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TEMPORAL PATTERNS OF REPEATED ACUTE STRESS AFFECT STRESS SEVERITY

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ABSTRACT

Chronic stress has become a growing concern in the modern world, yet since Hans Selye conducted his rat experiments and discovered the negative effects of chronic stress in the 1930s, researchers are still unsure about when acute stress becomes chronic stress and the key characteristics of chronic stress that govern severity. This study was created to investigate whether or not the temporal pattern of acute stressors is an important characteristic that affects physiological and psychological responses to chronic stress. Specifically, we tested whether it is more stressful to experience multiple stressors in a short amount of time (ie. within an hour), or if it is more stressful to experience the same intensity, duration and frequency of stressors spread across the day (ie. across 8hrs). Sixty three male young adult Sprague Dawley rat were used in this study, with 42 rats administered chronic mild stress (CMS) for 4 weeks and 21 as non-handled control animals. Of the 42 chronic mild stress animals, 21 had three CMS stressors administered within an hour (this group is the clumped group), while the other 21 animals had three CMS stressors administered spread across 8 hrs (this group is the distributed group). Clumped animals showed significant differences in weight gain and sucrose preference compared to the control group while distributed animals did not differ in any measurements compared to the control group, suggesting that stressors are more detrimental to health when they are clumped together.

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Finally I would like to thank my family and sandy dan for the emotional support throughout the writing of this thesis.

Chapter 1

Introduction

The effects of chronic stress are a growing concern for the modern world. According to the American Psychological Association, 20 percent of Americans report experiencing extreme stress in 2012. At that time, 66 percent of Americans believed that stress impacted their physical health negatively while 63 percent believed the same for their mental health (9).

At first glance, the effects of stress can seem contradictory. Researchers have agreed that when an organism is exposed to a short-lived stressor, it becomes better prepared in the face of potential challenges (6). While the animal experiences acute stress, glucocorticoids are released into the blood stream to make glucose more readily available for use, the cardiovascular system responds by increasing heart rate and blood pressure preparing the animal to response to the challenges (21), and the immune system increases leukocyte trafficking to cope with potential injuries (2). This type of stress is responsible for stimulating adaptive physiological responses associate with the "fight or flight" response and is necessary for everyday functioning.

However if stressors are present for prolonged periods of time, ranging from days to years, acute stress becomes chronic. The initially beneficial response can lead to detrimental effects on the organism as chronic stress can lead to allostatic load (a state when the body's catabolic activity out weights its anabolic activity) which can lead to impaired immunity, insulin resistance, obesity and neurodegeneration in brain tissues (21). Atrophy in the hippocampus and hypertrophy in the amygdala brain regions are observed in individuals suffering from psychological disorders such as depression and anxiety (6) (21). A recent meta-analysis has

found that chronic stress and telomere length are negatively correlated (10). This is especially troubling in today's society, as non-life threating but stressful events continuingly activate the stress response. As it has been shown that stress response can even be activated by worrying about the future or recreating (in the mind) past stressful events (27).

The detrimental effects of chronic stress are evident. Yet since Hans Selye conducted his rat experiments and discovered the negative effects of chronic stress in the 1930s, researchers are still unsure about when acute stress becomes chronic stress and the key characteristics of chronic stress that governs response severity. Some have proposed to view chronic stress as a response to a series of acute stressors, thus severity of acute stress might play a role in chronic stress severity (27). Previous research has examined how the duration, intensity and frequency of an acute stressor affect the stress response. However, the temporal dynamics, or the pattern of acute stresses across the day, has not been given much attention by the scientific community. To further our understanding of this important component of chronic stress, this study was designed to determine whether or not the temporal pattern of acute repeated stressors, is a determinant of physiological stress severity.

Before we discuss other possible stressor characteristics, we should first differentiate *stress*, *stressor* and *stress response*. Stress is a broad, generic term and is unspecific. Stressor is any specific challenge presented to an organism. Stressors can be physical, for example a leg injury, or psychological, such as the thought of an upcoming examination. Stress response is defined as an organism's response when confronted with a stressor. An example of aphysiological response would be rats producing elevated circulating corticosterone concentration after exposed to a stressor, such as predator odor, which functions to mobilize stored energy.

Potential characteristics of chronic stress that might affect stress severity

Possible stressor characteristics that were examined in previous studies include the intensity of stressor, duration of stressor and previous exposure to similar stressors. One way to determine stressor severity is through behavioral measures, however behavior does not necessarily reveal underlying physiological changes, which may influence an organism's health (11). Thus it is best to couple behavioral observations with physiological measures, such as monitoring stress hormones, in particular glucocorticoid regulation.

The hypothalamic- pituitary- adrenal axis (HPA) is one system that governs stress responses and the key hormones are glucocorticoids and adrenocorticotropic hormone (ACTH). To examine stress hormone regulation in animals, previous studies have examined basal and peak ACTH and corticosterone levels (one type of glucocorticoid) and the time it takes for hormonal levels to return to baseline (i.e., recovery) (1, 2, 28). studies also suggest that for acute stress, increased intensity of stress is associated with higher corticosterone and ACTH peak and prolonged stress recovery time, in other words, it takes longer for hormonal levels to return to baseline levels after a stressor (1, 2).Other research has shown that intensity of a stressor does not affect the peak of a stress response but could affect recovery rate after an acute stressor (1, 28). However, the duration of a stressor does not seem to have much effect on stress recovery rate (1). Previous experience with a stressor can shorten recovery time when an animal is exposed to a similar stressor. A single exposure to a similar stressor is enough to reduce recovery time, however this reduction in recovery time is less significant when chronically stressed animal are exposed to a novel stressor (1, 4, 3). Researchers have suggested that the severity of a stressors might be positively correlated to total hormonal production following stressor exposure, often measured as area under the curve of an HPA activation graph (1, 27).

Finally rodents that experienced chronic mild stress, compared to their control group do not seem to have alterations in stress response peak nor recovery time, but rather often show elevated basal corticosterone production (18, 23, and 24).

Chronic mild stress

A modified version of the chronic mild stress (CMS) paradigm was used to induce the stress response in this experiment. CMS was developed for the purpose of producing depression-like symptoms among rodents through daily exposure to mild stressors for 3-4 weeks, stressors typically last from 1 to 12 hours (7). Common CMS stressors include procedures such as tilted cages, food and water deprivation, water immersion, noise, light etc.

Processes affected by chronic stress

Glucocorticoid circadian rhythm:

Studies have shown that circadian rhythm disturbances are linked to development of depression, as patients suffering from depression often show altered circadian rhythms and sleep disturbances (16).The circadian rhythm is a 24 hour biological cycle that all organisms inherently possess and can be monitored by corticosterone concentrations across the day (17). It governs several physiological activities and its effect on hormonal secretion and sleep wake can directly affect results obtained for this study. Previous studies have shown that chronically stressed individual often show lower corticosterone levels in the morning and higher corticosterone levels in the evening compared to their control counterparts (22). Given these prior findings on the

effects of chronic stress on the glucocorticoid circadian rhythm, this study used morning and evening basal glucocorticoids production as indicators of chronic stress severity.

Body weight:

Studies have showed that weight loss can also produce an index of stress severity as change in body weight is closely related to stressor intensity (18, 12, and 13). After experiencing chronic stress, adult rats often show reduced appetite, leading to weight loss. Body weight loss seems to be an enduring effect as findings have shown that weight loss remained significant in adult Sprague-dawley rats 40 days after the completion of chronic stress administration (12).

Behavior measurements:

Stress severity can also be measured through depression-like and anxiety-like behavior. Depression and anxiety like symptoms are measured using sucrose preference and elevated plus maze respectively.

Sucrose preference test were used to measures the extent of depression-like behaviors by measuring anhedonia, an inability to experience pleasure. Perhaps due to the high comorbidity of depression and anxiety, rodents going through CMS might also exhibit more anxiety-like related behavior as well as less exploratory behavior in the elevated plus maze (EPM) (7). All these phenotypic changes can be reversed after 3-4 weeks of anti-depressant treatment (5). For logistical reasons, a less severe version of CMS was used for this study instead of the traditional hours-long stressors. Stressors for this study were modified to be 15 minutes long to better manipulate temporal characteristics of chronic stress. Food and water deprivation were avoided

for this study since body weight is speculated to be a potential moderator of sucrose consumption (8).

The effects of CMS on elevated plus maze behaviors is less clear compared to sucrose preference. Previous studies assess anxiety-like behavior based on the amount of time spent in open arms and time in closed arms, and usually CMS would cause rats to show increased anxiety-like behavior. In some studies instead of anxiety like symptoms, CMS would induce anti-anxiety like symptoms among its subjects (5). Recent studies have found that the rat's circadian rhythm is especially important for determining wither a rat would have a depressive profile or an anomalous profile (14, 15). Specifically, when CMS was administered during the rat's light phase (thus disrupting circadian rhythm) rats showed more anxiety/depressive-like symptoms, while dark phase administration would have antianxiety-like effects on rats tested.

