 0.18, P = 0.52]. When ACTH peak corticosterone levels were controlled for, the relationship disappeared [R = 0.37, P = 0.06].

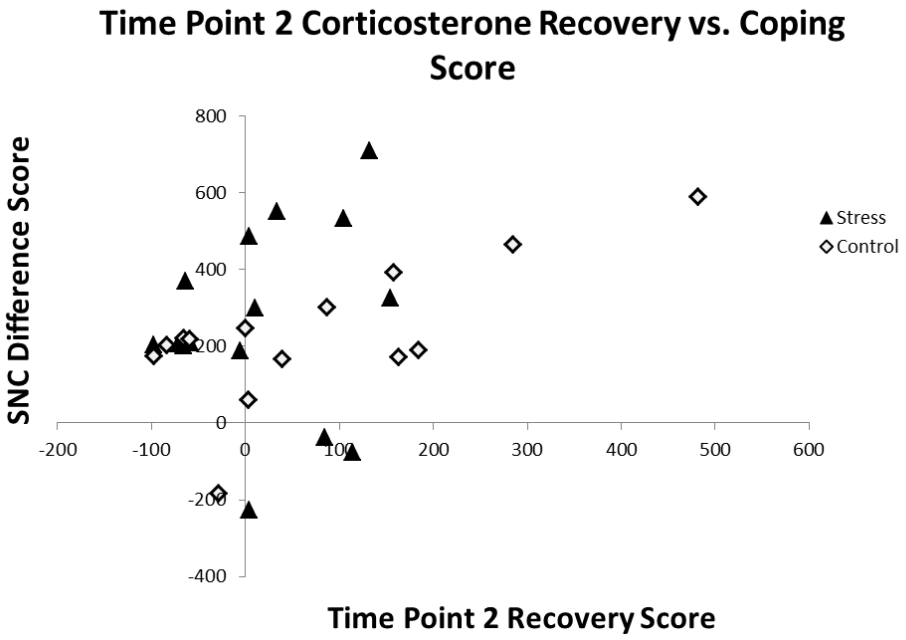

Figure 8: Correlation of time point 2 corticosterone recovery and SNC difference score

3.10. Recovery Score and Reactive Scope Correlation

The time point 1 recovery score (ACTH peak – ACTH recovery) was strongly related to the change in reactive scope due to stress [R = 0.49, P < 0.01, see Figure 9]. This correlation was present in control rats [R = 0.83, P = 0.00] but not in stress rats. The correlation in stress rats was trending in the opposite direction[R = -0.47, P = 0.08]. However, this trend is quite possibly the result of an outlier rat in the stress group.

