THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

INTERCOLLEGE PROGRAM OF NEUROSCIENCE

THE EFFECTS OF CHRONIC ADOLESCENT STRESS ON PHYSIOLOGY AS A FUNCTION OF TIME

LINDSAY BACIK
SPRING 2014

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Science
with honors in Neuroscience

Reviewed and approved* by the following:

Victoria Braithwaite
Professor of Fisheries and Biology
Thesis Supervisor

Ping Li
Professor of Psychology and Linguistics
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Animals that experience stressful life events undergo psychological, behavioral, and physiological changes. Adolescence is a critical period of development due to increased neuroplasticity in the adolescent brain, and research has focused on the effects of adverse experiences during this time on the adult phenotype. The effects of adolescent stress depend on a range of factors including the duration of the stress exposure and the temporal relationship between stress onset, offset, and physiological response. The present study investigated the differences in blood composition in rodent models at two separate time points as a measure of physiological change. Rats exposed to chronic adolescent stress (e.g. predation simulation, isolation, crowding) were compared to control animals maintained in regular standard housing throughout development. Blood composition collected at a time point allowing for rest after the stress paradigm showed greater neutrophil to lymphocyte ratios ($F_{1,45}$=19.574, P<0.001), lower lymphocyte count ($F_{1,45}$=37.268, P<0.001), lower absolute white blood cell count ($F_{1,45}$=18.010, P<0.001), higher absolute red blood cell count ($F_{1,45}$=11.150, P=0.002), and lower absolute platelet count ($F_{1,45}$=53.966, P<0.001). These results showed that with time, the physiological response to stress changes significantly.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

Special thanks to the Huck Institute of the Life Sciences and the Eberly College of Science for support. This project was funded, in part, with the Summer Undergraduate Discovery Grant. I would like to thank Dr. Victoria Braithwaite and Lauren Chaby for their guidance throughout the duration of this project. I would also like to thank the members of the Braithwaite group, especially Amy Hirrlinger, James Lim, and Tessa Samuels for their assistance in data collection. Finally, I would like to thank Dr. Ping Li for serving as my honors thesis advisor.

Chapter 1

Introduction

The Prevalence of Stress

Chronic stress is widely apparent in today's society, and has been linked to negative human health consequences including neuroendocrine changes and effects on immune function (Gurfein et al., 2012). Stressful life events are known to result in changes that include negative effects on general health, susceptibility to disease, and duration and intensity of illness (Cohen et al., 2007). While stress management is clinically promoted for well-being, there is little evidence of a mechanistic link between stress and disease (Gurfein et al., 2013).

The living conditions of today's society involve a variety of high-stress situations. One particular demographic change that may increase the population's exposure to stress is the increase in urbanization across the world. While only 30% of the world's population lived in urban environments in 1950, this number is expected to grow to almost 70% by 2050 (Krabbendam et al., 2005). Studies have shown that overcrowding associated with urban lifestyles can induce stress and illness. In addition, those brought up in cities are at an increased risk of depression, anxiety, and schizophrenia (Krabbendam et al., 2005).

A neuroimaging study investigating the specific brain regions that respond to environment-related stressful stimuli measured brain activation while participants engaged in a social-stress test. This led to an increase in heart rate, blood pressure, and salivary levels of cortisol, a stress-associated hormone (Krabbendam et al., 2005). Two particularly interesting brain regions activated by the test were the amygdala and the perigenual anterior cingulate cortex (pACC), both structures known to be related to emotional responses and stress processing

(Krabbendam et al., 2005). Participants who had spent more time in large cities had reduced connectivity between these regions, and reduced amygdala-pACC connectivity has been associated with a genetic risk for mental illness (Krabbendam et al., 2005). This information highlights the prevalence of stress in modern society and the importance of preventing stress from contributing to mental illness in the population.

The Stress Mechanism

In addition to serving as a contributing factor to the development of mental illness, stressors have been shown to have negative effects on immune response functions. The vertebrate stress system mediates the stress response via effectors including corticotrophin-releasing hormone (CRH), arginine vasopressin, the propiomelanocortin-derived peptides alpha-melanocyte-stimulating hormone and beta-endorphin, the glucocorticoids, and the catecholamines norepinephrine and epinephrine (Charmandari, 2005). Failure of this system to respond appropriately contributes to many endocrine, immune, and psychiatric disorders.