Hypothesis of this study

The current study investigated whether or not the temporal pattern of acute stressors is an important characteristic that affects physiological and psychological responses to chronic stress. Specifically, we tested whether it is more stressful to experience multiple stressors in a short amount of time (ie. within an hour), or if it more stressful to experience the same intensity, duration and frequency of stressors spread across the day (ie. across 8hrs). Physiological and behavioral responses to those 2 temporal patterns of chronic stress were measured in adult male rats. Each rat was exposed to the same stressors in the same order for the same amount of time to control for stressor intensity, frequency and duration. Animals were exposed to either temporally clumped or distributed stressors, with clumped stressors occurring within an hour of each other

and distributed stressors occurring in the same order but with several hour gaps between each stressor. Below are the three hypothesis that were tested.

Null hypothesis:

The temporal pattern of daily, repeated stressors has no effect on an organism's behavioral or physiological responses to chronic stress.

Threshold Hypothesis:

Because minor acute stressors lead to a moderate elevation in corticosterone production that lasts about 60 minutes beyond stressor completion, we hypothesized that repeated acute stressors will only lead to effects of chronic stress when they occur in rapid succession where corticosterone production is extended above or longer than a certain acute threshold. Whereas when acute stress are administered in a distributed manner, earlier physiological stress responses will dissipate before the subsequent stressor lead to chronic physiological actuation. Thus, It may be more detrimental to an organism's physiological and behavioral health to experience multiple stressors in a short amount of time than to have the stressors spread out across the day.

Exhaustion Hypothesis:

Because the organism must remain alert throughout the day when stressors are distributed across the day, we hypothesized that distributed repeated acute stressors will lead to greater signs of chronic stress than clumped stressors. Thus it is more detrimental to an organism's behavior

and physiological health to have stressors spread throughout the day than going through all stressors in a short amount of time within each day.

These hypothesis were tested by measuring hedonic-related and anxiety-like behaviors and physiological measures before and after chronic stress exposure. If the null hypothesis is supported, we expect that hedonic-related and anxiety-like behaviors will be elevated, weight gain would be impaired and there would be an impaired circadian rhythm in the clumped condition animals relative to control animals. If hypothesis 2 is supported, we expect elevated hedonic-related and anxiety- like behaviors and impaired weight gain and glucocorticoid circadian rhythm in the distributed condition animals relative to the control animals. Since there are no past studies on this specific topic, the confirmation of either of the above hypothesis will further our understanding of whether acute stressor temporal dynamics are important predictors of chronic stress.

Chapter 2

Method

Animals and overall design:

63 male Sprague Dawley rats (2 months old) were ordered for this study. Rats were singled housed in standard (20 × 26 × 46 cm) rat-sized cages with corn cob bedding. Conditions within the colony room were controlled at 40% humidity and 21° C. All rats were provided with unrestricted food and water, a wooden chewing block and a clear red cylinder. Rats were housed in a reverse light cycle, with lights off at 8:00 hr and on at 20:00 hr and lighting automatically controlled by the housing facility. To monitor weight gain, rats were weighed weekly. The study was divided into four stages (Fig. 1 is an illustration of the study timeline.). Procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (IACUC No. 45196).

Pre-CMS

Rats were handled at the beginning of the study to habituate them to human handling. To control for initial behavioral profiles, rat behavior was tested at the beginning of the study. Behavior tests include elevated plus maze, sucrose preference test, novel physical test and novel social test. Rats were then assigned to one of the following three groups: Clumped Stressors,

Distributed Stressors or no treatment Control, with equal numbers of high and low exploratory rats in each group as defined by latency to engage novelty in the novelty tests.

CMS

Rats were given chronic mild stressors according to treatment groups. Each day, Clumped rats were given three stressors in one session (1 hr), while Distributed rats received the three stressors separately, one in the morning, one in the afternoon and one in the evening. No stressors were administered to the controlled rats. To measure basal corticosterone concentrations, blood samples were collected from the lateral tail vein once a week at 2 time points: once in the morning (am) at the end of the lights on phase, and once in the evening (pm) at the beginning of the lights on phase.

Post-CMS:

To assess change in behavior after CMS treatment, a second round of behavior testing was conducted, with the same behavior tests used during the pre-CMS stage. The second day CMS ended, rates were challenged with an antigen. 3 days later acute corticosterone responses were tested at the end of this stage, carried out during the last three hours of their dark phase.

Tissue collection:

33 days after acute corticosterone response was tested, rats were euthanized and body tissues were collected for later analysis.

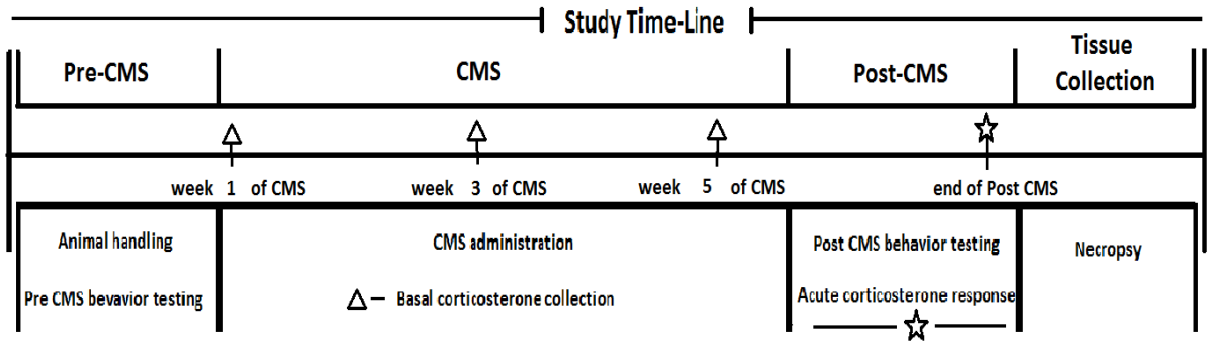

Figure 1: Study time line

Chronological order of events for this study. Triangles (△) indicate basal corticosterone measures stars (☆) indicate measure of acute corticosterone response. Procedures with no markers indicate they were administered frequently throughout the stage indicated.

Pre-CMS stage:

Elevated plus maze:

To measure anxiety-like behavior, rats were tested on the elevated plus maze (EPM). The EPM is a black polypropylene cross-shaped maze elevated 61 cm above the ground, with two sheltered arms (10x50 cm with 40 cm walls) and two unsheltered arms (10x50 cm). Rats were placed in the center of the maze and allowed to move freely for five minutes the test was conducted in red light, and an overhead video camera recorded all behavior for later coding. Anxiety was quantified as time spent in closed arms, thus more time spent in closed arms was interpreted as more anxious.

Sucrose preference test:

To measure depression-like symptoms, rats were tested in the sucrose preference test (SPT). Sucrose concentration had been set at 1%, in addition another bottle containing regular tap water is used as a control. Results are generally analyzed as Sucrose consumption/ total liquid consumption (5). The less sucrose consumed, the more depress the rat is. Sucrose preference test usually lasts 12 hours and before administration, there is usually a 20 hour food and water deprivation period. Studies that include such a deprivation period often show a large change in percent sucrose consumed (5). However, it is possible to show a statistically significant reduction in sucrose preference without such food and water deprivation, though the difference is typically much smaller, usually less than 10% (19, 20). Bottles were left on the cage for 24 hours and the amount of fluid lost in each bottle recorded at the end of 24 hours. Half way through the test (12 hours in), bottles were switched sides to control for potential side preferences. Lower sugar-water/ tap-water consumption ratio indicates a higher level of anhedonia, one component of depression

Novel physical and Novel social:

To assess rat temperament, animals were put into a 120 x 120 x 46 cm square arena, filled with bedding. A Plexiglas cover minimized threatening stimuli and allowed behavior to be recorded using an overhead camera. The novel physical arena contained unfamiliar rat-sized objects placed in three corners of the arena. Willingness to explore was assessed based on the number of interactions and the latency to approach novel objects. The novel social arena, contained a cage with an unfamiliar rat and an empty cage in opposite corners of the arena.

Novel stimulus rats were chosen from animals not included in this study. The outcomes of these two tests were used to assign animals into different treatment groups to control for initial differences in behavior profile.

CMS stage:

Chronic Mild Stress (CMS) stressor:

A modified version of the unpredictable chronic mild stress protocol was used (7). To avoid habituation, we used six different stressors: wet cage, wet bedding, tilted cage, novel cage, noise, and strobe light. Novel cage consisted of rats being put into a clean empty rat cage identical to their original housing cage. Wet cage involved rats being placed in a new cage filled with 2 cm of room temperature tap water. For wet bedding, the cage was filled with 2 cm of wet corn cob bedding. In tilted cage, strobe light and noise, rats remained in their home cage and their cages were either tiled on one side at a 45° angle, exposed to white strobe light, or 80 decibels of white noise. Each stressor lasted 15 minutes and was administered in red light, in a room separate from the housing colony room.

