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The Persistent Behavior And Physiological Effects Of Stress

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THE PERSISTENT BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF STRESS

By

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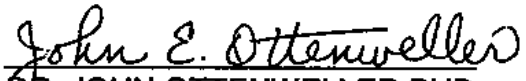

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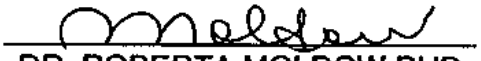

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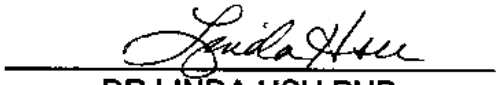

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Abstract

Stress can persistently affect the physiology and behavior of the rat. Here we addressed whether exposure to inescapable stress persistently affects adrenocortical function and the circadian rhythms of core body temperature and activity. Rats were outfitted with telemeters and placed in either a shoebox cage or a metabolic cage for urine collection (depending on the experimental protocol), thereby allowing non-invasive measurements of urinary corticosterone (CORT), temperature and activity. In Experiment 1, rats were housed in shoebox cages and exposed to 3 daily sessions of tailshock stress. In Experiment 2, rats were housed in metabolic cages and exposed to 3 daily sessions of stress. Finally, in Experiment 3, rats were exposed to only one day of stress while being housed in metabolic cages. The results demonstrate that stress consistently leads to persistent elevations in core body temperature, decreased behavioral activity and increased urinary CORT. Moreover, exposure to a single traumatic stressor leads to similar if not more persistent changes in the variables measured. These experiments demonstrate the utility of these measures for monitoring chronic stress responses and suggest that complex interactions among these respondents may govern persistent responses to stress.

Introduction

Overview:

Stress is a psychophysiological process that involves the mobilization of the body to respond to any situation that the brain deems to be a threat. The acute physiological responses to stress including, increased heart rate, increased glucocorticoids and increased epinephrine has been well documented over the past 50 years. Only in recent years, has the documentation of the lingering or chronic effects of stress gained interest in the manifestation of and susceptibility to disease.

Studying stress can be relatively problematic because most of the assessment techniques such as blood collection tend to be invasive, hence leading to more stress. The intention of this research is to attempt to measure the persistent or chronic effects of stress through biological markers that are actually non-invasive such as core body temperature, behavioral activity and urinary corticosterone levels.

Stress: An Introduction:

Stress is the generalized response of the body to any factor or condition that overwhelms or threatens the body's compensatory mechanism to maintain homeostasis (Sherwood, 1997). It is a subject that has been the focus of a great deal of research since Hans Selye's pioneering work in 1936 where he suggested that stress is the non-specific response of the body to any demand made upon it (Selye, 1936). A stressor is what causes the stress response, no

matter if it is pleasant or noxious; what matters is the intensity and the body's need for readjustment (Selye, 1973).

Selye's work talks of a generalized reaction model to stress known as the General Adaptation Reaction. In this model there are three stages. The first stage, known as the General Alarm Reaction, occurs within 6-48 hours after a severe stressor exposure. During this period, there is a decrease in size of the thymus, spleen, lymph nodes, liver and fat tissue. There is also a loss of muscle tone, decrease in body temperature, erosion of the gastric lining and edema. The second stage occurs 48 hours after stressor exposure and this stage is defined by an enlarging of the adrenal glands, a decrease in edema, an increase in thyroid size, a decrease in growth hormone and gonadotropin, and a decrease in milk production. The final stage occurs only if the stress continues and is defined as a state of exhaustion (Selye, 1946).

It has been suggested (Levine and Ursin, 1991) that stress is an integral part of an adaptive biological system. Both behavioral and physiological responses to a stressor are required by both animals and humans to exist in a frequently changing environment. Complete freedom from stressors would lead to death (Selye, 1973).

In an attempt to define stress, researchers have come up with a definition composed of three components. These are the input (the stressor), the processing system and the output (stress response) (Levine and Ursin, 1991). The stress response can be further defined as described by Selye's General

Adaptation Model or simply as an acute and chronic response to stress (Ursin and Olff, 1993). On an acute level, there may be certain physiological changes such as an increase in heart rate, an increase in blood pressure, an increase in respiration, pupillary dilation, pallor and perspiration. This response may be generalized as the "flight or fright" response (Cannon, 1914; Cannon and Paz, 1911). On the chronic side, release of slower acting hormones such as cortisol may act to dampen the acute response and return the organism to its physiological norm (Ursin and Olff, 1993). Chronic stress states also place strain on the physiological systems that maintain homeostasis and lead to "chronic wear and tear" (McEwen and Stellar, 1993). Additionally, chronic exposure to stress leads to increases in glucocorticoids, which in turn may lead to neuronal death of cells within the brain, specifically the hippocampus (Sapolsky, 1996).

Adaptation to stressors can occur on three levels: nervous, immunological and hormonal (Selye, 1973). This adaptation is no longer referred to as homeostasis, but rather heterostasis (Selye, 1973) or allostasis (McEwen and Stellar, 1993) since after stressor challenge the body's physiological steady state balance has been changed. Almost all stress responses are mediated by the hypothalamus (Sherwood, 1997) or more specifically the Hypothalamic-Pituitary-Adrenal Axis (HPA-axis) (Willner, 1993) and the sympathetic nervous system (Khansari, et al., 1990). Khansari et al. (1990) also suggested that most if not all neuroendocrine functions are affected by stressors. Both the sympathoadrenal

and HPA axes are activated which leads to a negative effect on the immune system (Hiramoto, et al., 1999).

It is difficult to prove if stressful situations have direct negative effects on the organism. Hence, some researchers have resorted to an animal model to look at the pathophysiological effects of repeated stressor exposure (Pitman, et al., 1988; Ottenweller, et al., 1989; Ottenweller, et al., 1992). Recently, a disorder known as Posttraumatic Stress Disorder (PTSD) has been identified as a behavior or syndrome linked to extreme stressor exposure (McEwen and Stellar, 1993). PTSD has been found in up to 58% of individuals exposed to traumatic events and maintains characteristic symptoms such as hypervigilance and exaggerated startle for extended periods of time (APA, 1994). PTSD sufferers also have been found to have increased catecholamine/cortisol ratios (McEwen and Stellar, 1993).

The Endocrine Response to Stress:

When an individual or organism is exposed to a stressor, there are prominent responses in two physiological systems. On one hand, the sympathetic response can occur which leads to dilation of pupils, an increase in heart rate and constriction of blood vessels (Zigmond, et al., 1995). This response is mediated by epinephrine a catecholamine that is released from the adrenal medulla. Epinephrine can either act as a potent vasoconstrictor through its own action or secondarily through the renin-angiotensin-aldosterone pathway. It also can affect the pancreas resulting in an increase in glucagon and a

decrease in insulin (Sherwood, 1997). Both glucagon and insulin work in conjunction to increase and mobilize the glucose stores during stressor exposure.

Alternatively, is the response of glucocorticoids mediated through the Hypothalamic-Pituitary-Adrenal Axis. Within this pathway are a number of key players that act to mediate the endocrine response to stress. Stress, whether emotional or physical, causes the hypothalamus to release Corticotrophin Releasing Hormone (CRH). This hormone is secreted at the medial eminence of the hypothalamus from axons whose cell bodies are located in the paraventricular nucleus (PVN) of the hypothalamus. It then travels through the hypophyseal portal vessels to the anterior pituitary (Cullinan, et al., 1995). Numerous afferent fibers from the various parts of the brain converge at the PVN. Fibers from the amygdaloid nuclei mediate emotional, fearful and anxiety stressors. Additionally, nocioceptive pathways mediate painful stressors. When activated all of these afferent neurons lead to an increase in CRH levels. These stimulatory factors are counteracted by negative feedback within this system due to circulating levels of glucocorticoids (Ganong, 1995). Increases in CRH trigger an increase in adrenocorticotropin (ACTH) from the anterior pituitary (Axelrod and Reisine, 1984). When ACTH is released from the anterior pituitary, it travels to the adrenal cortex where it facilitates the release of cortisol. Cortisol's main purpose is proactive in that fat and protein stores are broken down while carbohydrate stores increase leading to the increased availability of glucose. In

the mouse and rat, the main glucocorticoid that plays the same role as cortisol is corticosterone (Marti and Amario, 1998). In order to measure the stress response in organisms, researchers often use either cortisol (in humans) (Mason, et al., 1986) or corticosterone (in rats) (Kley, et al., 1978).

ACTH has been proposed to be a hormone that can reflect the intensity of the stress. In other words, the greater the stress the greater the rise in ACTH (Marti and Amario, 1998). Until relatively recently the concept of habituation to a stressor has not gained interest. Marti and Amario in 1998 also suggested that through repeated stressor exposure ACTH responses are found to decrease.

Hayden-Hixson and Nemeroff in 1993 suggested that there exists another CRH system other than that located in the PVN and at the medial eminence of the hypothalamus. They suggested that this CRH system consists of diffuse neuronal circuits located in the hypothalamic, limbic, neocortical and brainstem areas which are activated by stress but play a non-endocrine role. This means that the CRH from these areas does not directly travel to the pituitary.

CRH plays two roles in the response to stress. The endocrine CRH response to stress (through the HPA axis) controls most of the peripheral responses to a stressor, whereas the non-endocrine CRH largely mediates the central response to a stressor. Different theories suggest that CRH actually plays the primary role in integrating the whole organism's response to stress or resisting the effects of stress (Munck, et al., 1984) as well as a belief that the

interaction between CRH and opioid peptides underlie successful adaptation to stress (Hayden-Hixson and Nemeroff, 1993).