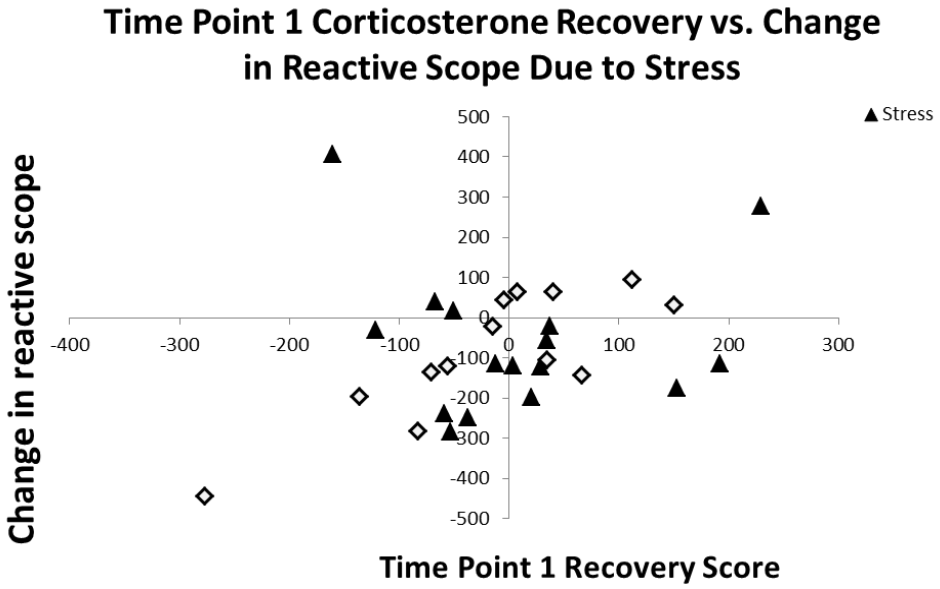

Figure 9: Correlation of time point 1 corticosterone recovery score and the change in reactive scope from time point 1 to time point 2

The recovery score left at time point 3 was strongly related to long term change in reactive scope [R = -0.37, P =0.04, see Figure 10].

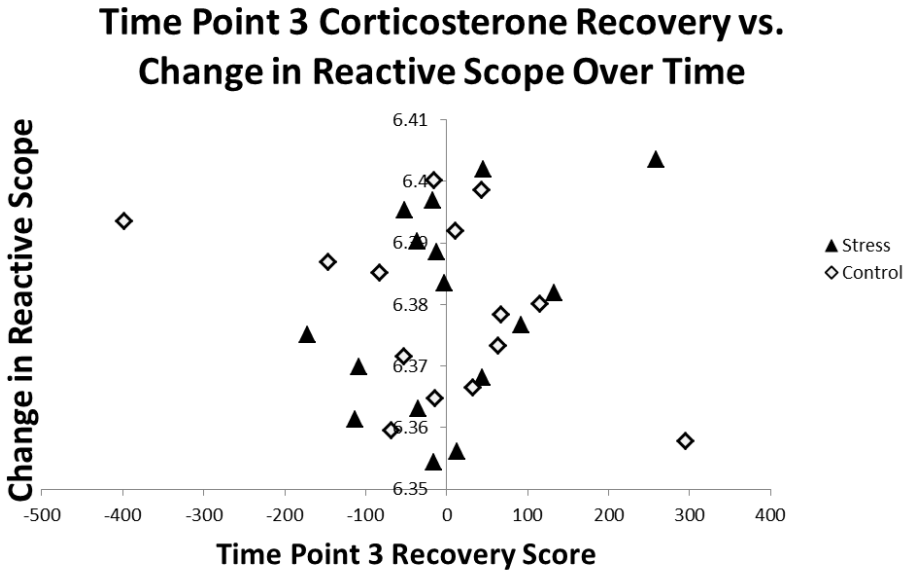

Figure 10: Correlation of corticosterone recovery and reactive scope change from time point 1 to 3

A higher reactive scope in rats was related to a larger recovery score during time point 2 and 3 [R = 0.70, P = 0.00] and [R = 0.51, P < 0.01] but not during time point 1 [R = 0.13, P = 0.51, see Figure 11].