Stressful events in vertebrates are commonly considered to involve stimulation of a glucocorticoid hormone response, which is mediated by the hypothalamic-pituitary-adrenal (HPA) axis (Koolhaas et al., 2011). Specific body systems affected by stress are the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic and parasympathetic nerve projections that innervate secondary lymphoid organs such as the spleen and lymph nodes (Koolhaas et al., 2011). Neuroendocrine factors known to have an effect on the reduction of immune system function following stress are elevated levels of glucocorticoids and catecholamines.

Life events involve a dynamic equilibrium termed homeostasis, but this state can be challenged by adverse forces or stressors, including intrinsic and extrinsic, as well as real or

perceived negative stimuli. Thus, stress can be defined as a state of threatened homeostasis, and the mind and body react to stress by activating certain behavioral and physiological central nervous system responses (Charmandari, 2005). If these responses are prolonged or excessive, the effect can have negative outcomes for certain physiological functions, including the immune response. The way in which animals respond to stressors can be adaptive under some situations, and can help to promote survival. Behavioral adaptation in response to stress includes increased alertness and vigilance, improved cognition and focus, and physical adaptations that are characterized by a redirection of energy to body sites that most require it for functioning and increased metabolism and cardiovascular tone. However, these adaptive changes may become excessive, and chronic exposure to stress may contribute to the development of pathology (Charmandari, 2005).

Several studies have demonstrated the negative effects of chronic stress on physiology. An experiment performed by Cohen et al. (1991) showed that in humans, chronically stressed individuals were more susceptible to experimental rhinovirus infection than were non-stressed subjects. A similar study showed accelerated human immunodeficiency virus (HIV) progression in subjects undergoing chronic stress (Leserman, 2000). In addition, research with animal models has demonstrated that those exposed to chronic stress experience enhanced tumor growth when compared to control subjects (Sapolsky, 1985; Thaker et al., 2006).

The severity of each condition depends on factors including genetic predisposition to that specific disorder, the exposure of the individual to adverse environmental factors, and the timing of stressful events. Owing to increased neuroplasticity in the brain at these times, critical periods of development are subject to an elevated vulnerability to stressful events. These critical periods include prenatal life, infancy, childhood, and adolescence (Charmandari, 2005).

Adolescent Stress

The behavior and physiology of mammals can be affected by negative life experiences, and the timing of these negative experiences in relation to stages of development is important. Response to aversive stimuli during adolescence results in a longer and more intense hormone response than normal during adulthood (McCormick et al., 2010). In adolescence, the neural structures associated with processing stress are still undergoing change and maturation (McCormick et al. 2010). The duration of changes following stressful adolescent stimuli can differ, with some responses diminishing with time and some having lasting affects (McCormick et al., 2012).

Previous studies linking the long-term effects of adolescent stress to physiological functioning have focused on the HPA axis but have yielded mixed results. One study reported that rodents exposed to 6 weeks of stressors during adolescence had blunted HPA response when exposed to a new stressor 5 days later (Goliszek et al., 1996). However, another study reported that rodents exposed to 4 weeks of adolescent stressors had lower corticosterone concentrations approximately 2 weeks later (Toth et al., 2008).

Chaby et al. (2013) investigated the consequences of adolescent stress on long-term adult phenotype by examining decision making, cognitive bias, coping response, and exploratory behavior using a rodent model. Rats exposed to unpredictable adolescent stress were compared to control animals kept in regular standard housing, and the results showed an increase in the negative cognitive bias, a short-term increase in boldness behaviors, an altered coping response, and accelerated decision making in the stress condition. These results highlight the long-term impacts of stress during adolescence on behavior and cognition, influencing the way that animals make decisions, interpret ambiguity, respond to adverse events, and interact with novel environments (Chaby et al., 2013). These data, taken together with previous literature involving

the relationship between stress and disease, suggest that exposure to chronic adolescent stress may negatively impact the body's physiological functioning long into adulthood.

While stress has been shown to contribute to the severity of disease, there is little research investigating the mechanistic link between the timing of adverse events and physiological responses. While several studies show the effects of stress on HPA function, behavior, and learning, very little research has examined the possible relationship between adolescent stress and blood composition, a measure commonly used to indicate immune function or dysfunction in the medical field. In addition, the long-term effects of adolescent stress and blood composition are largely unknown. The current study therefore focused on the effects of chronic adolescent stress on blood composition in rats at two separate time points; the first was immediately following the period of stressful stimuli presentation, while a second time point allowed for a period of rest prior to blood composition being screened. These data were taken to shed light on the differential effects of adolescent stress on physiology at different points in the lifespan.