Since CRH has been demonstrated to play a role in the stress response, drug development research has attempted to block the CRH adenylate cyclase activity which thereby decreases ACTH release (Schulz, et al., 1996; Arvanitis, et al., 1999). Unfortunately these CRH antagonists are peptides that can not be administered peripherally and therefore, they had no therapeutic usefulness. However, recently, CP-154,526 or Antalarmin has been discovered which is a selective non-peptide antagonist of CRH receptors. It can be administered peripherally and it crosses the blood brain barrier to block the actions of CRH in the brain. In animals, it can be given intraperitoneally and has been shown to block the increase in ACTH levels produced by a mild stressor exposure (Deak, et al., 1999).

The Thermal Response to Stress:

It has been suggested that changes in core body temperature is a sensitive marker for the acute response to stress (Deak, et al., 1997). There is also evidence that changes in circadian rhythms may be responsible for numerous affective disorders (Meerlo, et al., 1996). When a rat is exposed to stress there is an increase in core body temperature (Kluger, et al., 1987; Gordon, 1990; Long, et al., 1990; Briese and Cabanac, 1991; Kant, et al., 1991; Gordon, 1993; Harper, et al., 1996; Meerlo, et al., 1996; Deak, et al., 1997; Meerlo, et al., 1997; Dymond and Fewell, 1998). This effect has also been found

in mice (Groenink, et al., 1993; Zethof, et al., 1994) and humans (Marazziti, et al., 1992).

Research has shown that exposure to inescapable stress versus escapable stress leads to pronounced differences in the temperature changes due to stress. Essentially, those exposed to inescapable stress exhibit more persistent changes to their temperature (Kant, et al., 1991). This rise in temperature has been classified as a fever. Briese and Cabanac (1991) found that this increase in temperature was due to an increase in the thermoregulatory set point, not an increase in activity. Moreover, they found that the ambient temperature did not alter the fever (Briese and Cabanac, 1991; Long, et al., 1990). Kluger and O'Reilly (1987) further clarified that the stress-induced hyperthermia was in fact a fever by intracerbroventricular administration of sodium salicylate and intraperitoneal injection of indomethacin. Both of these drugs clearly attenuated the fever response. Furthermore, it has been discovered that these thermoregulatory changes are in fact not due to changes in circadian pacemaker function (Meerlo, et al., 1997) and in part may result from the thermogenic effects of CRH (Rothwell, 1990; Rothwell, et al., 1991; Morimoto, et al., 1993).

The Behavioral Effects of Stress:

Behaviorally, stress in humans may lead to a withdrawn character as well as a decrease in activity (APA, 1994). In the laboratory, measurement of activity in rats can be done in numerous ways, most predominantly with running wheel

activity (Desan, et al., 1988). However, the radiotelemetric recording devices used for temperature recording also have the ability to measure activity. Researchers have demonstrated with social defeat stress that activity is decreased after stressor exposure, but these are not persistent effects (Meerlo, et al., 1996; Meerlo, et al., 1996; Meerlo, et al., 1997).

Stress Effects on Urine:

In the clinical setting, assessment of adrenal function in Post Traumatic Stress Disorder (PTSD) has been performed by blood samples of cortisol. However, more recently, analysis of the urine has also been found to be as effective (Mason, et al., 1986). Assay of urinary cortisol is a powerful clinical tool to evaluate adrenal function (Kley, et al., 1978). Bitter and Neilsen (1972) looked at urinary corticosterone levels in response to stress in the rat. They found that this measure can assess the response to stressor exposure. Additional research has suggested that urinary corticosterone in stressed animals is elevated immediately after stressor exposure, as well as for several days afterwards (Brennan, 2000).

The Purpose:

In animal stress research, there is often a discrepancy between the organism's delicately balanced physiological processes and the crude techniques used to study them (DeBoer, 1990). The following research attempts to study the physiological and behavioral effects of the stress response without causing additional stress. There are three experiments that are included. The first

experiment looks at the persistent effects of three days of stressor exposure on temperature and activity. Since temperature has been suggested to be a good marker of the acute stress response, we wanted to look at its ability to assess the persistent effects. The second experiment looks at the persistent effects of three days of stressor exposure on temperature, activity and urinary corticosterone levels. The inclusion of urinary corticosterone levels allows us to see how the stressor effects the secretion of corticosterone and if there are persistent effects. None of the prior literature has ever measured these three variables (temperature, activity and urinary corticosterone) together. The second experiment also attempted to use one of the non-peptide CRH antagonists in order to block the endocrine response to stress. Finally, experiment three involves one day of stressor exposure. In this experiment, the persistent effects of a single stressor exposure on body temperature, activity and urinary corticosterone were assessed to determine if they were similar to those after three stressor exposures.

Finally, this research studies the chronic effects of multiple stressors or a single traumatic stressor. Since Post Traumatic Stress Disorder (PTSD) is attributed to multiple exposures to stressors or a single very traumatic stressor, it is appropriate to see how this research attempts to model this devastating disorder. Perhaps by understanding the physiology behind PTSD, we can develop treatments to ameliorate the symptoms.

Materials & Methods

Experiment 1: *Persistent Changes In Body Temperature and Activity After 3 Stressor Exposures*

A. Animals

Thirty-five male Sprague-Dawley rats from Charles River (Wilmington, DE) were housed individually in shoebox cages (8" x 16" x 8"). Rats were allowed free access to Lab Diet 5001 Pellet Rodent Chow and tap water. They were maintained on a 12-hour photoperiod with light onset at 8:00 am. The rats were kept under these conditions for 4 weeks before the start of the experiment.

B. Procedures

At the start of the experiment rats were ranked by body weight (body weights ranged from 365-500g) and then randomly assigned to two groups: those that did not receive stress (n=14), the non-stress group, and those that received three days of chronic stress (n=21), the stress group.

Upon assignment to groups, rats were anesthetized with 70% Nembutal and implanted intraperitoneally with telemeters obtained from Data Science International (St. Paul, MN) to record core body temperature and activity. A small incision was made in the abdomen and the transmitters were implanted. The abdominal wall was sutured with 3-0 chromic gut, and the skin was stapled with surgical staples. After surgery was completed, rats were allowed to recover for one week prior to data acquisition. The shoebox cages rested on bases that recorded the signal from the implanted transmitters.

Baseline temperature and activity recordings were obtained every 2 minutes from the rats for four days prior to the initiation of the stress protocol. The stress rats were housed in different quarters than the non-stress group. The stress group was exposed to stress on three consecutive days. For the stress group, stress was initiated at the same time each day (11:00 am). Stress rats were removed from their home cages and brought to the stress room where they were restrained in plastic tubes and tail electrodes were attached. Once secured, 2-mA constant current shocks (166 msec out of every 200 msec) were given for 3 seconds every 3 minutes until 40 shocks had been delivered (approximately 2 hours). The stress protocol was the same as outlined by Ottenweller, et al. (1989) and was approved by the Institutional Animal Care and Use Committee at the VA New Jersey Healthcare Center in East Orange, New Jersey. After the stress session, the tail electrodes were removed and the rats were returned to their home cages. Also, during the stress session control rats were removed from their cages and transferred to another room. They were returned to their home cages at the same time the stress animals were returned to their cages.

The stress regimen outlined above was repeated for three consecutive days. Upon the conclusion of the stress days, animals remained in their shoebox cages for 10 days. During this time activity and temperature were constantly logged every 2 minutes.

C. Data Analysis

In order for data to be processed, it had to first be reduced. For each animal 720 data points were collected per day for both temperature and activity. This totaled approximately 10,000 data points for each animal for temperature (Figures 1a and 1b). The data were filtered using the software Lowess with a filtering value of f=10/length of the file and a delta of 0 and iterations equal to 1.