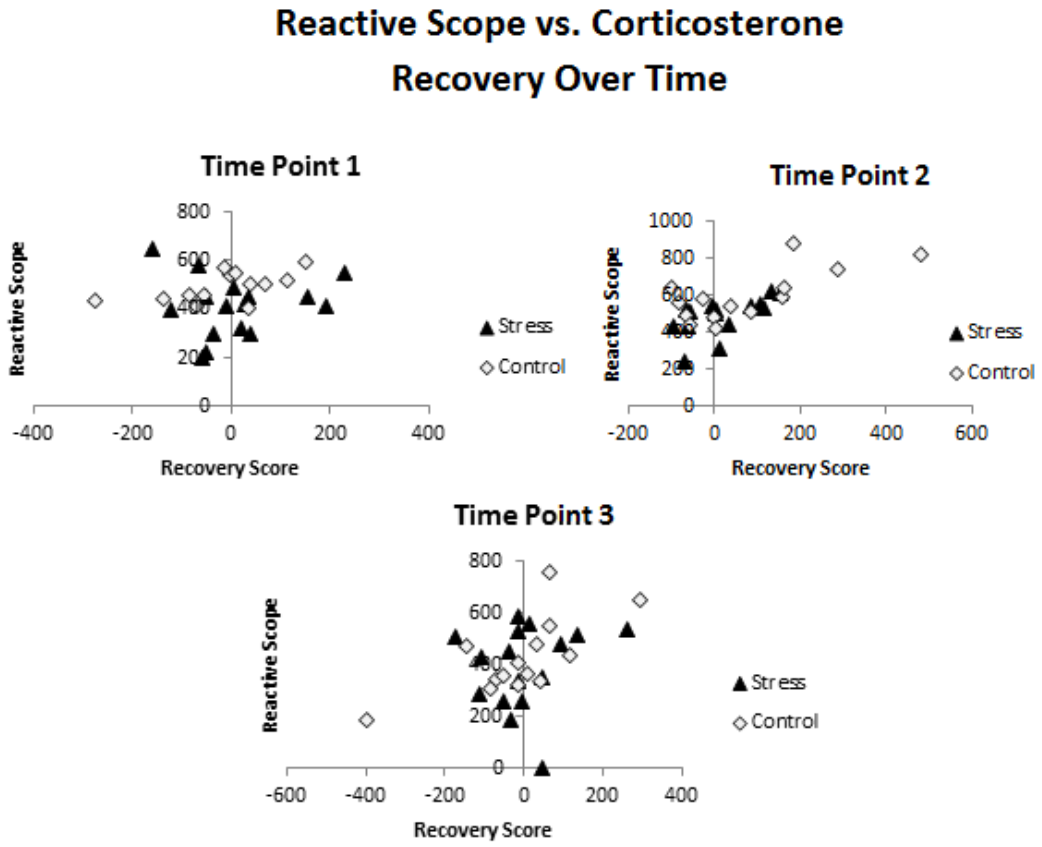

Figure 11: Correlation of recovery score and reactive scope over time

3.11. ACTH Peak and Change in Reactive Scope Correlation

Time point 1 ACTH peak corticosterone levels were related to the change in reactive scope over time. Rat's with higher ACTH peak corticosterone at time point 1 had a greater decrease in reactive scope at time point 2 and 3 [R = 0.41, P < 0.03] and [R = 0.48, P < 0.01]. The ACTH peak corticosterone level taken at each time point was related the reactive scope at

that time point. The higher the ACTH peak corticosterone level the larger the reactive scope at time point 1, 2, and 3 [R = 0.72, P = 0.00], [R = 1.0, P = 0.00], and [R = 0.96, P = 0.00].

3.12. ACTH Peak and SNC Difference Score Correlation

ACTH peak corticosterone levels at time point 2 did not relate to SNC difference scores in the rats [R = 0.14, P = 0.47]. However, when separated into groups, a correlation between ACTH peak levels and SNC difference scores was found in the control rats [R = 0.57, P = 0.04] but not the stress rats [R = -0.05, P = 0.86].

4. Discussion

The purpose of this thesis study was to determine if the behavioral coping response of rats could be linked to range of corticosterone production. The coping response of each rat was determined using a successive negative contrast test. SNC tests have been successful in determining individual coping responses of rats (Mitchell & Flaherty, 1998; Burman et al., 2008; Chaby et al., 2013). The range of corticosterone production was based on the reactive scope model developed by Romero (Romero et al., 2009). It was hypothesized that the rats with the greatest decrease in reactive scope of corticosterone release would act the most abnormally (least like the control group) during the SNC coping test.