Three CMS stressors were randomly scheduled for each day. Every day consisted of 3 stress sessions, each lasting 90 minutes. Sessions were either in the morning, afternoon or evening and were at least 135 minutes apart from one another. Clumped animals received all three stressors within 90 minutes with 5 minute breaks between stressors, thus undergoing all stressors in one of the 3 daily time slots. To control for circadian rhythm as a potential confound, Clumped rats were divided into three different groups, each group received stressors during a different time slot (morning, afternoon or evening). Distributed animals were exposed to one

stressor per time slot. Figure 2 is a diagram illustrating the CMS administration process. Rats in their home cage were placed on a cart and wheeled into another room for stressor exposure. Each stressors lasted 15 minutes and rats were wheeled back to the colony room between each stressor. During this break, experimenters would prepare for the next stressor. To control for handling effect, control rats were handled by experimenters 1 minute each day during breaks between stressors.

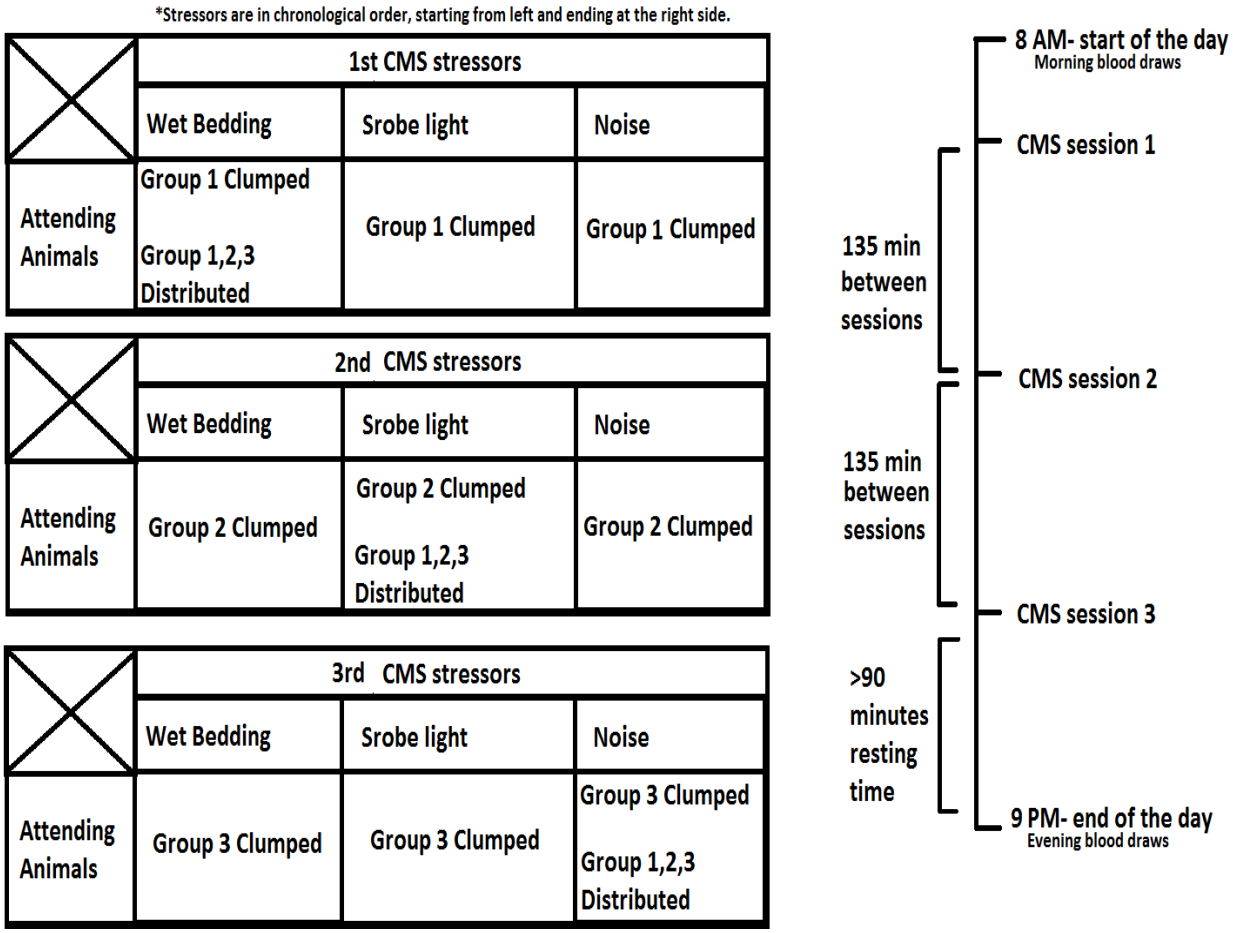

Figure 2: Sample CMS schedule

CMS organization. Each day consisted of three 15-minute stressors. Between each stressor, were 5 minutes breaks during which all animals were rolled from CMS room back to colony room. Blood draws were performed at the beginning and at the end of the day once a week. Animals were allowed to rest for more than 90 minutes before the evening blood collection.

Basal corticosterone collection:

Blood was collected at the beginning of each week and on the last day of stress administration. Blood was collected from the lateral tail vein at 8:00 hr (morning) and 21:00 hr (evening) in a separate room from the colony room and in white light. Evening blood draws were collected at least 90 minutes after the last CMS session so that rats had enough time for corticosterone levels to return to baseline. Rats were transported in their home cage to the blood collection room one-by-one and restrained by one of the collectors while the two other experimenters collected blood with a syringe and recorded time (each collection took an average of 2 minutes per rat). Blood draws were typically conducted on the left tail vein, but if unsuccessful blood was taken from the right tail vein. Blood samples were preserved on ice and spun to separate serum and stored at -80 °C to be analyzed after the end of the study. Each blood sample had to be more than 5 microliters in order to be analyzed effectively. Rats were weighed at the end of each collection and returned to their home cage.

Post-CMS stage

Acute corticosterone response:

Acute corticosterone responses were measured from 17:00 h to 20:00 h in a room separated from the colony room under white light. Rats were brought to the procedure room one at a time. A baseline blood sample was collected then rats were put into restrainers for 15minutes and returned to their home cage. After the 15 minute restraint rats were allowed to rest until the second and third blood draws at 30 minutes and 105 minutes after the baseline was collected.

Blood was collected the same way as for basal corticosterone measures. At the end of blood sampling, weights were recorded. Pre-CMS behavior tests were conducted again during Post-CMS stage.

Tissue collection

Necropsy:

Rats were brought to the necropsy room one at a time and euthanized using carbon-dioxide overdose. Blood was collected from the heart using a syringe and to measure basal corticosterone levels and telomeres length for another study. Brain tissue was also collected for another study.

Statistical Analysis

Repeated measures ANOVAs were used to analyze the effect of CMS treatment on body weight and acute corticosterone stress response. For weight analysis, weight on days 7, 14, 21 and 27 were analyzed with treatment group as the independent factor. Day 34 body weight was not included because a lipopolysaccharide immune challenge was administered prior to day 34, (not included in this particular study) and caused a sharp drop in body weight among all animals. To control for initial individual differences in weight, day 1 weight values were used as a covariate. For acute stress response, corticosterone levels at 0, 30 and 105 min after the beginning of restraint was the dependent variable and treatment group was the independent variable.

Univariate ANOVAS were used to analyze SPT, EPM and basal corticosterone data. For SPT, percent sucrose consumption was the dependent variable, for EPM, open arm time was the dependent variable and for basal corticosterone levels morning and evening basal corticosterone levels were the dependent variables. CMS treatment group was the independent variable for the above analyses. To control for initial individual behavioral differences, pre-CMS measures of each outcome variable were used as covariates for SPT and EPM and basal corticosterone level analyses.

Distributions of each measure were examined for normality and data points above or below 2.5 standard deviations were not included. Skewed distributions were log-transformed (using natural log). For viewing convenience, graphs in the result sections are displayed using raw data. Results were said to be significant if $P<0.05$.

Chapter 3

Results

Sucrose preference test

Percent sucrose solution consumed on the last day of CMS was compared for the two CMS and control groups, controlling for pre-CMS sucrose consumption as a covariate. Three animals were dropped from this analysis as outliers. Clumped rats consumed less sucrose than the control group (mean difference= -3.3% $P<0.05$), and the distributed group was not significantly different from either the control or clumped groups ($F_{2, 57} = 3.34$ and $P<.05$; Figure 4).