Blood Composition

Blood is the medium that transports gases, nutrients, hormones, and waste throughout the mammalian body. The circulatory system works to maintain homeostasis within the body by delivering oxygen rich blood from the heart to somatic cells. The plasma of blood contains the specialized red blood cells, white blood cells, and platelets, which can serve as indicators of health and physiological responses to the environment as the relative ratio of these can change.

Red blood cells are the principal carriers of hemoglobin molecules, which bind about 97% of all oxygen in the body for delivery to the body for proper functioning. White blood cells, also called leukocytes, are a vital source of defense against external organisms and serve to

remove dead cells and tissue debris that would otherwise lead to problems within the body. The five classes of leukocytes include lymphocytes, neutrophils, eosinophils, basophils, and monocytes, and specific blood composition of each individual sub-component may shed light on health of the individual. Some infections stimulate the release of a large number of protective leukocytes, causing a rise in the number of white blood cells. Some infections, however, result in a reduced white blood cell count and a reduced ability to fight other infections. A leukocyte of particular importance to the immune response is the lymphocyte. Many studies have documented the presence and quantity of tumor-infiltrating lymphocytes (TILs) as a correlation factor with increased survival. A recent study by Robins et al. (2013) demonstrates an association between higher TIL counts and improved survival among women with ovarian cancer. These results are consistent with previous studies showing that the immune response is a meaningful prognostic factor in oncology cases. In addition, recent studies indicate the importance of neutrophil (N) to lymphocyte (L) ratios on disease prognosis. For example, high N:L ratios are associated with poor prognosis in sarcoma patients (Balta et al., 2013). Platelets serve to replace damaged areas of blood vessels as a defense against dangerous loss of blood and therefore serve as a vital blood component for overall health.

The present study investigated the differences in complete blood count between animals exposed to chronic adolescent stress and control animals maintained in regular standard housing. The blood counts here were compared at two separate time points as a possible indicator of immune function. It was predicted that animals exposed to the stress condition would show significant differences in blood composition compared to the animals kept in the control condition. In addition, it was hypothesized that these results would be consistent across time points, as found in previous literature where adolescent stress results in long-term phenotypic changes.

Chapter 2

Methods

Table 1. Contact List for Experiment

First Name	*Last Name*	*Phone Number*
Lindsay	Bacik	(412) 580-8692
Lauren	Chaby	(717) 715-7285
Victoria	Braithwaite	(814) 865-4675

Subjects and Housing

Seventy-four male Sprague Dawley rats from Harlan Lab in Maryland were obtained at 21 days of age and were given 9 days upon arrival to allow the animals to settle. Housing for animals was done in pairs, with two rodents in each 20cm x 45cm plastic cage. Each cage was lined with corn cob bedding and contained enrichment items. This basic enrichment included two 2.5cm x 2.5cm x 8cm pine wood blocks and two 7.6cm diameter PVC tubes that were hung from the wire cage top. Cage tops were filled with standard rat chow (LabDiet®) and tap water in water bottles was available at all times. The facility was kept at 20-21^{o}C and 41-42% relative humidity. Animals were kept on a 12:12 reversed light/dark cycle. Procedures were approved by the Pennsylvania State University IACUC committee, protocol #44459.

Adolescent Chronic Unpredictable Stress

Nineteen cages of pair housed rats (n=38) were randomly assigned to the stress condition while eighteen cages (n=36) were assigned to the control condition. Stressors were presented to the stress condition animals daily from 30 to 70 days of age. According to past studies, the adolescent changes occurring in male rodents are believed to conclude around 55 days of age (Spear, 2000). Some studies include a sub-adult period to ensure full exposure of the adolescent stage to stressful events (Schmidt et al., 2007). For this reason, animals were stressed until 70 days of age in the present study.

To ensure that stressors were presented in a chronic and unpredictable manner, physical, social, and predation stressors were presented randomly across the light/dark cycle. Six rest days were randomly incorporated into the schedule (see Table 2).