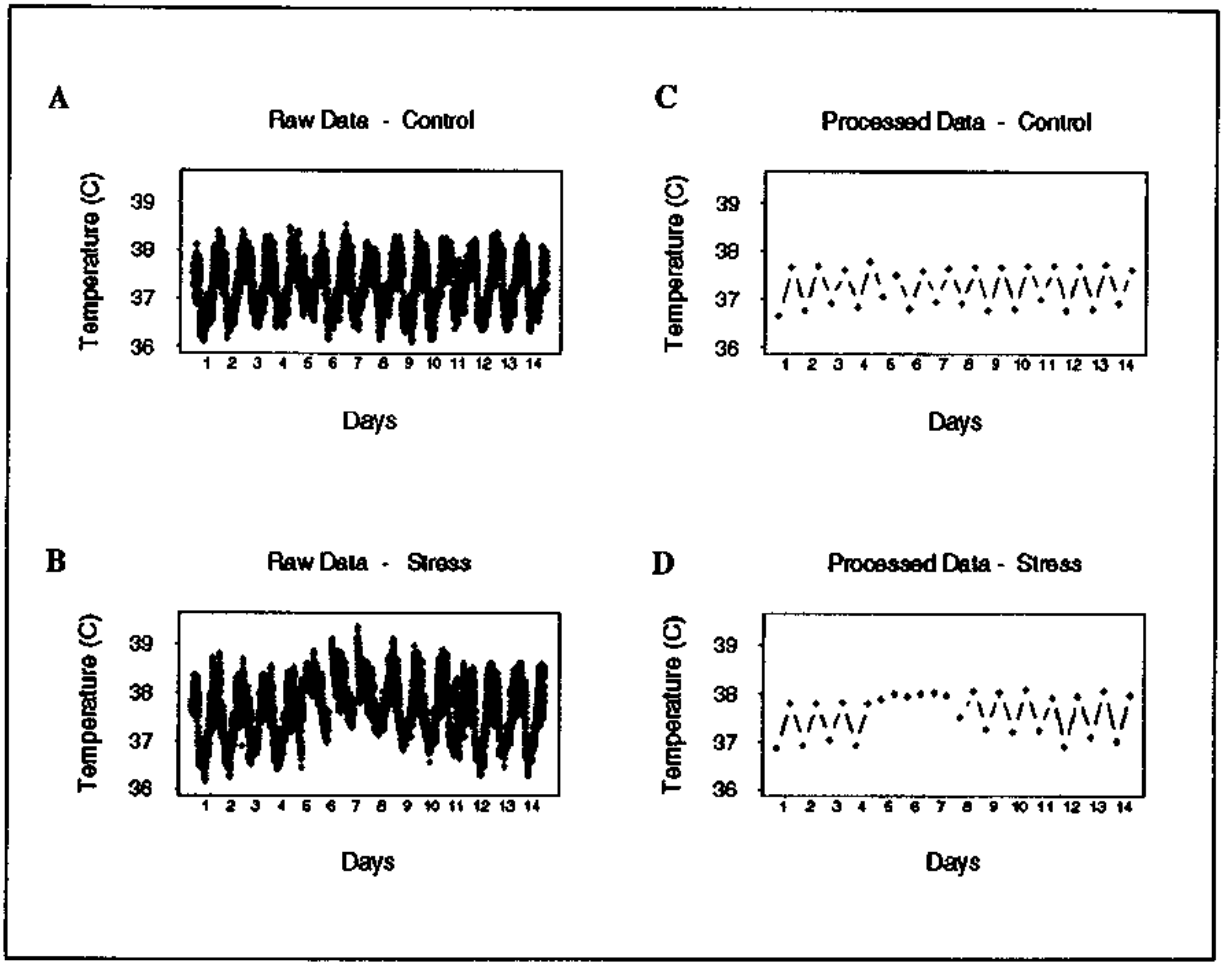

Figure 1: Examples of a raw and processed file for both stressed and control animals. A and B are the raw files for control and stressed animals respectively. C and D are the filtered mean versions of same plots.

The data were then collected into 12-hour bins and a mean was taken for both the night and the day bins (Figures 1c and 1d). Activity data were measured in the same frequency as temperature, however values were simply summed into 12-hour bins for night and day.

Statistical comparisons of the stress group versus the non-stress group were performed using an Analysis of Variance (ANOVA) for repeated measures with group as a between animal measure and days as a within measure. The daytime and nighttime temperatures and activity were analyzed with separate ANOVA's. A priori comparisons between the stressed rats and the controls on each day were made using Dunn's test to control for multiple comparisons. All data are presented as means $\pm$ standard errors of the mean.

Experiment 2: Persistent Changes in Body Temperature, Activity and Urinary Corticosterone After 3 Stressor Exposures.

A. Animals

Sixteen male Sprague-Dawley rats from Charles River (Wilmington, DE) were housed individually under the same conditions as Experiment 1.

B. Procedures

After a 4-week period, the rats were weighed and ranked by body weight (body weights ranged from 475-568g). They were randomly assigned to four groups: those that did not receive stress (n=3) but received a vehicle (90% Saline: 5% Emulfor: 5% DMSO), those that did not receive stress but received the CRH antagonist CP-154,526 (20mg/kg) (n=3), those that received three days of stressor exposure but no CRH antagonist (n=5) (only vehicle), and finally,

those that received three days of chronic stress (n=5) as well as the CRH antagonist. After being assigned to groups the rats were placed in metabolic cages from the Nalgene Company (Rochester, NY) (Figure 2). The metabolic cages were suitable for rats ranging from 300-850g. The rats were allowed to habituate for 5 days prior to telemeter surgery. Throughout the experiment, rats were allowed free access to Lab Diet 5001 Powder Rodent Chow and tap water.

Rats were implanted with temperature / activity telemeters as described above. They were allowed to recover for 5 days prior to data acquisition. The protocol followed that described above with temperature and activity measured for 4 days pre-stress, 3 days during stress, and 7 days post-stress. On stress days, all 16 rats received either treatment with a CRH antagonist or vehicle. The injections of vehicle and antagonist were given intraperitoneally 15 minutes prior to stress. The drug demonstrated no effects on blocking the stress response. Therefore, this data is presented in the results section, but statistical comparisons focused on the differences between stressed and control animals.

C. Urine Collection

In addition to measuring temperature and activity, urine was also collected throughout Experiment 2. The purpose of the urine collection was to measure and assess the changes in the urinary corticosterone (CORT) levels in response to stress. The metabolic cages allowed for feces and urine separation in such a way that urine was accumulated in a cup. The urine was collected at 11:00 am each day. Urine volumes were measured and urine was centrifuged for 15

minutes to separate any contaminants of food and feces. The supernatant was poured off into clean tubes and frozen at -40 °C until further analysis. Urine samples were taken for 1 pre-stress day, 3 stress days and 6 post-stress days.

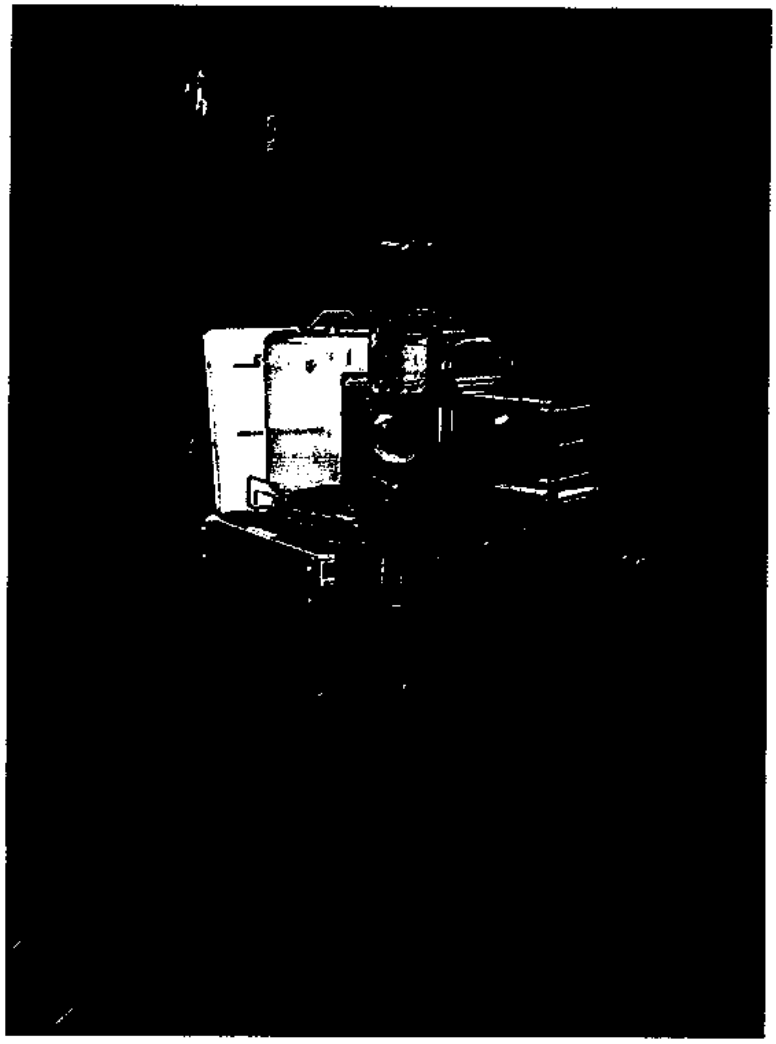

Figure 2: A picture of the typical metabolic cages used in Experiments 2 and 3.

The urine samples were analyzed using the ImmuChem Double Antibody Corticosterone I^{125} radioimmunoassay (RIA) kit from ICN Biomedicals (Costa Mesa, CA) as described previously (Ottenweller, et al., 1989; Brennan, et al., 2000). Urine samples were diluted 1:20 with assay diluent, and 100 microliter samples were assayed for CORT levels. Serial dilutions of urine samples paralleled the standard curve over its linear portion, and 95.1 $\pm$ 9.2% (mean $\pm$ sem; 9 samples) of unlabeled corticosterone added to urine samples as detected by RIA. Thus, direct assay of unextracted urinary CORT using the ICN RIA kit appears to be an excellent noninvasive method for assessing overall adrenocortical activity. Creatinine levels were measured using a kit from Sigma (St. Louis, MO; #0400-100) and 24-hour urinary CORT levels are presented as ng/mg creatinine to correct for differences in urine excretion.

D. Data Analysis

Temperature and activity data were reduced and analyzed as described above. The urine data were analyzed using an ANOVA model similar to that described above for temperature and activity with group serving as a between animal measure and days as a within measure. Post Hoc tests were conducted with Dunn's tests (T_D's) and $p<.05$ was used as the rejection criterion.