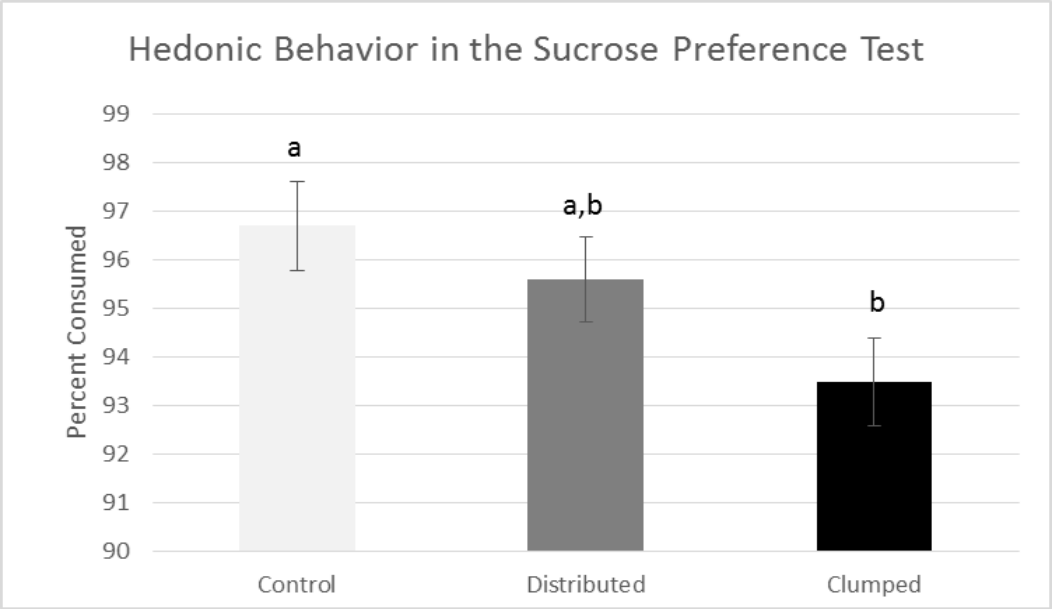

Figure 3: Percentage sucrose consumption

Estimated marginal means of SPT percent sucrose consumption on the last day of CMS (with pre-CMS SPT sucrose consumption as covariate). Letters indicate groups that are statistically significantly different from one another.

Weight gain:

Body weights from day 7 to 27 were analyzed, using day 1 body weight as a covariate. Among the three groups, clumped animals gained the least weight while control animals had gained the most weight since the beginning of stress administration. Between group differences were significant among all groups (Figure 5, $F_{2, 59}$ =9.87, P< 0.01, Clumped vs Control groups, P< 0.02, Clumped vs Distributed, P <0.05, Distributed vs Control).

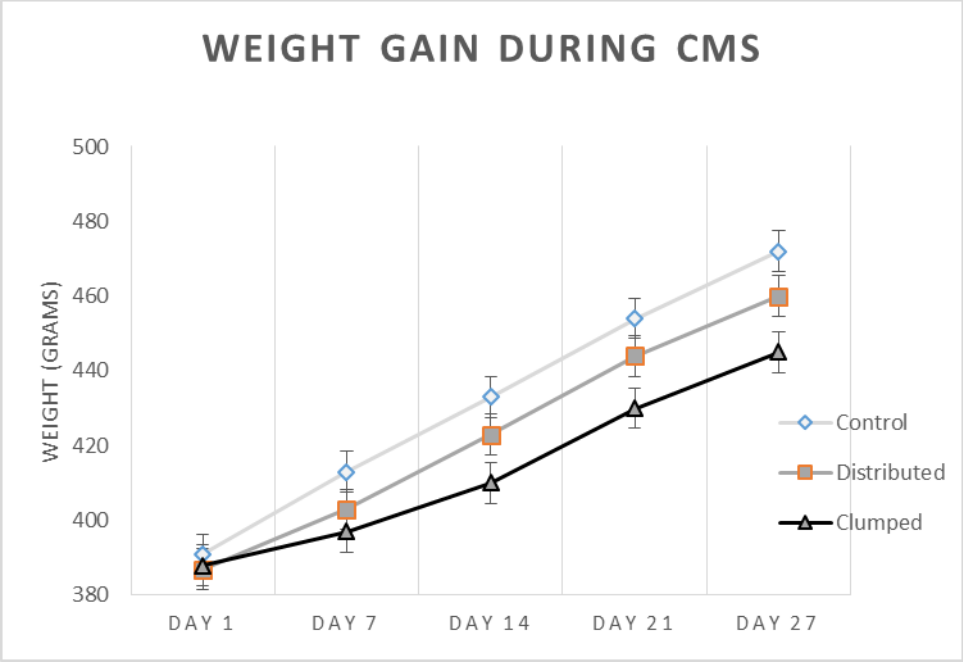

Figure 4: Body weight across CMS administration

Estimated marginal mean body weights from day 7 to day 27(with day 1 body weights as covariate). All groups showed significant difference between each other.

Elevated Plus Maze:

Open arm time on the elevated plus maze after CMS was analyzed for all groups while using pre-CMS open arm time as a covariate. There were no significant differences among the three groups. ($F_{2, 61}$ =0.58, P>0.10; Figure 6)

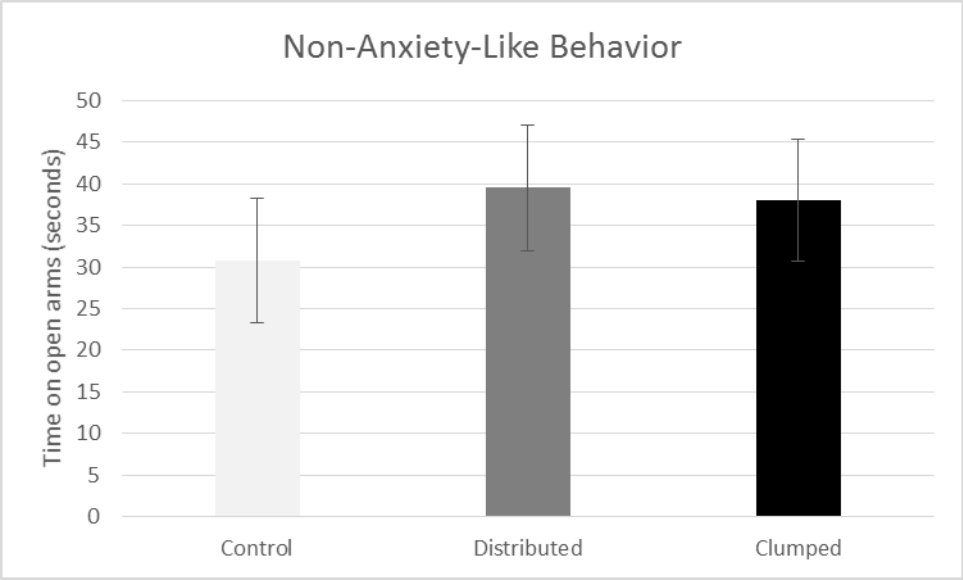

Figure 5: Post-CMS time in open arms

Post-CMS EPM open arm time, with Pre-CMS EPM open arm time as covariant.

Basal Corticosterone:

Morning and evening basal corticosterone levels on the last day of CMS were analyzed using corticosterone values on the first day of CMS as a covariate. There were no significant differences among groups for either morning or evening samples (morning samples, $F_{2,61}=1.21$, $P>0.10$, evening samples, $F_{2,62}=0.42$, $P=.66$). For clarity, Figures 7 and 8 show the raw data for morning and evening corticosterone levels instead of the log values that were used in statistical analyses.

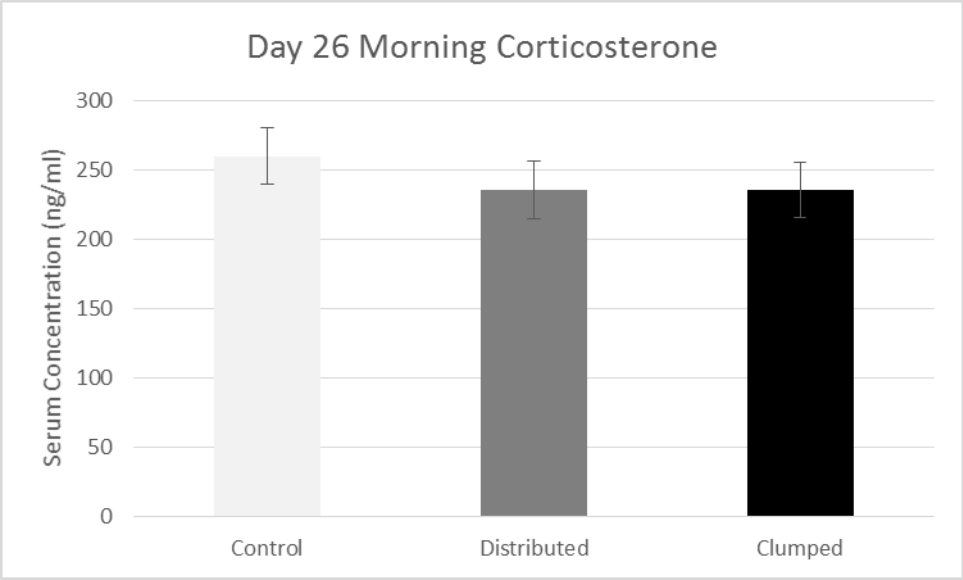

Figure 6: Week 5 CMS morning Corticosterone concentration

Day 26 morning basal serum corticosterone concentration with standard error bars. There were no significant differences among groups.