Table 2: Chronic Unpredictable Stressors

Physical Stressors	
Cage Tilt	Cages were tilted for 6 hours at a 30^{o} angle (Harding et al., 2004; Zurita et al., 2000).
Damp Bedding	Bedding was dampened with 200ml of water on 2/3 of the cage's bedding, and after 6 hours rat pairs were transferred to a clean home cage (Harding et al., 2004; Zurita et al., 2000).
Smaller Cage	Rat pairs were removed from the standard home cage (25cm x 46cm x 20cm) and placed in a cage 25% of this volume for 4 hours (Doyle et al., 2011)
Predation Stressors	
Cat Hair	Rat pairs were transferred to a procedure room and one packet of real cat fur was placed in a plastic container which was then placed in each cage. After 30 minutes, the stressor was removed and rat pairs were transferred to clean home cages in the housing room.
Feline Vocalizations	Rat pairs were transferred to a procedure room and recordings of feline vocalizations were played for 30 minutes. Rat pairs were then transferred to clean home cages in the housing room.

Fox Urine	Rat pairs were transferred to a procedure room and standard fox urine was sprayed onto cotton swabs which were placed in plastic containers which were then placed into each cage. After 30 minutes, the stressor was removed and rat pairs were transferred to clean home cages in the housing room.
Social Stressors	
Crowding	For 4 hours, 2 rat pairs were combined into one clean home cage, with combinations balanced over repeated trials (Harding et al., 2004; Doyle et al., 2011; Zurita et al., 2000).
Foreign Bedding	Rat pairs were transferred to an empty cage of another rat pair and housed for 12 hours (Harding et al., 2004).
Isolation	Rats were separated from their cage mate and housed in a clean cage with enrichment (7.6cm diameter PVC tube and 2.5cm x 2.5cm x 8cm pine wood block) for 1.5 hours (McCormick et al., 2012; Zurita et al., 2000).

*Reproduced in part from Chaby et al (2013).

Throughout stressor exposure, the weight and physical appearance of both stress and control condition animals was monitored closely. Rats were weighed weekly and weights were plotted to ensure normalized growth across the cohort. Rats were examined for physical changes in coat color, size, and general appearance. One rat in the control condition began to show signs of poor growth and changes in appearance including glassy eyes and thin coat texture. The animals' condition was immediately reported to the veterinary technician, who diagnosed hydroencephaly and carried out euthanasia to prevent further suffering.

To control for the effects of experimenter handling on the condition of the animals, control rats were handled twice weekly for 5 minutes each. This procedure ensured that the stress and control animals had equal exposure to contact with humans and the rats were acclimated to the stresses associated with handling.

Blood Collection

Blood was collected from animals at two separate time points. At 71 and 72 days of age, one day after the offset of the stressor paradigm, blood was taken from 25 animals assigned to the time point one condition. At 106 and 107 days of age and 35 days after the stressor paradigm had finished, blood was taken from the 25 animals assigned to the time point two condition. There were three experimenters in the procedure room at all times during blood collection and one experimenter remained outside of the procedure room to transfer animals from the housing room. This experimenter was careful to avoid exposure to the blood collection environment to ensure that control animals and stress animals not involved in blood analysis were not exposed to cues from the procedure room.

Rats were separated from their cage mate and transferred to the procedure room individually and in a random order. The animal was placed in an air-tight container attached to a tube delivering isoflurane anesthesia for approximately three minutes until reflex responses were inhibited. The animal was placed on a procedure table connected to a mask delivering additional isoflurane via the animals' snout. The hind leg of the animal was shaved free of fur and the saphenous vein was pricked with a standard injection needle. Approximately 200uL of blood was collected in a micro bullet tube and stored on ice while the remaining blood collections were carried out. At the end of each procedure day, blood was centrifuged at and separated into plasma and red blood cell components. Blood was then analyzed with the Hemavet 950 for a complete blood count.

Data Analysis

Data for lymphocyte, neutrophil, white blood cell, red blood cell, platelet counts, and neutrophil to lymphocyte ratio conformed to the assumptions for parametric analysis. Data for monocyte, basophil, and eosinophil count were natural log transformed to achieve normality. To compare the stress and control groups within the two time points an independent samples two tailed t-test was run on all outputs. To investigate whether time point had an effect on blood composition, all data were evaluated with a mixed model with time point and stress/control condition as fixed effects. Only significant findings are shown in the figures. Analyses were run in SPSS v. 21 and values are reported as means + standard deviation.