Experiment 3: *Persistent elevations in Body Temperature, Activity, and Urinary Corticosterone After 1 Stressor Exposure*

A. Animals

Sixteen male Sprague-Dawley rats from Charles River (Wilmington, DE) were housed under the same conditions as in the previous two experiments.

B. Procedures

After a 2 week period, the rats were weighed and ranked by body weight (body weights ranged from 335-388g). They were randomly assigned to two groups: those that did not receive stress (n=8), the non-stress group, and those that received one day of stress (n=8), the stress group. After being assigned to groups, the rats were placed in metabolic cages. The rats were allowed to habituate for 5 days prior to telemeter surgery. Throughout the experiment, rats were allowed free access to Lab Diet 5001 Powder Rodent Chow and tap water.

Rats were implanted with temperature / activity telemeters as described above. They were allowed to recover for 5 days prior to data acquisition. The protocol followed those described for the previous experiments except that the rats were exposed to only one day of stress and there was no CRH antagonist used. Temperature and activity were measured for 4 days pre-stress, 1 day of stress, and 7 days post-stress.

C. Urine Collection

The urine was collected as described in Experiment 2. Urine samples were taken for 2 days pre-stress, 1 stress day and 6 post-stress days. The urine samples were assayed and analyzed as described above.

D. Plasma Collection

Plasma samples were taken by tail nick from both stress and non-stress rats 24 hours post stressor exposure. Blood was collected in heparinized hematocrit tubes and centrifuged. The plasma was then removed. Plasma samples were stored in fresh tubes frozen at -40°C until analyzed. CORT analysis was done by radioimmunoassay (RIA) using the ICN assay kit described in detail previously (Ottenweller et al., 1989).

E. Data Analysis

Data were reduced and analyzed as described above, except there was only one day of stress.

Results

Experiment 1:

Experiment 1 compared the responses of temperature and activity to three days of inescapable tail shock stress with temperature and activity in non-stressed controls.

Temperature

The daytime temperature change can be seen in Figure 3. Data are represented as changes from a pre-stress average due to variability between rats in their instrumentation. The Pre-Stress mean for the non-stressed group was 37.06°C with a standard error of the mean (SEM) of $\pm$ 0.16°C which was similar to the with a Pre-Stress mean of 36.88 $\pm$ 0.13°C for the stress group. There was not a significant stress effect on the first day of stressor exposure. However, stress increased daytime temperature on days two and three of stressor exposure, $F(1,33) = 26.6$, $p<0.001$ and $F(1,33) = 68.2$, $p<0.001$, respectively. Also during Experiment 1, there was a significant stress by day interaction during recovery $F(2,206) = 46.7$, $p<0.001$. Further statistical analysis indicated that for days 1, 2 and 3 following the last stressor exposure temperature was significantly elevated in the stressed rats compared to the non-stressed rats ($p<0.05$). The stress day 1 mean is not as high as the other stress day means because daytime values included three hours of pre-stress temperature data.

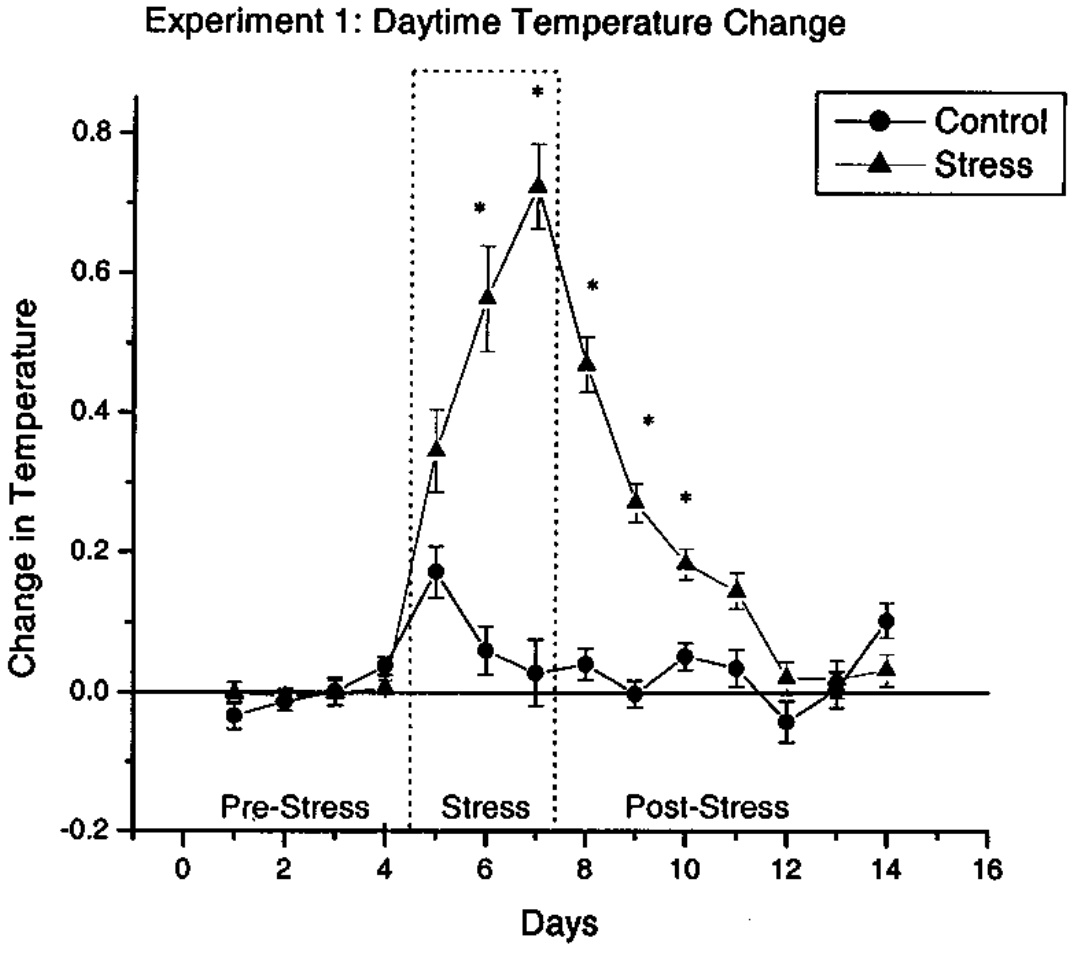

Figure 3: Daytime temperature changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$.

The nighttime temperature changes are represented in Figure 4. The Pre-Stress mean was 37.84 $\pm$ 0.16°C for the non-stress rats which was similar to a mean of 37.64 $\pm$ 0.14°C for the stress group. Again, stress elevated body temperature on days 2 and 3 of stressor exposure $F(1,33) = 7.16$, $p<0.05$ and $F(1,33) = 13.15$, $p<0.01$, respectively. There was a nonsignificant decrease in the mean temperature for both control and stress means the evening following the first day of stress. There was a significant stress by day interaction during recovery, $F(2,31) = 36.31$, $p<0.001$. Nighttime temperature means in the stress group where greater than those in the control group for three evenings following the last stressor exposure, $p<0.05$.

Activity

The daytime activity change can be seen in Figure 5. Data is represented as a percentage of a Pre-Stress average because of the variability in activity count recording. The daytime Pre-Stress mean for the non-stressed rats was 316 counts with a SEM of $\pm$ 53 counts. The daytime Pre-Stress average for the stress animals was 270 $\pm$ 38 counts. These means were not significantly different. Stressor exposure did not have any significant effects on the daytime activity counts ($p>0.1$). There were no significant stress effect or stress by day interactions, $F(1,31) = 0$, $p>0.1$ and $F(2,206) = 0.11$, $p>0.1$, respectively.