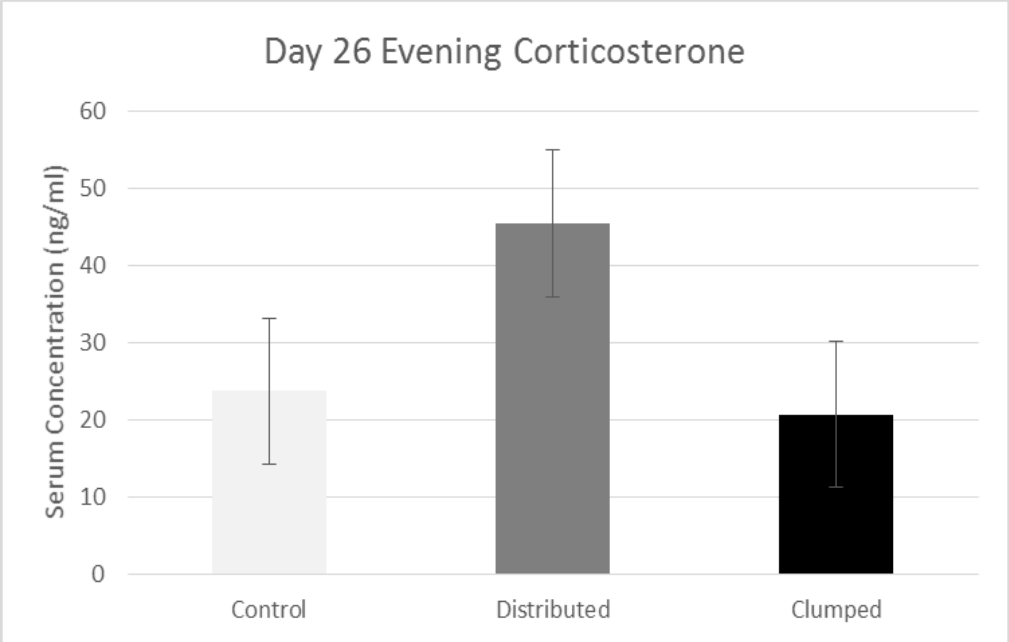

Figure 7: Week 5 CMS evening Corticosterone concentration

Day 26 evening basal serum corticosterone concentration with standard error bars. There were no significant differences among groups

Acute stress response:

Serum corticosterone concentration at 0, 30 and 105 min after acute restraint was compared across the three groups (Figure 9, 0 min represents the baseline, 30 min represents the peak of corticosterone production and 105 min represents the return to baseline. There were no significant differences among the three treatment groups ($F_{2, 59} = 0.26$, $P>0.10$). Additional analysis shows that there were no differences between each time point. For clarity, figure 9 shows the raw data instead of their log values.

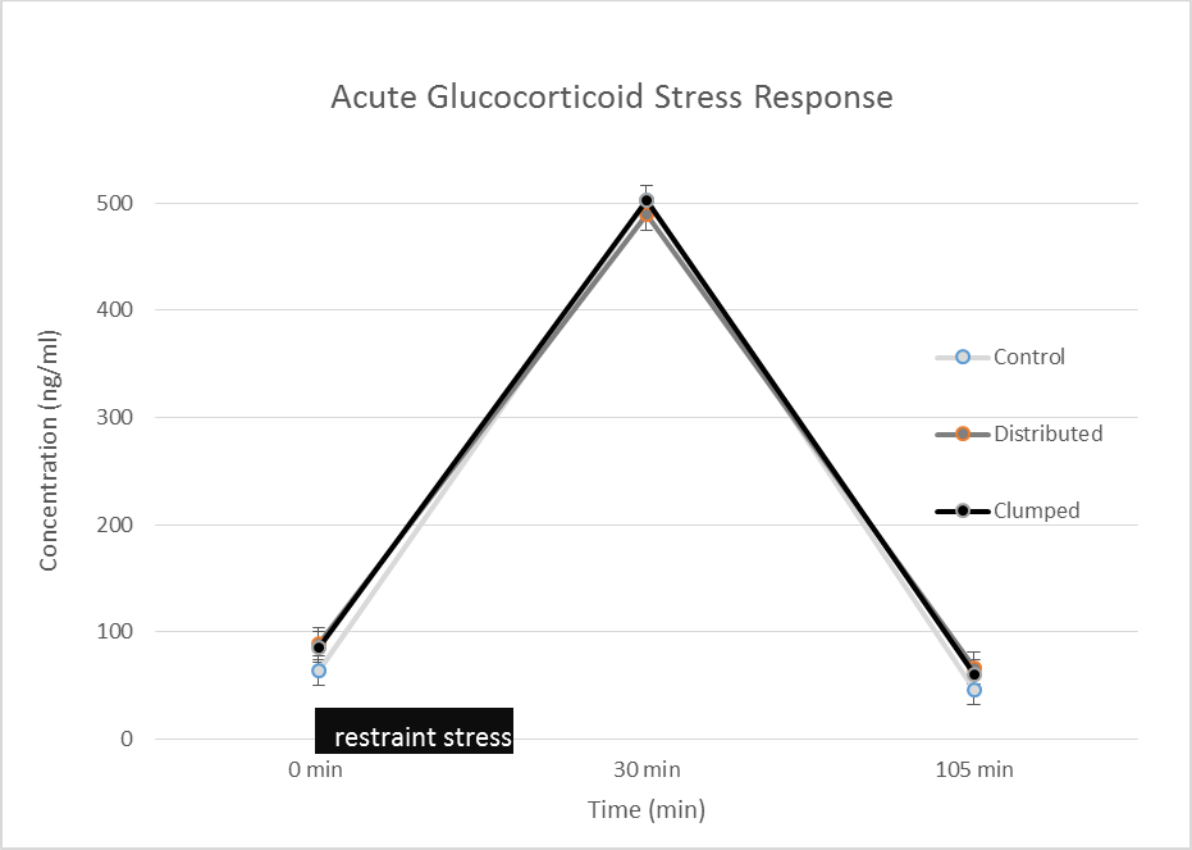

Figure 8: Acute Corticosterone stress response

Mean serum corticosterone concentration before, during and after acute restraint stress (15 min). Error bars represent S.E.M. There were no significant differences among the three groups at each time point.

Chapter 4

Discussion

Hypothesis and supporting results:

Rats that were exposed to daily acute stressors that all occurred within an hour (i.e. the clumped rats) drank 3% less sucrose water than rats that were not exposed to daily stressors. This same decrease in sucrose water consumption was not seen in rats that were exposed to the same stressors spread across the daily active period (i.e. the distributed rats). These results suggest that when regular daily stressors are experienced within a short period of time within the day lead to low-level anhedonia but that this does not occur when similar stressors are distributed over the day. Individuals facing multiple challenges in a short amount of time will have more difficulty coping and adjusting, increasing likelihood of developing mental disorders. Using human examples, a person who has to shopping for groceries, file taxes and pick up kids from school within a couple of hours is worse off (health wise) than a person that has the whole day to complete said objectives. These result are in line with hypothesis 2, stating that it is more detrimental to mental health if an animal experiences repeated multiple stressors in a short amount of time rather than spread out across the day.

Body weight data showed that clumped animals gained the least weight during CMS while distributed animals gained more weight than clumped animals but also less than the control animals. Impaired weight gain is often caused by reduced food intake or reduced appetite (12), suggesting that clumped grouped animals consumed the least amount of food compared than

their control and distributed counterpart. In humans, anorexia is often accompanied by symptoms of depression and the occurrence of depression in anorexia nervosa patients is often associated with negative outcomes (30). These results on weight gain also show support for hypothesis 2, suggesting that clumped rats have experienced more stress than the other groups, and this lead to more detrimental health outcomes.

There were no significant differences in elevated plus maze behavior and /or restraint stress and basal corticosterone levels among groups. These results support the null hypothesis, stating that the temporal dynamics of stress does not influence stress- and health-related physiological processes, however the treatment groups did not differ from the control group, and thus an alternative explanation is needed.

Results from both weight gain and sucrose preference test suggest that clumped animals experienced the most negative outcomes. These results support the speculation that there might be a short-term stress response threshold that must be reached to bring out the detrimental effects of chronic stress. Some researchers have proposed to view chronic stress as a continuum instead of a dichotomy and conditions that cause longer periods or more frequent stress response activation lead to more chronic stress-like responses (27). The timing of these stressors across the day has some influence on the overall severity of these stressors on health-related behavior and physiology. Acute stressors spread out over time may not cause the organism to reach a threshold, which might allow the effects of acute repeated stressors to dissipate before any long-term effects accumulate.