Chapter 3

Results

The neutrophil to lymphocyte ratio was greater in the T2 subject group than in the T1 animals ($F_{1,45}$=19.574, P<0.001, see Figure 1). No significant differences in N:L ratio were found between the stress and control subject groups ($F=_{1,45}$0.276, P=0.602). The lymphocyte count was greater in the T1 subject group than in the T2 animals ($F_{1,45}$=37.268, P<0.001, see Figure 2). No significant differences in lymphocyte count was found between the stress and control conditions ($F_{1,45}$=0.716, P=0.402). The absolute white blood cell count was greater in T1 animals than T2 animals ($F_{1,45}$=18.010, P<0.001, see Figure 3). There was no significant difference in white blood cell count between the stress and control groups ($F_{1,45}$=1.616, P=0.210). The absolute red blood cell count was greater in T2 animals than T1 animals ($F_{1,45}$=11.150, P=0.002, see Figure 4). There was no significant difference in red blood cell count between the stress and control groups ($F_{1,45}$=2.530, P=0.119). The absolute platelet count was greater in T1 animals than T2 animals ($F_{1,45}$=53.966, P<0.001, see Figure 5). There was so significant difference in platelet count between the stress and control groups ($F_{1,45}$=0.844, P=0.363).

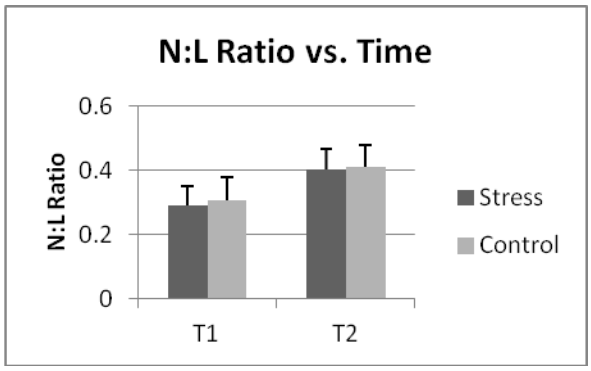

Figure 1: Neutrophil to Lymphocyte Ratio vs. Time

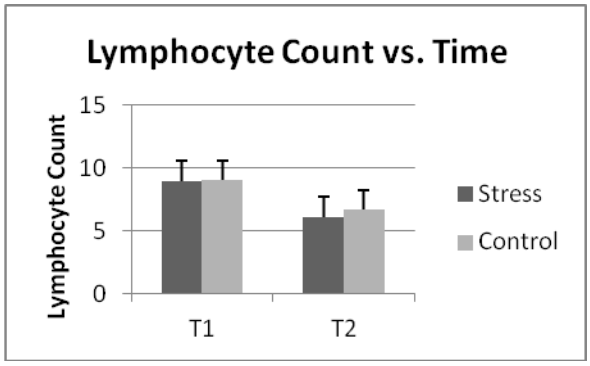

Figure 2: Lymphocyte Count vs. Time

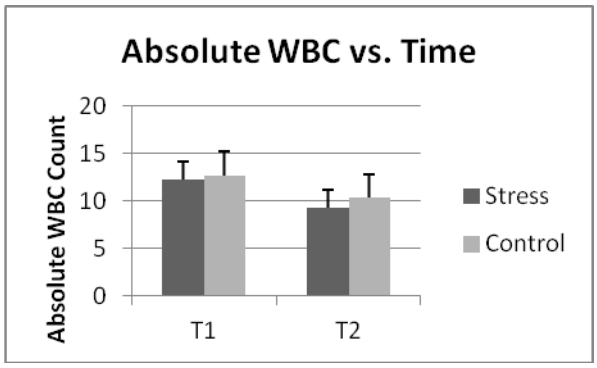

Figure 3: Absolute White Blood Cell Count vs. Time

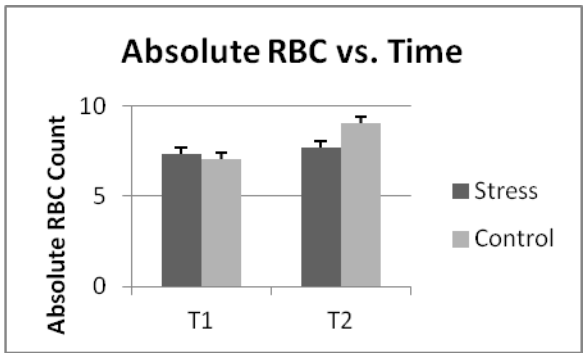

Figure 4: Absolute Red Blood Cell Count vs. Time

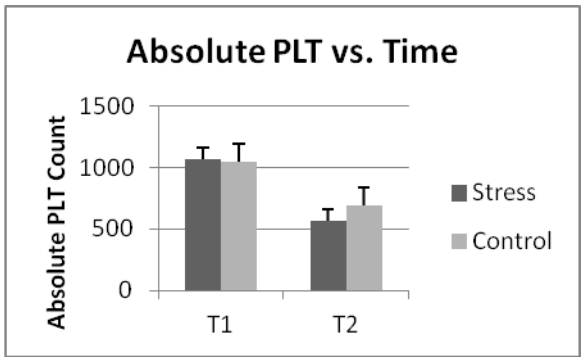

Figure 5: Absolute Platelet Count vs. Time

Chapter 4

Discussion

The results do not support our hypothesis that animals exposed to the stress condition would show significant differences in blood composition compared to control animals. The rats showed no differences between stress and control conditions. In addition, our results do not support our hypothesis that measures would be consistent from T1 to T2. Rather, there were significant differences in N:L ratio, lymphocyte, white blood cell, red blood cell, and platelet counts across time.

Previous studies linking the negative effects of chronic stress to physiology point to increased susceptibility to rhinovirus infection, accelerated progression of human immunodeficiency virus, and enhanced tumor growth in stress compared to control subjects (Cohen, Tyrrel, and Smith, 1991; Lesserman, 2000; Sapolsky, 1985; Thaker et al., 2006). The present study shows no differences between stress and control subjects with respect to blood composition. These results indicate that while differences may exist in physiology between the two groups, more specific measures are needed to provide conclusive evidence of these differences. Future studies could focus on one particular indicator of immune function, such as specific cytokines or interleukins, and compare this measurement between control and stress groups to investigate a linkage between stress exposure and disease.