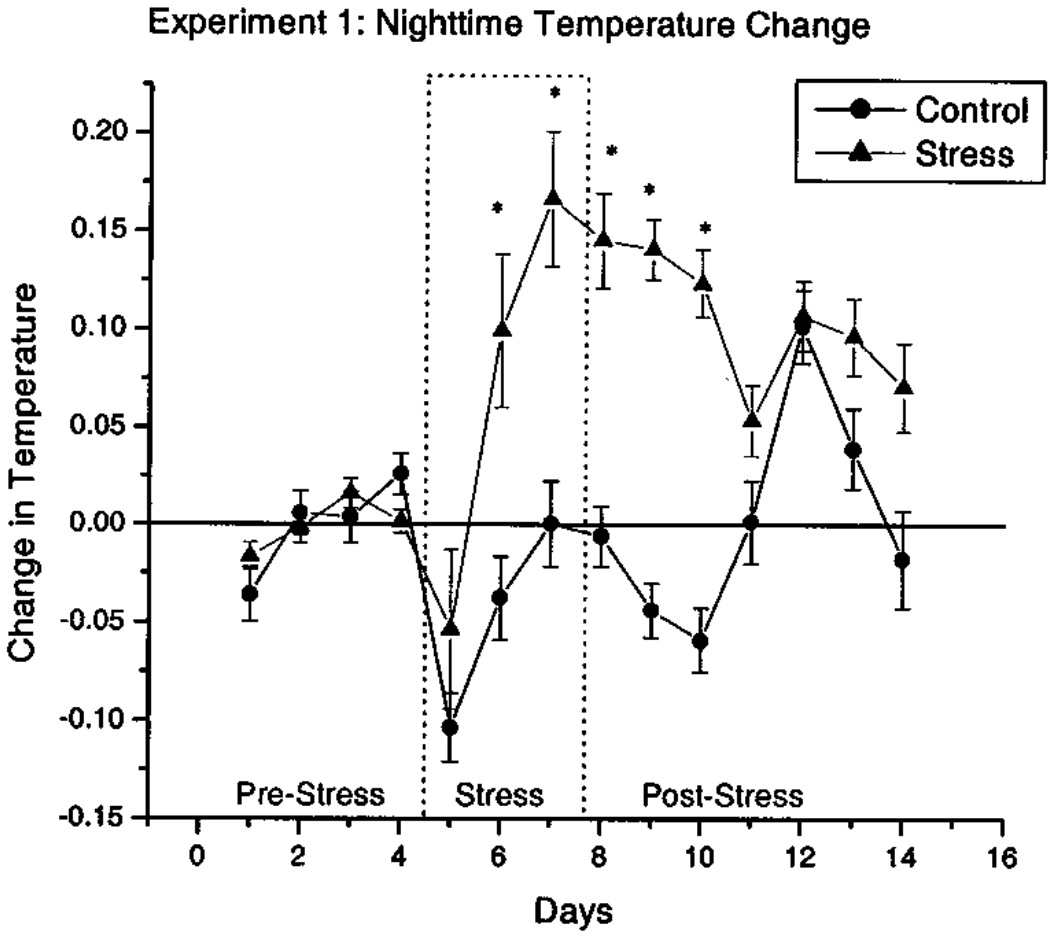

Figure 4: Nighttime temperature changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$.

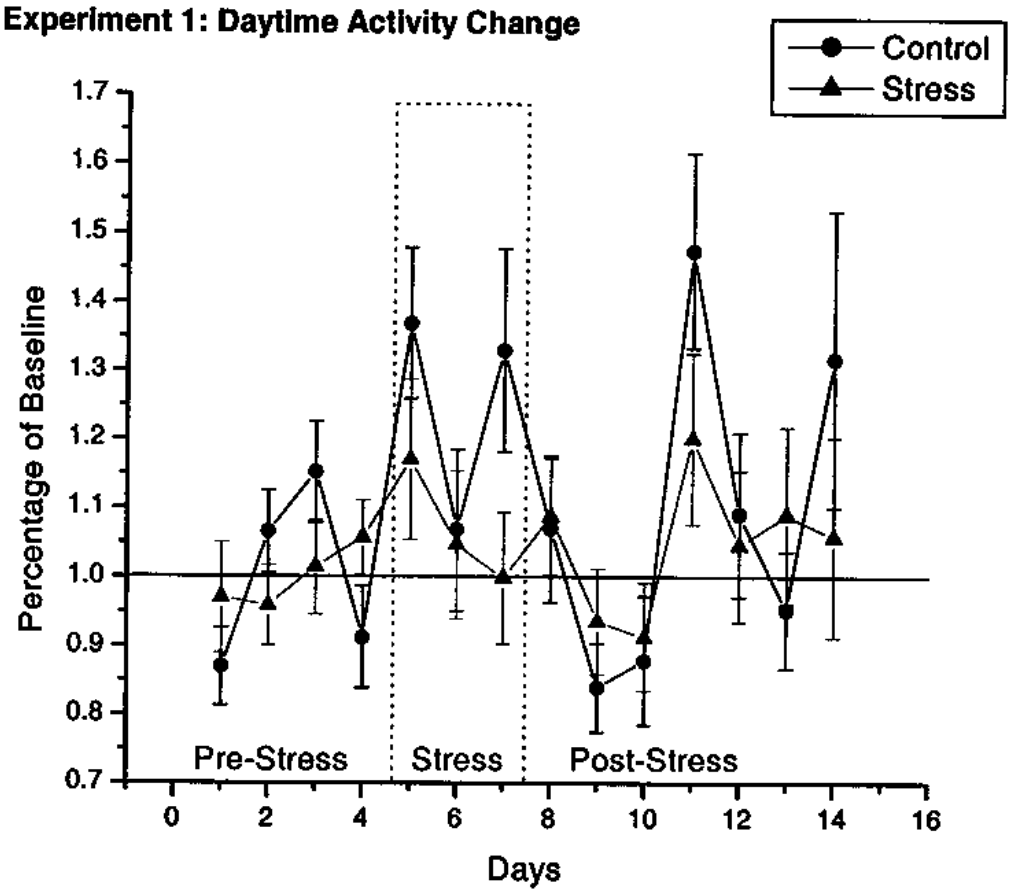

Figure 5: Daytime activity changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure.

The nighttime activity changes are represented in Figure 6. The Pre-Stress mean for the control rats was 1105 $\pm$ 117 counts which was similar to that for stressed animals which was 1013 $\pm$ 118 counts. The nighttime activity mean for the stressed animals was significantly lower than the control animals on each day of stress, $F(1,30) = 21.12$, $p<0.001$, $F(1,30) = 37.37$, $p<0.001$, $F(1,30) = 38.88$, $p<0.001$, for days 1,2 and 3 respectively. However, the decrease in activity for the stressed animals was not chronic in nature and recovered to the level in control animals on the first day after the stressor exposures.

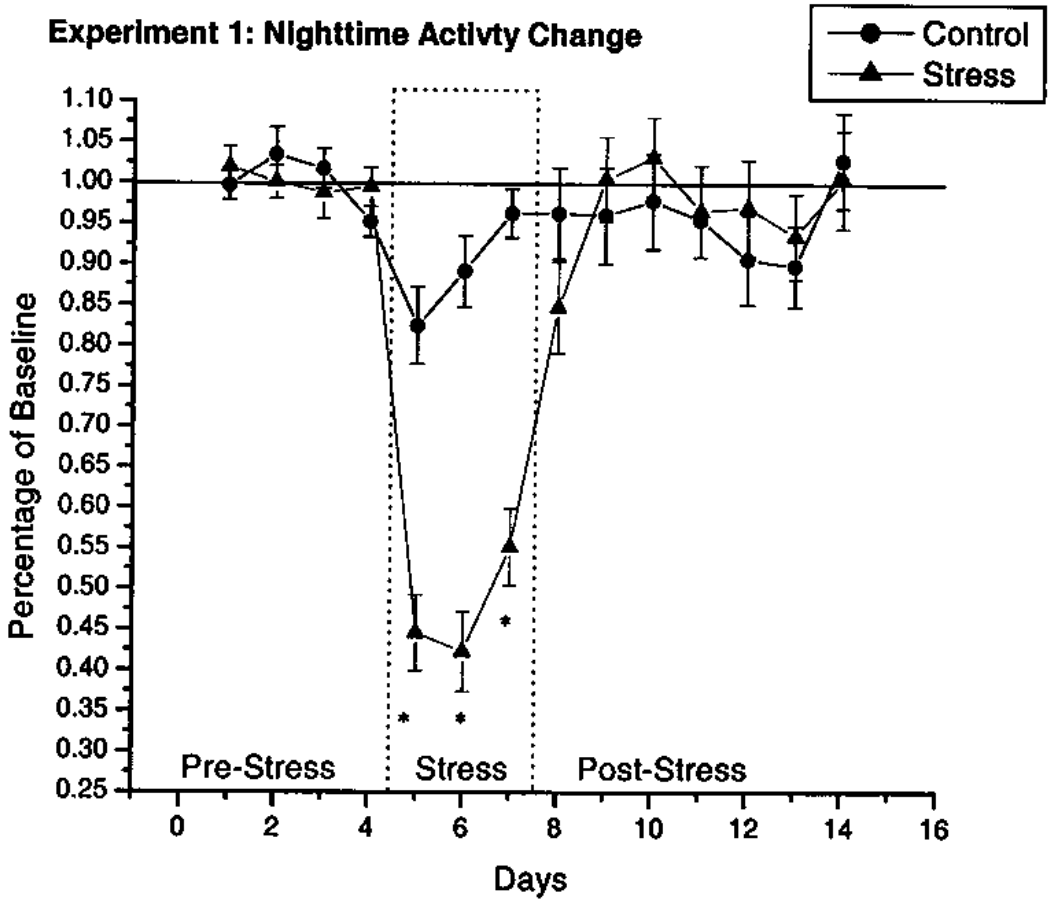

Figure 6: Nighttime activity changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$.

Experiment 2:

Experiment 2 compared the responses in temperature, activity and urinary corticosterone for rats exposed to three days of inescapable tail shock stress with those of non-stressed controls.

Prior to each stressor exposure some of the control and stress rats received an injection of CP-154,526 or DMSO vehicle. The drug injections had no effects on urinary corticosterone levels, body temperature or activity. Therefore, data from vehicle-and drug-treated rats were pooled and analyzed as stress versus non-stress rats. Figures 7 through 11 present temperature, activity, and urinary corticosterone for the experiment as originally envisioned in a 2 X 2 experimental design. The most important aspect of these figures is that the drug had no effect on any of the variables, so we will present and discuss the findings collapsed across drug treatment groups.