An alternative explanation might be found from the conservation of resources theory developed by Stevan E. Hobfoll (31). The theory views stress dynamics in an economy-like manner. Individual would gather resources, these resources can be physiological such as stored

fat psychological such as emotional support or a physical good such as money. To cope with a challenge, individual would use/consume previously stored resources. However once stored resources become depleted, negative health outcomes would soon follow (Such as burnout or depression). In the case of this study, clumped stressors is worse health wise because rats are consuming resources at a faster rate than the rat can replenish it as these rats are confronting stressors in a shorter amount of time.

In addition to the procedures reported in the methods section, rats also underwent lipopolysaccharide immune response. Due to time constraint, the innate inflammatory response to this immune challenge has not been analyzed yet. However, once analyzed, it may provide additional insight on the effects of stressor temporal dynamic on immune processes related to health. Brain tissue collected during necropsy has also not been analyzed and will undergo further analysis after completion of this thesis to determine if neurogenesis and/or neurotransmitter function was altered by clumped or distributed CMS.

Comparison to previous researches and study limitations:

Compared to previous CMS studies, one limitation of this study is that the protocol exposed rats to much shorter stressors. Initial concerns involved whether or not rats were actually experiencing significant levels of stress during the study. However, results from weight loss and sucrose preference test indicates that even with a milder stress protocol, the animals did experience some level of response to the protocol. The effects that we observed were in line with previous CMS studies in which animals show impaired weight gain and reduced sucrose intake (5) Compared to sucrose consumption results from other studies, treatment effects in the current

study were much smaller. However, as shown in the introduction, other studies that didn't employ a 20 hr food/water starvation period prior to post-CMS sucrose preference testing only showed small but significant differences between treatment and control groups. If a more severe CMS protocol was used, treatment group effects might have been more potent.

Previous research has shown that chronic mild stress does not greatly affect acute stress responses, but rather that CMS can influence basal corticosterone production (18, 23, 24). Our data follows a similar quadratic trend shown in the previous studies (low basal corticosterone values, high corticosterone levels 30 minutes following a challenge, and low corticosterone levels approximately 90 minutes after challenge completion). However, we did not find a difference in basal corticosterone levels among CMS and control rats. Furthermore, analysis of morning and evening corticosterone levels during CMS confirms that there were no differences in basal corticosterone levels across group. There are no other studies that have measured the effects of CMS on morning and evening corticosterone baseline levels. However from previous results on depressed human patients, which are thought to have similar symptoms as CMS rodents, morning corticosterone levels are usually suppressed while evening corticosterone levels are elevated (22). These results were not replicated in our study.

One potential explanation for this lack of difference might have been due to the frequent blood sampling that we employed in our protocol. We sampled blood every week, while most other studies usually collected blood twice, once at the beginning of the study an again at the end of the study. The frequent blood sampling protocol that we used might have masked treatment differences in corticosterone levels, as blood collection is considerably more stressful than the mild stressors that we used for the CMS protocol. During the last couple of blood sample collection, many animals showed considerable amounts of distress, struggling and squeaking

more compared to the first week of sampling. These responses were noted in control as well as treatment animal.

Typically after CMS treatment, animals usually show increased or decreased anxiety-like behavior (5,25). However, neither of these effects were seen in this study. Although older studies typically conducted CMS during the rat's dark (active) phase (as was done in our study), some of the more recent studies have shown that administrating CMS during the rat's light (inactive) phase is a more effective way of inducing depression-like and anxiety-like behavior (25). However, since prior studies indicate that sucrose preference is the behavioral response that is most often affected by CMS (5), our current lack of results from the elevated plus maze may not be surprising. In addition to anxiety-like measures, elevated plus maze can be used to examine locomotive activities, these locomotive activities can be used to measure circadian rhythm (26). Thus elevated plus maze locomotive data might help determine whether or not CMS caused a deviated circadian rhythm, which was not observed in the corticosterone measures.

Our study also only used male rats as subjects. Prior studies that included both female and male rats have shown that female rats are more susceptible to the chronic mild stress protocol (29). This may be a result of the fact that female rats tend to show a stronger corticosterone response and more behavioral change than male rats. If female rats are more susceptible to CMS and if the result of the current study hold true, we might expect that the effects of stress temporal dynamics might be better observed in female rats. For females, estrogen cycle disruption might also be used as a potential indicator of stress severity.

Implication and future directions:

When reducing workload is not an option , knowing that it might be more stressful to undergo multiple stressors in a short amount of time than having stressors distributed across a longer period of time might help managers and school administrators to plan activities and distribute work more effectively, reducing work related stress and reducing burnout. Future research directions should focus on determining whether there is a temporal threshold after which exposure to multiple, rapidly repeated acute stressors lead to symptoms of chronic stress.

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Appendix A

Study Events List

Title	Start(date and time)	End(date and time)
receive rats	03/18/2014 00:00	03/19/2014 00:00
weigh & handle	03/19/2014 00:00	03/24/2014 00:00
Weigh rats with Alexander and Mary after class	03/19/2014 09:30	03/19/2014 11:00
Weigh rats with Alexander and Jess	03/20/2014 10:00	03/20/2014 12:00
Weigh and handle rats	03/21/2014 10:00	03/21/2014 11:30
Handle/weight rats-RAC	03/22/2014 11:30	03/22/2014 13:00
weigh/handle rats	03/23/2014 13:00	03/23/2014 14:30
EPM testing	03/24/2014 00:00	03/27/2014 00:00
G1 EPM	03/24/2014 13:30	03/24/2014 17:00
SPT testing	03/25/2014 00:00	03/28/2014 00:00
G1 SPT	03/25/2014 09:00	03/25/2014 10:00
G2 EPM	03/25/2014 13:30	03/25/2014 17:00
G2 SPT	03/26/2014 09:00	03/26/2014 10:00
practice blood with rats	03/26/2014 09:30	03/26/2014 11:00
G3 EPM	03/26/2014 13:30	03/26/2014 17:30
NP testing	03/27/2014 00:00	03/30/2014 00:00
G3 SPT	03/27/2014 09:00	03/27/2014 10:00
practice blood with rats	03/27/2014 10:00	03/27/2014 11:30
NP G1	03/27/2014 13:00	03/27/2014 17:00
remove and record G3 SPT	03/28/2014 09:00	03/28/2014 10:00
CMS 'dry run'	03/28/2014 10:00	03/28/2014 12:00
NP G2	03/28/2014 12:00	03/28/2014 16:00
NP G3	03/29/2014 12:30	03/29/2014 16:30
NS testing	03/30/2014 00:00	04/02/2014 00:00
NS G1	03/30/2014 13:00	03/30/2014 17:00
NS G2	03/31/2014 13:30	03/31/2014 17:30
weigh rats	04/01/2014 00:00	04/02/2014 00:00
Remove SPT bottles	04/01/2014 09:00	04/01/2014 10:00
Weigh rats	04/01/2014 10:00	04/01/2014 11:30
NS G3	04/01/2014 13:00	04/01/2014 17:00
cage tilt(CT), Water immersion (WI), Noise (N),	04/02/2014 00:00	04/03/2014 00:00
CMS & blood	04/02/2014 00:00	04/05/2014 00:00
G1 Blood	04/02/2014 08:00	04/02/2014 10:00
CMS - cage tilt D1,cage tilt, water immersion, noise C1	04/02/2014 10:30	04/02/2014 12:00