Chaby et al. (2013) demonstrated the long-term impacts of stress during adolescence on behavior on cognition, specifically, that stress affects the ways in which animals make decisions, interpret ambiguous situations, respond to adverse events, and interact with novel environments. Their results confirm that in adolescence, the neural structures associated with processing

stressful stimuli are plastic, and that the duration of these changes have lasting effects. In the present study, there is a significant difference in blood composition between the animals from T1 and the animals from T2. Since blood collection of T1 animals was performed immediately after a period of adolescent stress and blood collection of T2 animals was performed 35 days after the end of the stress paradigm, it is possible that significant changes occurred in the physiology of the animal from T1 to T2.

Previous studies have indicated the importance of the timing of negative life experiences in relation to stages of development. Goliszek et al. (1996) showed a blunted HPA response in rodents 5 days after exposure to a stressor, while the experiment performed by Toth et al.(2008) showed lower corticosterone concentrations 2 weeks after adolescent stress. Results of the present study align with previous work and suggest that long-term physiological changes are occurring in the rodent between 1 day post-adolescent stress and 35 days post-adolescent stress. In one experiment performed by Schmidt et al. (2007), a prolonged stress procedure ranging from adolescence into adulthood resulted in elevated corticosterone as far as a year after the stress exposure. Based on this finding, it is possible that the changes observed in the present study would persist further into adulthood than measured. Future experiments could include blood collections in late adulthood to examine whether the observed changes do indeed continue past the period measured in the current study.

The present study failed to provide conclusive evidence for a difference between stress and control animals in terms of their blood composition. One way in which the study could be improved in the future is by introducing a manipulation check to investigate whether there were any confirmatory signs that the stressed animals were indeed experiencing more stress than the control animals. While stressors were presented strategically to this group and great caution was taken to avoid control animals from being exposed to any stressors, it might be helpful to assay whether the stressors actually caused those rats assigned to the stress condition to become

significantly more stressed than those assigned to the control condition. Future studies could use changes in body composition as a possible marker of stress response. Gurfein et al. (2012) used magnetic resonance imaging to detect changes in body composition, and found that animals living under "calm" conditions gained greater body mass than control animals. Therefore, it is possible that animals living under stress conditions would have lower body mass than control animals.