The SNC test which introduced the rats to a high reward (32% sucrose solution) followed by a low reward (4% sucrose solution) showed that there was no difference in downshift of consumption between the stress and control groups (Figure 5). While there were variations in the SNC results between each rat there was not a significant difference between the groups. This result differs from that of a previous study which shows an increased sensitivity to reward loss in rats that experienced a period of chronic stress (Chaby et al., 2013). The difference between the Chaby et al. (2013) result and the one reported here may be due to the fact the rats tested by Chaby et al. experienced chronic stress during adolescence and, during this stage of development the production of glucocorticoids due to stress lasts longer and is more intense when compared with the adult hormone response (McCormick et al., 2010). Chaby et al. used a chronic stress period of 40 days rather than the 9 day period used in this study. This longer length of chronic stress could be responsible for the difference in SNC between control and stress rats.

Statistical analysis on basal corticosterone levels determined that the stress rats had lower basal corticosterone levels than the control rats immediately after the stress period. It is likely

that the stress rats were more accustomed to unexpected stimuli due to their period of chronic stress therefore they were less affected by the stress of the blood collection process. The control rats, that had experienced very little unexpected stimuli during their lifetime, had a more severe reaction to the blood collection process.

Statistical analysis on ACTH peak corticosterone levels determined that stress rats had lower ACTH peak corticosterone levels immediately after the stress period. This shows that the stress response resulted in a decrease in the maximum amount of corticosterone that a rat could produce. This concept of depreciating peak corticosterone levels due to a period of chronic stress is supported by previously published papers on wild animal stress response (Harlow et al., 1992; Boonstra et al., 1998). The ACTH peak corticosterone level in a rat is representative of a rat's homeostatic overload threshold. Therefore, the stress response resulted in a decrease in the homeostatic overload threshold of the rats. This decrease in the homeostatic overload threshold resulted in a smaller reactive scope range in the stress rats compared to the control rats immediately after stress (Figure 6). This result supports Romero's theory that chronic stress will ultimately decrease the reactive scope of corticosterone production in an animal (Romero et al., 2009). In addition to stress having an impact on the reactive scope range of rats, the general aging of both control and stress rats also had an effect their reactive scopes. Reactive scope is responsive to environmental conditions (Romero et al., 2009, and therefore varies through an individual's lifetime even under highly constant, controlled laboratory settings.

Each rat's unique corticosterone production was compared to their behavior during the SNC coping test. It was determined that the size of a rat's reactive scope of corticosterone production, after stress exposure, was strongly related to their success in the coping test (Figure 7). Rats that had a larger reactive scope range after the period of chronic stress consumed more sucrose on the second day of the downward sucrose shift. These rats that were able to maintain a larger reactive scope, despite the period of chronic stress were less affected by the downshift in

reward value. Stressed rats with smaller reactive scopes, however, became more frustrated during the SNC test presumably due to their reduced range of reactivity and, this led to a lower consumption of the sucrose solution after the downshift in the reward. This trend supports our hypothesis that a greater decrease in reactive scope relates to a more abnormal response in a behavioral coping test.

The opposite relationship between reactive scope and SNC success was found in control rats. Control rats with larger reactive scopes consumed less sucrose on the first day after the downward sucrose shift. This behavior is likely explained by the fact that the control rats had experienced very few unexpected, aversive stimuli prior to the SNCs test. By the nature of the experimental design, the SNC reward downshift was the most stressful experience they had ever encountered (Mitchell & Flaherty, 1998). Therefore those rats with the largest reactive scope range of corticosterone production could elicit the most extreme behavioral response to the SNC test. This notion was supported by the finding that control rats with larger ACTH peak corticosterone levels immediately after the stress period had larger SNC difference scores. The rats with the higher peak production had a more extreme negative response to the SNC test. This higher level of hormone production corresponded to a larger reactive scope range. Due to the higher hormone production both reactive scope and peak corticosterone production correlated to a more extreme behavioral response.