CMS 2 - water immersion D1	04/02/2014 14:15	04/02/2014 15:45
CMS noise D1	04/02/2014 18:00	04/02/2014 19:30
G1 Blood	04/02/2014 21:00	04/02/2014 22:00
G1 rats weighed and into clean cages, spin blood	04/02/2014 22:00	04/02/2014 22:30
D groups get, strobe light (SL) AM, New Cage (NC) wet bedding (WB), PM	04/03/2014 00:00	04/04/2014 00:00
G2 Blood	04/03/2014 08:00	04/03/2014 09:30
CMS1 C2,D1-2	04/03/2014 10:00	04/03/2014 11:30
CMS2 C3,D1-2	04/03/2014 13:45	04/03/2014 15:15
CMS3 C1,D1-2	04/03/2014 17:30	04/03/2014 19:00
G2 Blood	04/03/2014 21:00	04/03/2014 22:00
G2 rats weighed and into clean caging, spin blood	04/03/2014 22:00	04/03/2014 22:30
Noise(N), Water Immerse(WI), Cage tilt (CT)	04/04/2014 00:00	04/05/2014 00:00
G3 Blood	04/04/2014 08:00	04/04/2014 09:00
CMS C3,D1-3	04/04/2014 09:30	04/04/2014 11:00
CMS2 C1,D1-3	04/04/2014 13:15	04/04/2014 15:15
CMS C2,D1-3	04/04/2014 16:45	04/04/2014 18:15
G3 Blood	04/04/2014 21:00	04/04/2014 22:00
G3 rats weighed and into clean caging	04/04/2014 22:00	04/04/2014 22:30
CMS	04/05/2014 00:00	04/29/2014 00:00
Wet Bedding (WB), Noise (N), Strobe Light (SL)	04/05/2014 00:00	04/06/2014 00:00
CMS1 C3,D1-3	04/05/2014 11:00	04/05/2014 12:30
CMS2 C2,D1-3	04/05/2014 14:45	04/05/2014 16:15
CMS3 C1,D1-3	04/05/2014 18:30	04/05/2014 20:00
Wet Bedding (WB), Cage Tilt (CT), Water Immerse (WI)	04/06/2014 00:00	04/07/2014 00:00
CMS C1,D1-3	04/06/2014 10:00	04/06/2014 11:30
CMS2 C2,D1-3	04/06/2014 13:45	04/06/2014 15:15
CMS3 C3,D1-3	04/06/2014 17:30	04/06/2014 19:00
Strobe light (SL), New Cage), Water Immerse, (WI)	04/07/2014 00:00	04/08/2014 00:00
CMS C2,D1-3	04/07/2014 09:00	04/07/2014 10:30
CMS C1,D1-3	04/07/2014 12:45	04/07/2014 14:15
CMS C3,D1-3	04/07/2014 16:30	04/07/2014 18:00
Wet Bedding (WB), New Cage (NC), Noise (N)	04/08/2014 00:00	04/09/2014 00:00
blood	04/08/2014 00:00	04/11/2014 00:00
Blood G1	04/08/2014 08:00	04/08/2014 09:30
CMS C3,D1-3	04/08/2014 10:30	04/08/2014 12:00
CMS C2,D1-3	04/08/2014 14:15	04/08/2014 15:45
CMS C1,D1-3	04/08/2014 18:00	04/08/2014 19:30
Blood G1	04/08/2014 21:00	04/08/2014 22:00
Weigh rats & into clean caging	04/08/2014 22:00	04/08/2014 22:30
WI, S,CT	04/09/2014 00:00	04/10/2014 00:00
SPT	04/09/2014 00:00	04/12/2014 00:00

Blood G2	04/09/2014 08:00	04/09/2014 09:30
CMS C1,D1-3	04/09/2014 10:00	04/09/2014 11:30
CMS C3,D1-3	04/09/2014 13:45	04/09/2014 15:15
CMS C2,D1-3	04/09/2014 17:30	04/09/2014 19:00
SPT G1 bottles on	04/09/2014 20:00	04/09/2014 21:00
Blood G2	04/09/2014 21:00	04/09/2014 22:00
Weigh rats & into clean caging	04/09/2014 22:00	04/09/2014 22:30
SPT G1 water bottles OFF	04/09/2014 22:30	04/09/2014 23:00
N,NC,WI	04/10/2014 00:00	04/11/2014 00:00
Blood G3	04/10/2014 08:00	04/10/2014 09:30
CMS C2,D1-3	04/10/2014 09:30	04/10/2014 11:00
CMS C3,D1-3 & handle control rats	04/10/2014 13:15	04/10/2014 14:45
CMS C1,D1-3	04/10/2014 17:00	04/10/2014 18:30
SPT G2 water bottles on	04/10/2014 20:00	04/10/2014 21:00
Blood G3	04/10/2014 21:00	04/10/2014 22:00
Weigh rats & into clean caging	04/10/2014 22:00	04/10/2014 22:30
SPT G2 water bottles OFF	04/10/2014 22:30	04/10/2014 23:00
CT, S, WB	04/11/2014 00:00	04/12/2014 00:00
CMS C3,D1-3	04/11/2014 09:00	04/11/2014 10:30
CMS C2,D1-3 and handle control rats	04/11/2014 12:45	04/11/2014 14:15
CMS C1,D1-3	04/11/2014 16:30	04/11/2014 18:00
SPT G3 water bottles on	04/11/2014 20:00	04/11/2014 21:00
SPT G3 water bottles OFF	04/11/2014 22:30	04/11/2014 23:00
NC, WI, CT	04/12/2014 00:00	04/13/2014 00:00
CMS C1,D1-3	04/12/2014 11:00	04/12/2014 12:30
CMS2 (ADB,NRC) C3 and handle control rats	04/12/2014 14:45	04/12/2014 16:15
CMS C2,D1-3	04/12/2014 18:30	04/12/2014 20:00
N,WB,S	04/13/2014 00:00	04/14/2014 00:00
CMS C1,D1-3	04/13/2014 11:30	04/13/2014 13:00
CMS C2,D1-3 and handle control rats	04/13/2014 15:30	04/13/2014 17:00
CMS C3,D1-3	04/13/2014 19:30	04/13/2014 21:00
NC,WI, CT	04/14/2014 00:00	04/15/2014 00:00
CMS C3	04/14/2014 09:30	04/14/2014 11:00
CMS2 C1 and handle control rats	04/14/2014 13:30	04/14/2014 15:00
CMS 3 C2	04/14/2014 17:30	04/14/2014 19:00
blood	04/15/2014 00:00	04/18/2014 00:00
WB, N, S	04/15/2014 00:00	04/16/2014 00:00
Blood	04/15/2014 08:00	04/15/2014 09:30
CMS C2	04/15/2014 09:30	04/15/2014 11:00
CMS2, handle control rats	04/15/2014 13:30	04/15/2014 15:00
CMS 3 Alex Mary C3	04/15/2014 17:30	04/15/2014 19:00
Blood	04/15/2014 21:00	04/15/2014 22:30

Weigh rats & into clean caging	04/15/2014 22:30	04/15/2014 23:00
SPT	04/16/2014 00:00	04/19/2014 00:00
WI, CT, N	04/16/2014 00:00	04/17/2014 00:00
Blood	04/16/2014 08:00	04/16/2014 09:30
CMS C2	04/16/2014 10:00	04/16/2014 11:30
CMS2 C3 and handle control rats	04/16/2014 14:00	04/16/2014 15:30
CMS 3 C1 Mary and Andrew	04/16/2014 18:00	04/16/2014 19:30
SPT G1 bottles on	04/16/2014 20:00	04/16/2014 21:00
Blood	04/16/2014 21:00	04/16/2014 22:30
SPT G1 water bottles OFF	04/16/2014 22:00	04/16/2014 22:30
Weigh rats & into clean caging	04/16/2014 22:30	04/16/2014 23:00
S, WB, NC	04/17/2014 00:00	04/18/2014 00:00
Blood	04/17/2014 08:00	04/17/2014 09:30
CMS Alex Mary C3	04/17/2014 11:00	04/17/2014 12:30
CMS2 C2 and handle control rats	04/17/2014 15:00	04/17/2014 16:30
CMS 3 C1	04/17/2014 18:30	04/17/2014 20:00
SPT G2 water bottles on	04/17/2014 20:00	04/17/2014 21:00
Blood	04/17/2014 21:00	04/17/2014 22:30
SPT G2 water bottles OFF	04/17/2014 22:00	04/17/2014 22:30
Weigh rats & into clean caging & clean 4 additional cages (extra rats & sentinel)	04/17/2014 22:30	04/17/2014 23:00
CT, N, WB	04/18/2014 00:00	04/19/2014 00:00
CMS C1	04/18/2014 09:30	04/18/2014 11:00
CMS2 C3 and handle control rats	04/18/2014 13:30	04/18/2014 15:00
CMS 3 C2	04/18/2014 17:30	04/18/2014 19:00
SPT G3 water bottles on	04/18/2014 20:00	04/18/2014 21:00
SPT G3 water bottles OFF	04/18/2014 22:00	04/18/2014 22:30
NC, S, WI	04/19/2014 00:00	04/20/2014 00:00
CMS Alex Tierra C2	04/19/2014 11:30	04/19/2014 13:00
CMS C1,D1-3 and handle control rats	04/19/2014 15:30	04/19/2014 17:00
CMS C3,D1-3	04/19/2014 19:30	04/19/2014 21:00
N, WI, CT	04/20/2014 00:00	04/21/2014 00:00
CMS Alex Mary C2	04/20/2014 11:30	04/20/2014 13:00
CMS C3,D1-3 and handle control rats	04/20/2014 15:15	04/20/2014 16:45
CMS 3 C1	04/20/2014 19:00	04/20/2014 20:30
WB, NC, S	04/21/2014 00:00	04/22/2014 00:00
CMS C3	04/21/2014 09:30	04/21/2014 11:00
CMS2 C2 and handle control rats	04/21/2014 13:15	04/21/2014 14:45
CMS 3, C1	04/21/2014 17:00	04/21/2014 18:30
N, CT, WB	04/22/2014 00:00	04/23/2014 00:00
blood	04/22/2014 00:00	04/25/2014 00:00
Blood x3	04/22/2014 08:00	04/22/2014 09:30