Research such as that conducted in the present study aims to use animal models to provide insights that can be applied to humans and utilized in improving the medical treatment of various diseases. However, limitations exist in the degree to which results from animal models can be generalized to account for humans. One problem is that the caging conditions used in most laboratory environments act as a source of chronic mild stress. Not only does this influence how we can apply our results to human studies, but it also impacts the degree to which we can classify our control animals as being completely unexposed to stress. In some ways, it is inevitable that all animals involved in a study have experienced some stress during their development.

From this study and previous research, it is possible to conclude that the effects of adolescent stress depend on a great variety of factors, such as the age of the subject, the duration of the stress response, and the time between stress exposure and testing (McCormick et al., 2010). While it is understood that chronic stress is associated with negative health outcomes, neuroendocrine changes, and various negative effects on immune function, it is important that we investigate the specific mechanistic link between chronic adolescent stress and disease pathology in order to effectively treat those impacted by its deleterious effects. A great amount of research using animal models on the impact of stressors in adolescence is presently being conducted, but it is clear that more research is needed before definitive statements can be made regarding the specific connection between stress and physiology.

Chapter 5

Conclusions

To conclude, the present study resulted in significant differences between time points in measures of N:L ratio, lymphocyte, white blood cell, red blood cell, and platelet count. These results suggest the importance of the temporal relationship between stressor offset and stress response, an interesting point of study in the field of stress research.

Appendix A

Data Tables

Type III Tests of Fixed Effects for Stress vs. Control Analysis

Measure	F-statistic	Significance
Neutrophil Count	1.500	0.277
Lymphocyte Count	0.716	0.402
N:L Ratio	0.276	0.602
Absolute WBC Count	1.616	0.210
Absolute RBC Count	2.530	0.119
Absolute PLT Count	0.844	0.363

Type III Tests of Fixed Effects for T1 vs. T2 Analysis

Measure	F-statistic	Significance
Neutrophil Count	0.131	0.719
Lymphocyte Count	37.268	0.000*
N:L Ratio	19.574	0.000*
Absolute WBC Count	18.010	0.000*
Absolute RBC Count	11.150	0.002*
Absolute PLT Count	53.966	0.000*

*Statistically Significant Difference

Appendix B

Sample Means

Descriptive Statistics for Sample Means

Measure	T1 Mean	T2 Mean
Neutrophil Count	2.660	2.582
Monocyte Count	-0.706	-0.745
Basophil Count	-2.702	-2.736
Lymphocyte Count	8.975	6.371
N:L Ratio	0.297	0.405
Absolute WBC Count	12.465	9.781
Absolute RBC Count	7.168	8.349
Absolute PLT Count	1059.750	630.875

BIBLIOGRAPHY

Balta S., Demirkol S., Sarlak H., Kurt O. (2013). Elevated preoperative neutrophil/lymphocyte ratio is associated with poor prognosis in soft-tissue sarcoma patients': neutrophil to lymphocyte ratio may be predictor of mortality in patients with soft-tissue sarcoma. *British Journal of Cancer* 108, 2625–2626.

Chaby, L., Braithwaite, V., Cavigelli, S., Wang, K., White, A. (2013). "Long-term changes in cognitive bias and coping response as a result of chronic unpredictable stress during adolescence." *Frontiers in Human Neuroscience* 7, 328.

Charmandari, Evangelia (2005). "Endocrinology of the stress response". *Annual review of physiology* 67, 259.

Cohen, S., Janicki-Deverts, D., and Miller, G. E. (2007). Psychological stress and disease. *Journal of the American Medical Association* 298, 1685–1687.

Cohen, S., Tyrrell, D. A., Smith, A. P. (1991). Psychological stress and susceptibility to the common cold. *New England Journal of Medicine* 325, 606-612.

Doyle, R. E., Lee, C., Deiss, V., Fisher, A. D., Hinch, G. N., and Boissy, A. (2011). Measuring judgement bias and emotional reactivity in sheep following long-term exposure to unpredictable and aversive events. *Physiology & Behavior* 102, 503-510.
Glaser, R., Kiecolt-Glaser, J. K. (2005). Stress-induced immune dysfunction: implications for health. *Nature Reviews in Immunology* 5, 243-251.

Goliszek, A.G., Crawford, G.E., Lawrence, H.S., Bennett, J., Williams, F., Hurley, S.L. (1996). Effects of prepubertal stress on subsequent ACTH response to novel stress and CRH in male vs. female rats. *Stress Medicine*, 12, 199-204.