Temperature

The daytime temperature change can be seen in Figure 12. Data are presented as changes from a Pre-Stress mean as in Experiment 1. The Pre-Stress mean of 37.06 $\pm$ 0.04°C for the control rats was similar to that of 37.05 $\pm$ 0.03°C for the stress rats. The ANOVA revealed a significant stress effect on days 2 and 3 of stressor exposure $F(1,14) = 10.0$, $p<0.01$ and $F(1,14) = 47.4$, $p<0.001$, respectively. The temperature change was not significantly different for the stress and control groups on the first day of stressor exposure due to the

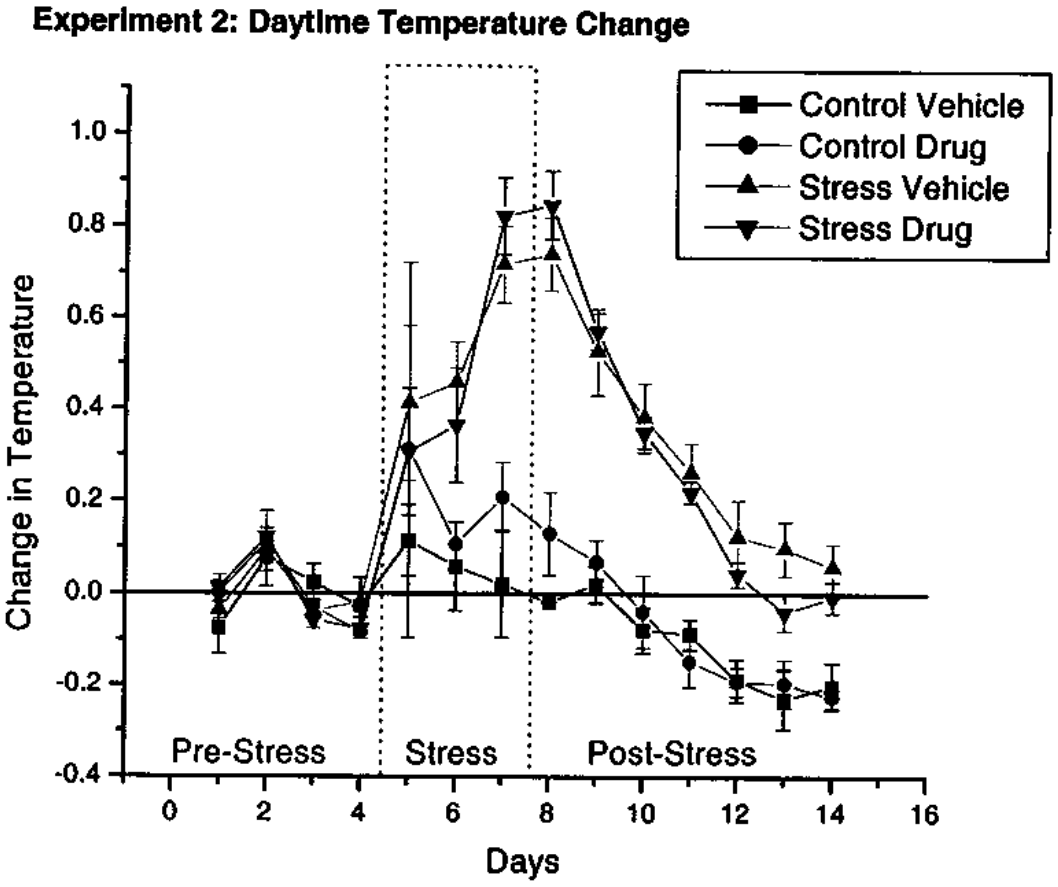

Figure 7: Daytime temperature changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure.

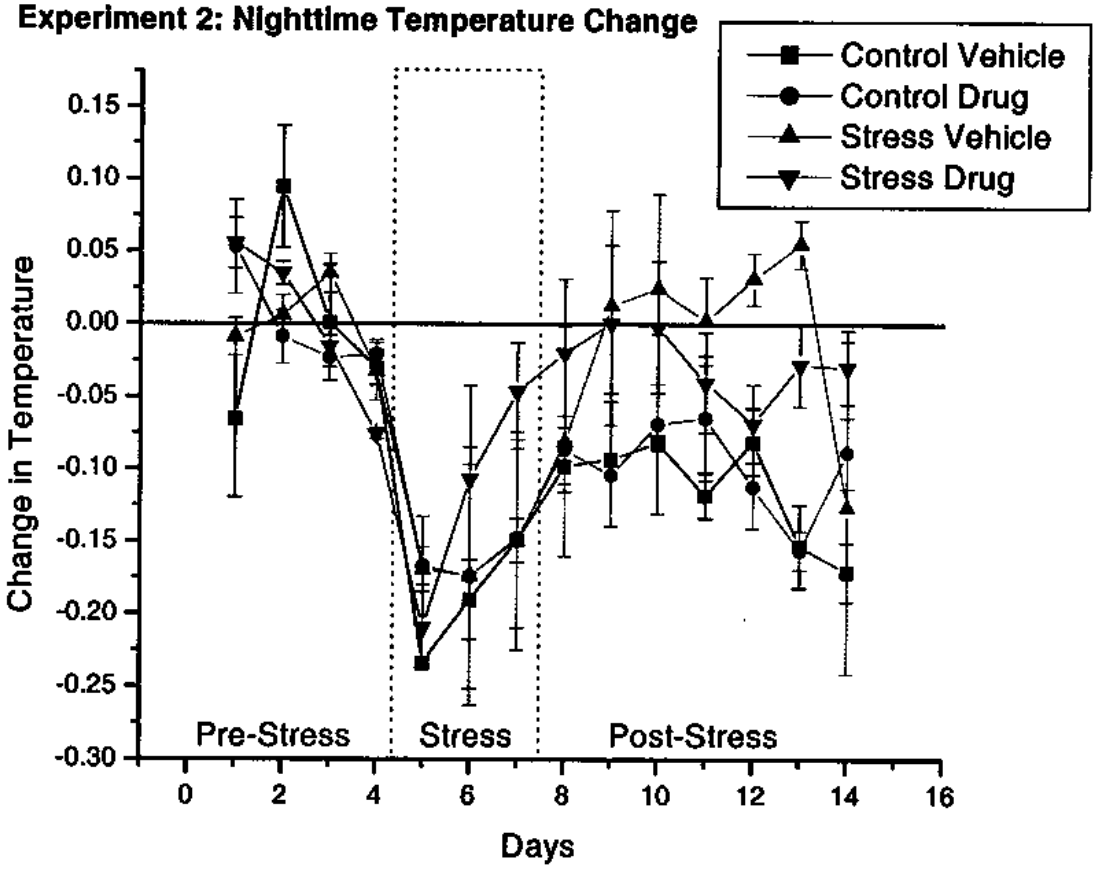

Figure 8: Nighttime temperature changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure.

Experiment 2: Daytime Activity Change

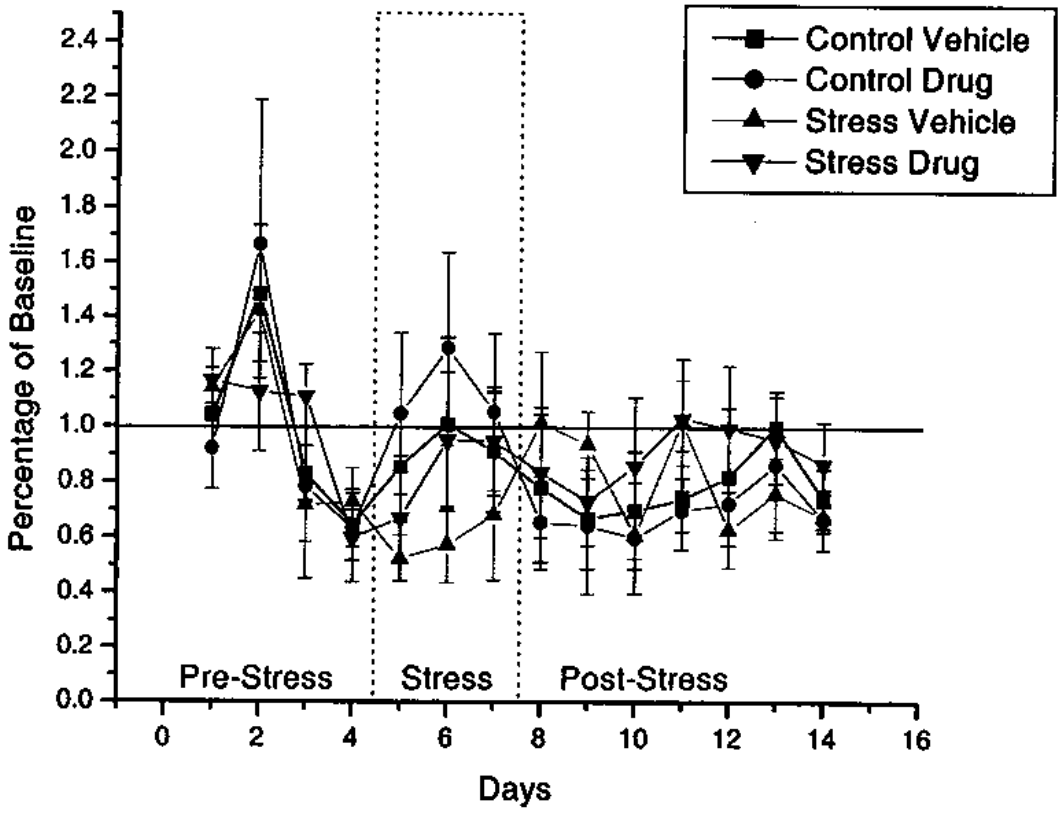

Figure 9: Daytime activity changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure.