CMS Alex Mary C1	04/22/2014 11:00	04/22/2014 12:30
CMS2, C3 and handle control rats	04/22/2014 14:45	04/22/2014 16:15
CMS 3, C2	04/22/2014 18:30	04/22/2014 20:00
Blood	04/22/2014 21:00	04/22/2014 22:00
Weigh rats & into clean caging	04/22/2014 22:00	04/22/2014 22:30
SPT	04/23/2014 00:00	04/26/2014 00:00
WI, S, NC	04/23/2014 00:00	04/24/2014 00:00
Blood x3	04/23/2014 08:00	04/23/2014 09:30
SPT G2 remove & weigh bottles	04/23/2014 08:30	04/23/2014 09:00
CMS C3	04/23/2014 10:00	04/23/2014 11:30
CMS2 C1 and handle control rats	04/23/2014 13:45	04/23/2014 15:15
CMS 3 C2	04/23/2014 17:30	04/23/2014 19:00
SPT G1 bottles on	04/23/2014 20:00	04/23/2014 21:00
Blood x3	04/23/2014 21:00	04/23/2014 22:00
Weigh rats & into clean caging	04/23/2014 22:00	04/23/2014 22:30
NC, WB, CT	04/24/2014 00:00	04/25/2014 00:00
Blood x3	04/24/2014 08:00	04/24/2014 09:30
SPT G1 water bottles OFF	04/24/2014 09:00	04/24/2014 09:30
CMS C2	04/24/2014 09:45	04/24/2014 11:15
CMS2 C1 and handle control rats	04/24/2014 13:30	04/24/2014 15:00
CMS 3 C3	04/24/2014 17:00	04/24/2014 18:30
SPT G2 water bottles on	04/24/2014 20:00	04/24/2014 21:00
Blood x3	04/24/2014 21:00	04/24/2014 22:00
Weigh rats & into clean caging	04/24/2014 22:00	04/24/2014 22:30
S, WI, N	04/25/2014 00:00	04/26/2014 00:00
SPT G2 water bottles OFF	04/25/2014 09:00	04/25/2014 09:30
CMS C1	04/25/2014 09:30	04/25/2014 11:00
CMS2, C3 and handle control rats	04/25/2014 13:15	04/25/2014 14:45
CMS 3, C2	04/25/2014 17:00	04/25/2014 18:30
SPT G3 water bottles on	04/25/2014 20:00	04/25/2014 21:00
CT, NC, WB	04/26/2014 00:00	04/27/2014 00:00
SPT G3 water bottles OFF	04/26/2014 09:00	04/26/2014 09:30
CMS C3	04/26/2014 11:30	04/26/2014 13:00
CMS2 C1 and handle control rats	04/26/2014 15:15	04/26/2014 16:45
CMS 3 C2	04/26/2014 19:00	04/26/2014 20:30
N, S, NC	04/27/2014 00:00	04/28/2014 00:00
CMS C2	04/27/2014 10:30	04/27/2014 12:00
CMS2 C1 and handle control rats	04/27/2014 14:15	04/27/2014 15:45
CMS 3 C3	04/27/2014 18:00	04/27/2014 19:30
CMS & blood	04/28/2014 00:00	05/01/2014 00:00
WB, CT, N	04/28/2014 00:00	04/29/2014 00:00
Blood G1	04/28/2014 08:00	04/28/2014 09:30

CMS C1	04/28/2014 09:30	04/28/2014 11:00
CMS2 C2 and handle control rats	04/28/2014 13:30	04/28/2014 15:00
Get SPT bottles ready as well as cages for changing	04/28/2014 15:00	04/28/2014 16:00
CMS 3 C3	04/28/2014 17:30	04/28/2014 19:00
Blood G1	04/28/2014 21:00	04/28/2014 22:00
Weigh rats	04/28/2014 22:00	04/28/2014 22:30
SPT G1 bottles on	04/28/2014 22:30	04/28/2014 23:00
NC, WI, S	04/29/2014 00:00	04/30/2014 00:00
Blood G2	04/29/2014 08:00	04/29/2014 09:30
CMS G2&3 only, C3,D2+3	04/29/2014 10:45	04/29/2014 12:15
CMS2 G 2 &3 only C1 and handle control rats	04/29/2014 14:30	04/29/2014 16:00
Label LPS tubes	04/29/2014 16:00	04/29/2014 17:00
CMS 3 C2 G2 and G3 only	04/29/2014 18:30	04/29/2014 20:00
SPT G1 bottles off	04/29/2014 20:30	04/29/2014 21:00
blood G2	04/29/2014 21:00	04/29/2014 22:00
Weigh rats & into clean caging	04/29/2014 22:00	04/29/2014 22:30
SPT G2 water bottles on	04/29/2014 22:30	04/29/2014 23:00
S, N, CT	04/30/2014 00:00	05/01/2014 00:00
LPS x2 save tail tips for DNA	04/30/2014 00:00	05/03/2014 00:00
Blood G3	04/30/2014 08:00	04/30/2014 09:30
LPS G1	04/30/2014 09:30	04/30/2014 20:30
CMS IMK C3 G3 only	04/30/2014 10:30	04/30/2014 12:00
CMS G3 only, C2,D2-3 and handle control rats	04/30/2014 14:00	04/30/2014 15:30
CMS G3 only	04/30/2014 17:45	04/30/2014 19:15
SPT G2 water bottles OFF	04/30/2014 20:30	04/30/2014 21:00
Blood G3	04/30/2014 21:00	04/30/2014 22:00
Weigh rats & into clean caging	04/30/2014 22:00	04/30/2014 22:30
SPT G3 water bottles on	04/30/2014 22:30	04/30/2014 23:00
LPS G2	05/01/2014 09:30	05/01/2014 20:30
SPT G3 water bottles OFF	05/01/2014 20:30	05/01/2014 21:00
LPS G3	05/02/2014 09:30	05/02/2014 20:30
acute CORT response to restraint (blood collection + 30 minute restraint)	05/03/2014 00:00	05/06/2014 00:00
restraint stress & blood G1	05/03/2014 17:00	05/03/2014 20:00
EPM testing	05/04/2014 00:00	05/07/2014 00:00
EPM G1	05/04/2014 13:00	05/04/2014 17:00
restraint stress & blood G2	05/04/2014 17:00	05/04/2014 20:00
EPM G2	05/05/2014 13:00	05/05/2014 16:00
restraint stress & blood G3	05/05/2014 17:00	05/05/2014 20:00
EPM G3	05/06/2014 13:00	05/06/2014 16:00
NP testing	05/07/2014 00:00	05/10/2014 00:00
NP G1 & into new cages	05/07/2014 13:00	05/07/2014 17:00

NP G2 & into new cages	05/08/2014 13:00	05/08/2014 17:00
NP G3 & into new cages	05/09/2014 13:00	05/09/2014 17:00
NS & weigh G1	05/10/2014 13:00	05/10/2014 17:00
NS testing	05/11/2014 00:00	05/14/2014 00:00
NS & weigh G2	05/12/2014 13:00	05/12/2014 17:00
NS & weigh G3	05/13/2014 13:00	05/13/2014 17:00
Change all rat cages	05/14/2014 13:00	05/14/2014 15:30
Change rat cages & weigh rats	05/21/2014 10:00	05/21/2014 12:00
Top off food + water for all rats	05/29/2014 14:00	05/29/2014 15:00
label/fill necropsy tubes without ID	05/29/2014 14:00	05/29/2014 17:00
sac (brains: neurogenesis, blood: baseline CORT & telomeres, adrenals)	06/05/2014 00:00	06/08/2014 00:00
necropsies - perfusion	06/05/2014 13:30	06/05/2014 17:30
necropsies - perfusion	06/06/2014 13:00	06/06/2014 18:00
necropsies - perfusion	06/07/2014 13:00	06/07/2014 18:00
necropsies - perfusion	06/09/2014 13:00	06/09/2014 18:00
necropsies - perfusion	06/10/2014 13:00	06/10/2014 18:00
necropsies - perfusions	06/11/2014 13:00	06/11/2014 18:00

ACADEMIC VITA

Alexander Bao
Permanent Address: 222 Qian Yun Rd, #71, Shanghai, China
School Address: 125 Banffshire Heights, State college, PA, 16803
Adb5400@psu.edu

Education:

The Pennsylvania State University
Major: Pre-medicine
Minor: Psychology

Research work experience:

Behavioral Neuroendocrinology Lab, State college, USA, 2012-2015
Work regular lab shifts and special hour shifts through the day to accommodate animal
Rodent handling including anesthetic, health check, injection, euthanizing, blood sampling, feces collection and autopsy
Experiment design and scheduling under mentor guidance.
Statistical analysis

Tiger Med, pharmaceutical company, Shanghai, China, June2013-August2013
English tutor
Help translate written documents

Professional experiences:

SunPointe Health, State College, PA, 2014- 2015
- Shadow Psychiatrists

Hershey Career Observation, **State College, PA, March2015-April2015**
-shadow a verity of medical specialties.

Honors:

Dean's list from 2011-2014
Recipient of Ruth E. Duffy Premedicine Endowment year 2013-2014
Schreyer Honors College Ambassador Travel Grant, 2014

Club membership:

Penn State Taiko Club.
-Art Designer