Each rat's recovery score (ACTH peak – ACTH recovery) was compared to their SNC difference score (consumption on the last high reward day – consumption on the first low reward day). These tests found a strong relationship between a rat's recovery score and their difference score immediately after stress treatment (Figure 8). Rats that were able to recover faster from a spike in hormone production had a more extreme response to the downshifted reward in the SNC test. This showed that rats with faster recovery scores immediately after stress were less successful at coping. When the rats were evaluated by treatment this correlation was not present

for the stressed rats showing that the control group was driving the correlation. Also when ACTH peak corticosterone levels were controlled for, this correlation was no longer significant in control rats. The disappearance of this correlation indicates that the ACTH peak value was likely the main contributing factor to the SNC results. This relationship can once again be explained by the fact that the control rats were naïve to stress at this point in their lives. Those with higher ACTH peak corticosterone levels could elicit the greatest negative behavioral response to the SNC test.

Each rat's recovery score was also compared to changes in reactive scope due to stress. The results found that the recovery score immediately before the stress period was strongly related to the change in reactive scope due to stress (Figure 9). Rats with a faster recovery score immediately before the stress period experienced a greater decrease in reactive scope immediately after the stress period. This correlation was present in control rats but not in stress rats showing that the control group was driving the correlation. The correlation in stress rats was actually trending in the opposite direction.

The recovery score left in the long term was strongly related to the long term change in reactive scope (Figure 10). Rats with faster recovery scores in the long term experienced a smaller change in reactive scope over time. Having a faster recovery score helps prevent the detrimental effects of chronic stress. Animals that can decrease their overstimulated corticosterone faster will spend less time in the homeostatic overload range. This result supports Romero's theory that maintained presence in the homeostatic overload range is what results in a decrease in reactive scope (Romero et al., 2009).

It was determined that a larger reactive scope in rats was related to a larger recovery score immediately after stress and in the long term but not immediately before stress (Figure 11). It is possible that this correlation occurred because of the period of chronic stress. Rats with

faster recoveries fared better during the stress period and therefore maintained larger reactive scopes.

Each rat's ACTH peak corticosterone level was compared to their change in reactive scope. The results showed that rat's with a higher initial corticosterone peak had a greater decrease in their reactive scope immediately after stress and in the long term. This result is likely due to the fact that those rats with higher initial peak values had further to fall. It is difficult to maintain such a high peak value therefore the peak production in these rats decreased to a more manageable level. It was also determined that higher peak values corresponded to larger reactive scopes at corresponding time points. This relationship is valid because a rat's ACTH peak corticosterone level makes up the upper threshold of its reactive scope. A larger peak production of corticosterone would correspond to a larger reactive scope.

5. Conclusion

The chronic stress period applied to the stress group rats resulted in decreased peak corticosterone production, decreased reactive scope of corticosterone production, and decreased success in the SNC coping test. These findings support the idea that there may be a link between corticosterone production and a coping response. A trend between reactive scope and SNC recovery score was only found after the stress period showing that stress does in fact cause individual variation in reactive scope and therefore coping ability.

Stress hormone recovery rate was also determined to be a good indicator of certain physical and behavioral characteristics: a faster recovery rate immediately before the stress period corresponded to an increase in reactive scope range, a faster recovery rate immediately after the stress period was related to decreased success during the SNC coping test, and a faster recovery rate in the long term was related to increased reactive scope over time. In conclusion, corticosterone levels and ranges hold great insight into individual ability to cope with stress. We found that certain levels and ranges were linked to changes in reactive scope and coping ability. By studying these trends, it may be possible in the future to develop screening tests that identify susceptibility to stress related diseases.

BIBLIOGRAPHY

Boonstra, Rudy et al. “THE IMPACT OF PREDATOR-INDUCED STRESS ON THE SNOWSHOE HARE CYCLE.” *Ecological Monographs* 68.3 (1998): 371–394.

Burman, Oliver H.P et al. “Sensitivity to Reward Loss as an Indicator of Animal Emotion and Welfare.” *Biology Letters* 4.4 (2008): 330–333.