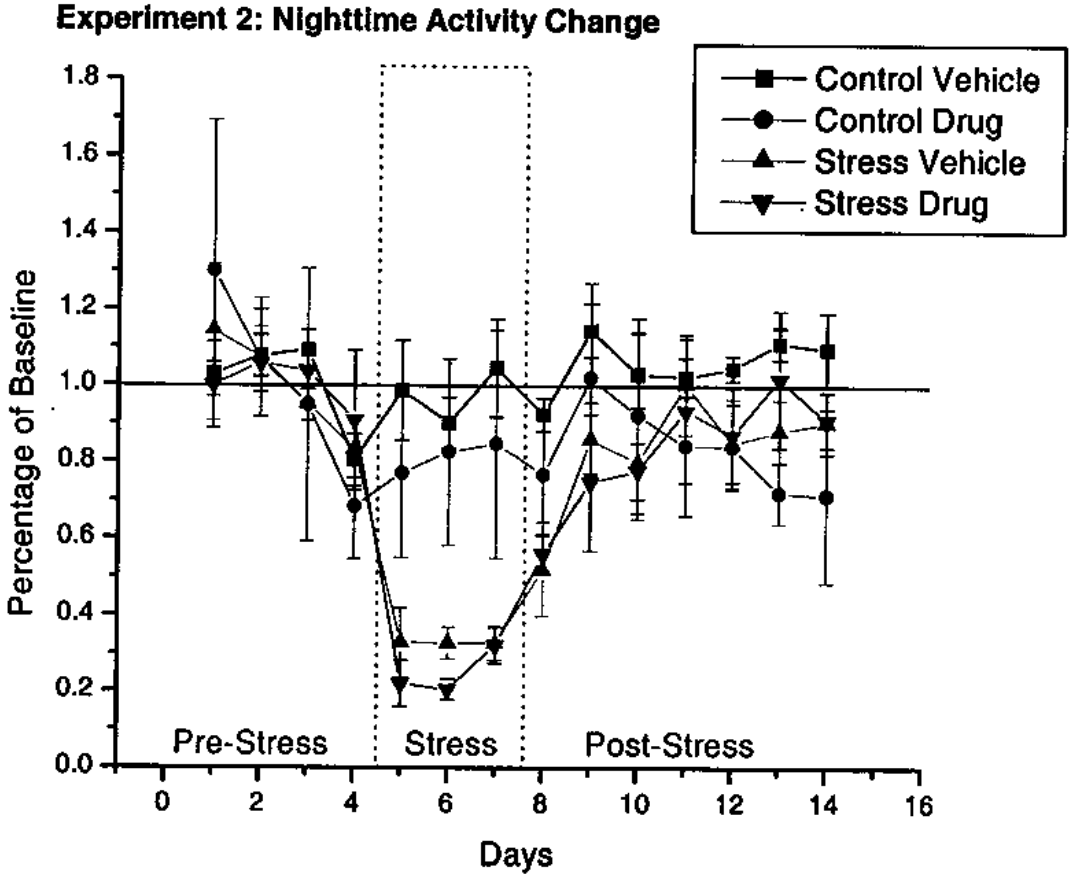

Figure 10: Nighttime activity changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure.

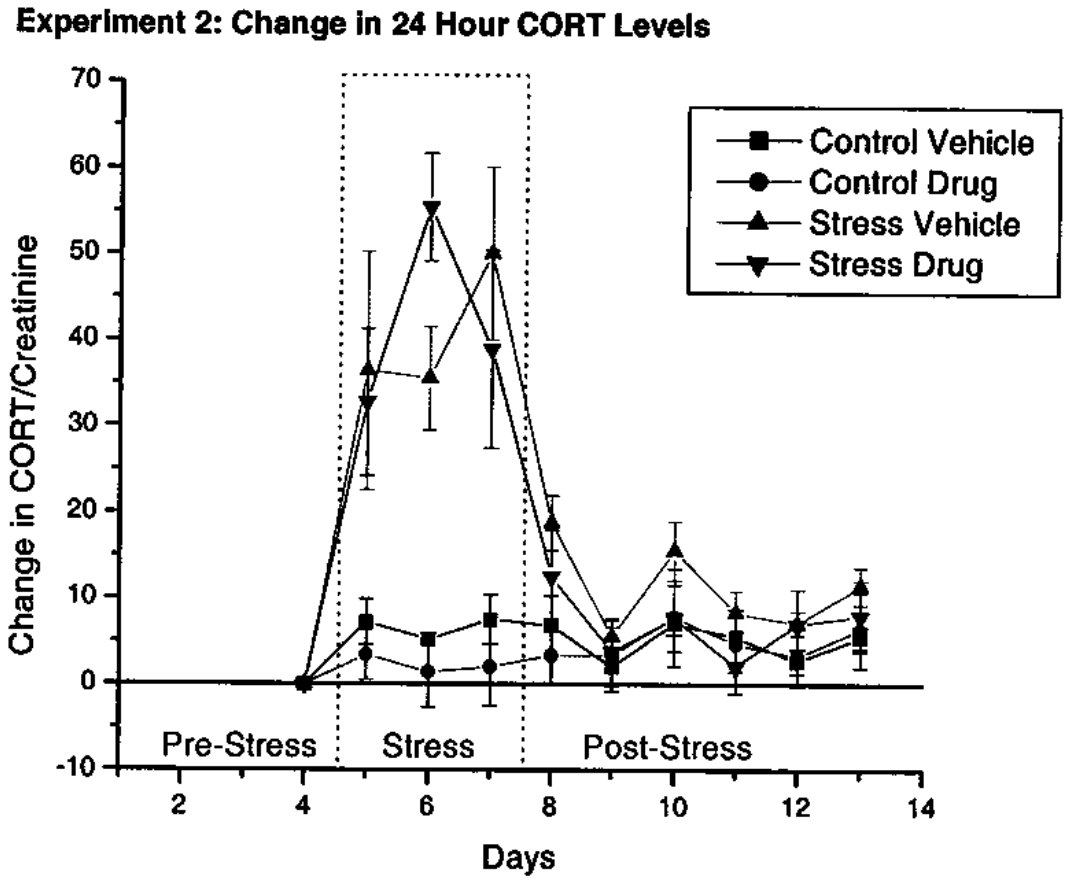

Figure 11: Changes in 24-Hour urinary corticosterone for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure.

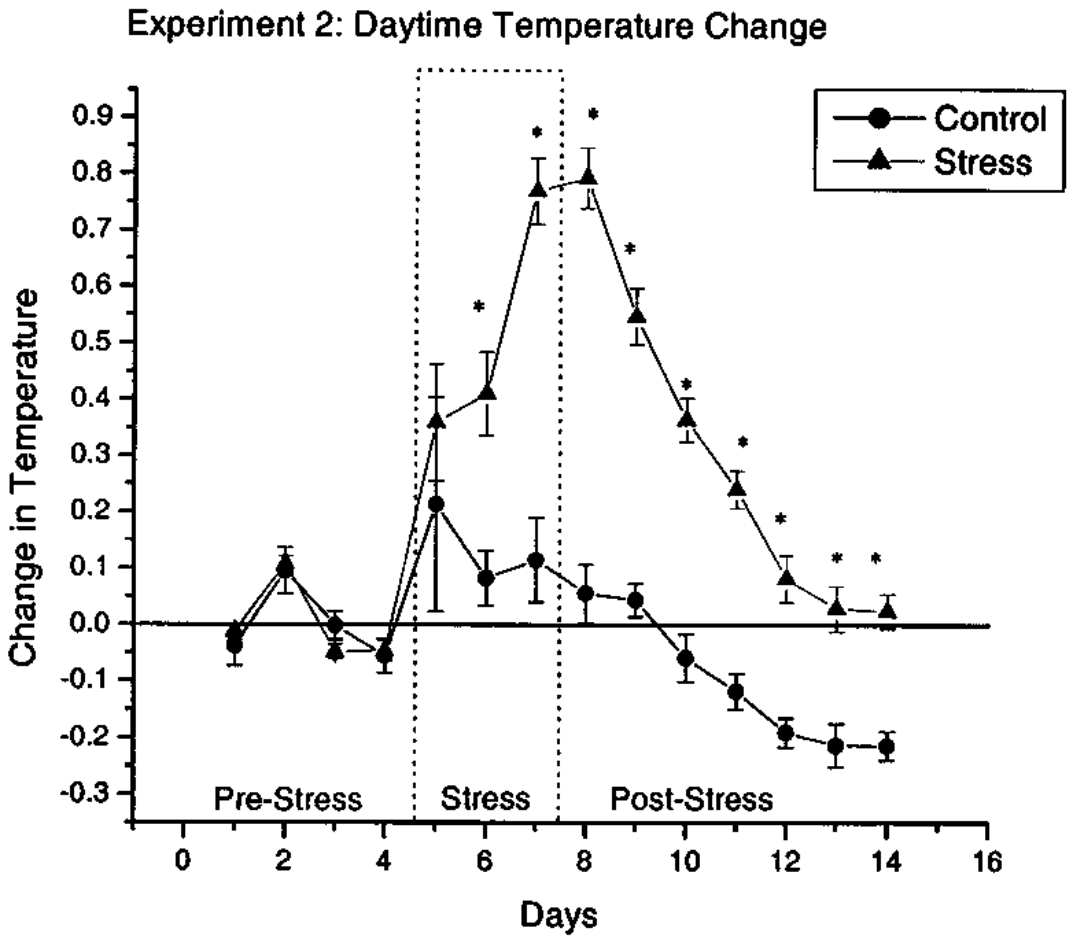

Figure 12: Daytime temperature changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$.

three hours of Pre-Stress data included in the Stress Day 1 mean. During the recovery period following stresor exposure there was a significant stress by day interaction $F(1,92) = 75.4$, $p<0.001$. Moreover, the temperature in stressed rats remained significantly elevated over that in control rats for 7 days after stressor exposure ($p<0.01$).