Gurfein, B.T., Stamm, A.W., Bacchetti, P., Dallman, M.F., Nadkarni, N.A., Milush, J.M., Touma, C., Palme, R., Di Borgo, C.P., Fromentin, G., Lown-Hecht, R., Konsman, J.P., Acree, M., Premenko- Lanier, M., Darcel, N., Hecht, F.M., Nixon, D.F. (2012). The Calm Mouse: An Animal Model of Stress Reduction. *Molecular Medicine* 18, 606-617.

Harding, E. J., Paul, E. S., and Mendl, M. (2004). Cognitive bias and affective state. *Nature* 427, 312.

Koolhaas, J. M., Bartolomucci, A., Buwalda, B., de Boer, S. F., Fluegge, G., Korte, S. M., Meerlo, P., Murison, R., Olivier, B., Palanza, P., Richter-Levin, G., Sgoifo, A., Steimer, T., Stiedl, O., van Dijk, G., Woehr, M., and Fuchs, E. (2011). Stress revisited: A critical evaluation of the stress concept. *Neuroscience and Biobehavioral Reviews* 35, 1291-1301.

Krabbendam, L., van Os, J. (2005). Schizophrenia. *Bull* 31, 795–799.

Lesserman, J (2000). The effects of depression, stressful life events, social support, and coping on the progression of HIV infection. *Current Psychiatry Reports* 2, 495-502.

McCormick, C. M., Mathews, I. Z., Thomas, C., and Waters, P. (2010). Investigations of HPA function and the enduring consequences of stressors in adolescence in animal models. *Brain and Cognition* 72, 73-85.

McCormick, C. M., Thomas, C. M., Sheridan, C. S., Nixon, F., Flynn, J. A., and Mathews, I. Z. (2012). Social instability stress in adolescent male rats alters hippocampal neurogenesis and produces deficits in spatial location memory in adulthood. *Hippocampus* 22, 1300-1312.

Sapolsky, R. M., Donnelly, T.M. (1985). Vulnerability to stress-induced tumor growth increases with age in rats: role of glucocorticoids. *Endocrinology* 117, 662-666.

Schmidt, M. V., Alam, D., Ganea, K., Harbich, D., Liebel, C., Sterlemann, V. (2007). Persistent neuroendocrine and behavioral effects of a novel, etiologically relevant mouse paradigm for chronic social stress during adolescence. *Psychoneuroendocrinology* 32, 417-429.

Thaker, P. H., Han, L. Y., Kamat, A. A. (2006). Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Natural Medicine* 12, 939-944.

Toth, E., Gersner, R., Wilf-Yarkoni, A., Raizel, H., Dar, D.E., Richter-Levin G. (2008). Age-dependent effects of chronic stress on brain plasticity and depressive behavior. *Journal of Neurochemistry* 107, 522-532.

Zurita, A., Martijena, I., Cuadra, G., Brandão, M. L., and Molina, V. (2000). 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, 163-171.

ACADEMIC VITA

Lindsay Caroline Bacik
Lib5114@gmail.com—(412) 580-8692

Current Address:
236-1 S. Barnard St.
State College, PA 16802

Permanent Address:
5066 Azalea Drive
Pittsburgh, PA 15236

Education

The Pennsylvania State University, University Park, PA 16802
Eberly College of Science and Schreyer Honors College
B.S in Science
Minors in Neuroscience and Psychology
Honors Thesis in Neuroscience
Expected Graduation Date: May 2014

Activities and Volunteer Experience

NCAA Division I Indoor/Outdoor Track and Field- 2010 to present
Best Buddies International Chapter Member- 2010 to present
The Children's Institute Volunteer- 2010 to 2011
Apollo Benefiting THON- Fundraising Chair 2012, Family Relations Chair 2013, Dancer in THON 2014
Global Medical Brigades- Nicaragua Brigade Member 2013
Mount Nittany Medical Center Emergency Department Volunteer- 2013 to present

Research Experience

Pennsylvania State University Research Assistant
Adolescent stress and cognitive development
Dr. Victoria Braithwaite, Principle Investigator

Honors and Awards

Schreyer Honors College- 2010 to present
Dean's List- 2011 to present
NCAA Scholar Athlete- 2012 to present
Undergraduate Discovery Research Grant- Summer 2013

Presentations and Publications

Long-term changes in cognitive bias and coping response as a result of chronic unpredictable stress during adolescence- 2013
Research Experience for Undergraduates presentation and exhibition- 2013