Chaby, Lauren E., Kayllie Wang, and Victoria A. Braithwaite. “Long-term Changes in Cognitive Bias and Coping Response as a Result of Chronic Unpredictable Stress During Adolescence.” *Frontiers in Human Neuroscience* 7 (2013): 328.

Doyle, Rebecca E et al. “Measuring Judgement Bias and Emotional Reactivity in Sheep Following Long-term Exposure to Unpredictable and Aversive Events.” *Physiology & behavior* 102.5 (2011): 503–510.

Fendt, Markus. “Exposure to Urine of Canids and Felids, but Not of Herbivores, Induces Defensive Behavior in Laboratory Rats.” *Journal of Chemical Ecology* 32.12 (2006): 2617–2627.

Flaherty, Charles F. *Incentive Relativity*. Cambridge University Press, 1999.

Gómez, Ma José et al. “Consummatory Successive Negative and Anticipatory Contrast Effects in Inbred Roman Rats.” *Physiology & behavior* 97.3-4 (2009): 374–380.

Harding, Emma J, Elizabeth S Paul, and Michael Mendl. “Animal Behaviour: Cognitive Bias and Affective State.” *Nature* 427.6972 (2004): 312.

Harlow, Henry J. et al. “Stress Response of Cougars to Nonlethal Pursuit by Hunters.” *Canadian Journal of Zoology* 70.1 (1992): 136–139.

Kendig, Michael D et al. “Predatory Threat Induces Huddling in Adolescent Rats and Residual Changes in Early Adulthood Suggestive of Increased Resilience.” *Behavioural brain research* 225.2 (2011): 405–414.

Koolhaas, J. M et al. “Coping Styles in Animals: Current Status in Behavior and Stress-physiology.” *Neuroscience & Biobehavioral Reviews* 23.7 (1999): 925–935.

McCormick, C M, and M R Green. “From the Stressed Adolescent to the Anxious and Depressed Adult: Investigations in Rodent Models.”*Neuroscience* 249 (2013): 242–257.

McEwen, B S. “Protective and Damaging Effects of Stress Mediators.” *The New England journal of medicine* 338.3 (1998): 171–179.

Mitchell, Colin, and Charles Flaherty. “Temporal Dynamics of Corticosterone Elevation in Successive Negative Contrast.” *Physiology & Behavior* 64.3 (1998): 287–292.

Piazza, P V, and M Le Moal. “The Role of Stress in Drug Self-administration.”*Trends in pharmacological sciences* 19.2 (1998): 67–74.

Romero, L. Michael, Molly J. Dickens, and Nicole E. Cyr. “The Reactive Scope Model - A New Model Integrating Homeostasis, Allostasis, and Stress.” *Hormones and Behavior* 55.3 (2009): 375–389.

Thanos, P K et al. “A Non-invasive Method for Detecting the Metabolic Stress Response in Rodents: Characterization and Disruption of the Circadian Corticosterone Rhythm.” *Physiological research / Academia Scientiarum Bohemoslovaca* 58.2 (2009): 219–228.

Zurita, A et al. “Early Exposure to Chronic Variable Stress Facilitates the Occurrence of Anhedonia and Enhanced Emotional Reactions to Novel Stressors: Reversal by Naltrexone Pretreatment.” *Behavioural brain research* 117.1-2 (2000): 163–171.

Øverli, Øyvind et al. “Evolutionary Background for Stress-coping Styles: Relationships Between Physiological, Behavioral, and Cognitive Traits in Non-mammalian Vertebrates.” *Neuroscience & Biobehavioral Reviews*31.3 (2007): 396–412.

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Schreyer Honors College Student Council

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- Social Chair (April 2011 – April 2012) Planned various events for the students of the Schreyer Honors College

Scholars International (Students for Sharing the Journey International)

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- Medicated, fed, cleaned and cared for the cats and dogs being boarded at the kennel
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