Figure 12 also shows a decrease in the post-stressor temperature means for the control rats below those in these rats during the Pre-Stress period. The reason for this decline is unclear and might be attributed to lower room temperatures post stressor.

The nighttime temperature changes are represented in Figure 13. The Pre-Stress mean was 37.90 $\pm$ 0.03°C for the control rats which was similar to 37.96 $\pm$ 0.04°C for stress rats. There was no significant stress by day interaction. For both groups (stress and control), there was a decrease in nightime temperature on each night post stressor exposure. This decrease after the injections in the non-stressed rats may have been due to the effects of the DMSO vehicle because the decrease was not significantly different between the groups $F(1,14) = 0.12$, $p>0.1$. During recovery, there was a significant stress by day interaction $F(2,92) = 3.17$, $p<0.05$. However, the stress animals' mean night temperatures were only marginally higher than the control animals for days 2 to 6 post stressor ($0.10<p>0.05$). As with the day temperatures, the elevations in night temperature appear to have lasted longer than those in Experiment 1.

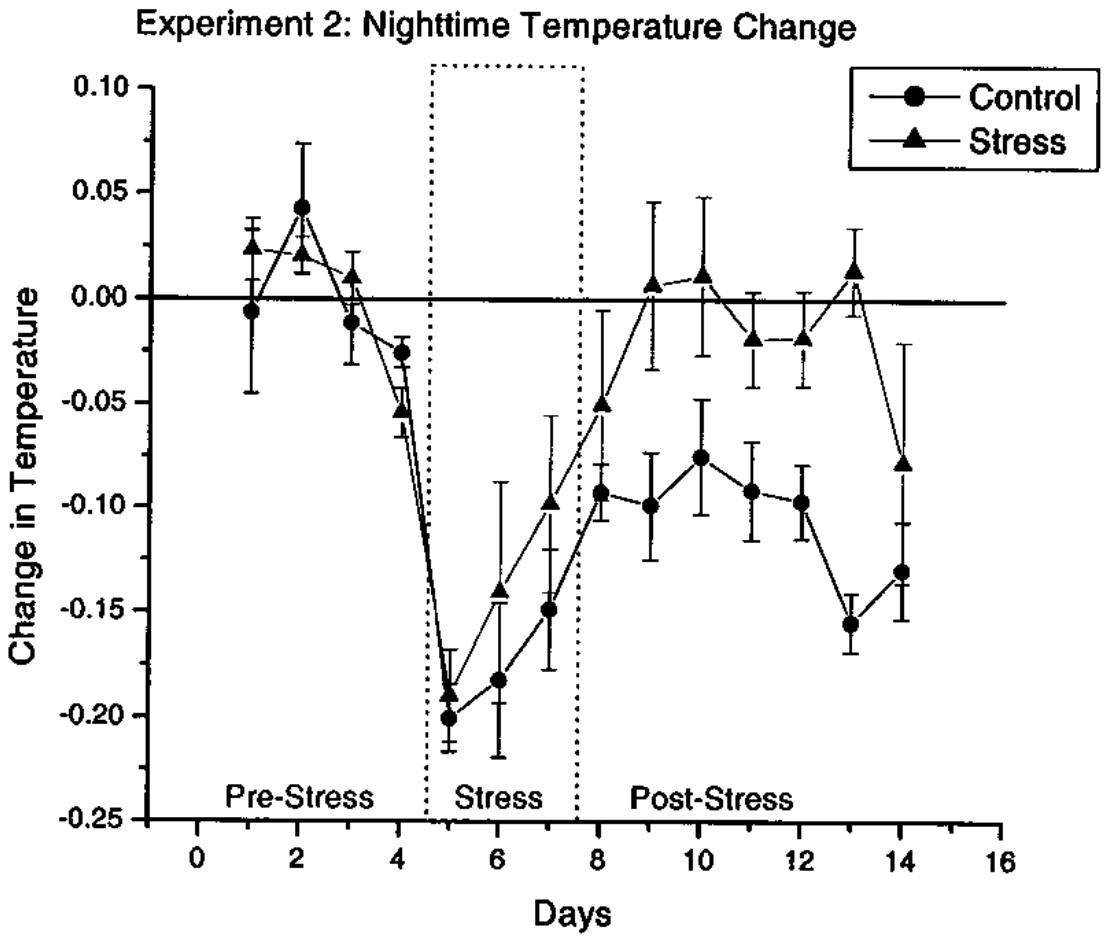

Figure 13: Nighttime temperature changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure.

Activity

The daytime activity change can be seen in Figure 14. Data is represented as a percentage of baseline similar to Experiment 1. The daytime Pre-Stress average for the control rats was 265 $\pm$ 52 counts which was similar to 177 $\pm$ 33 counts for the stress rats. There were no significant effects of stress during or after exposure to the stressors.

The nighttime activity change is reported in Figure 15. The Pre-Stress mean for the control rats was 653 $\pm$ 135 counts which is similar to 630 $\pm$ 123 counts for the stress rats. There was a significant stress effect on each of the stress days, $F(1,11) = 13.7$, 42.8 and 48.0, $p<0.001$, for the first, second and third days of stressor exposure, respectively. There was also a significant stress by day interaction during recovery, $F(2,92) = 6.23$, $p<0.05$, because the nighttime activity remained suppressed for one day post stress, $p<0.01$.

Urinary Corticosterone

The change in urinary corticosterone is represented in Figure 16. Data are presented as change from Pre-Stress means in order to maintain consistency in graphical representation. The Pre-Stress mean for the control rats was 28.99 $\pm$ 2.67 ng/mg creatinine and that for the stress rats was 26.86 $\pm$ 2.79 ng/mg creatinine. These means were not significantly different. The stress animals' mean CORT levels are significantly higher for each of the days of stress $F(1,14) = 8.3$, 35.6 and 15.9, $p<0.001$, for days one, two and three of stress, respectively. During recovery, there was a significant stress by day interaction, $F(2,76) = 4.44$,

$p<0.05$, because stress animals had higher urinary corticosterone than the control animals for one day after the stressor, $p<0.05$.

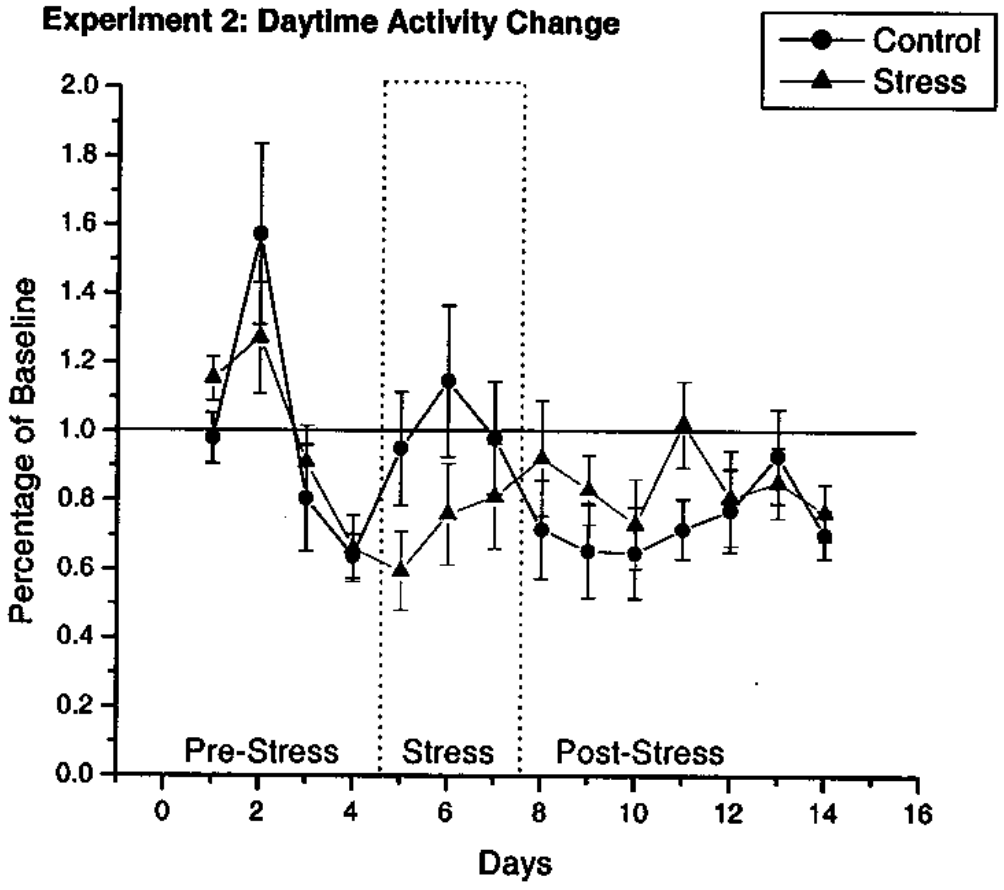

Figure 14: Daytime activity changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure.

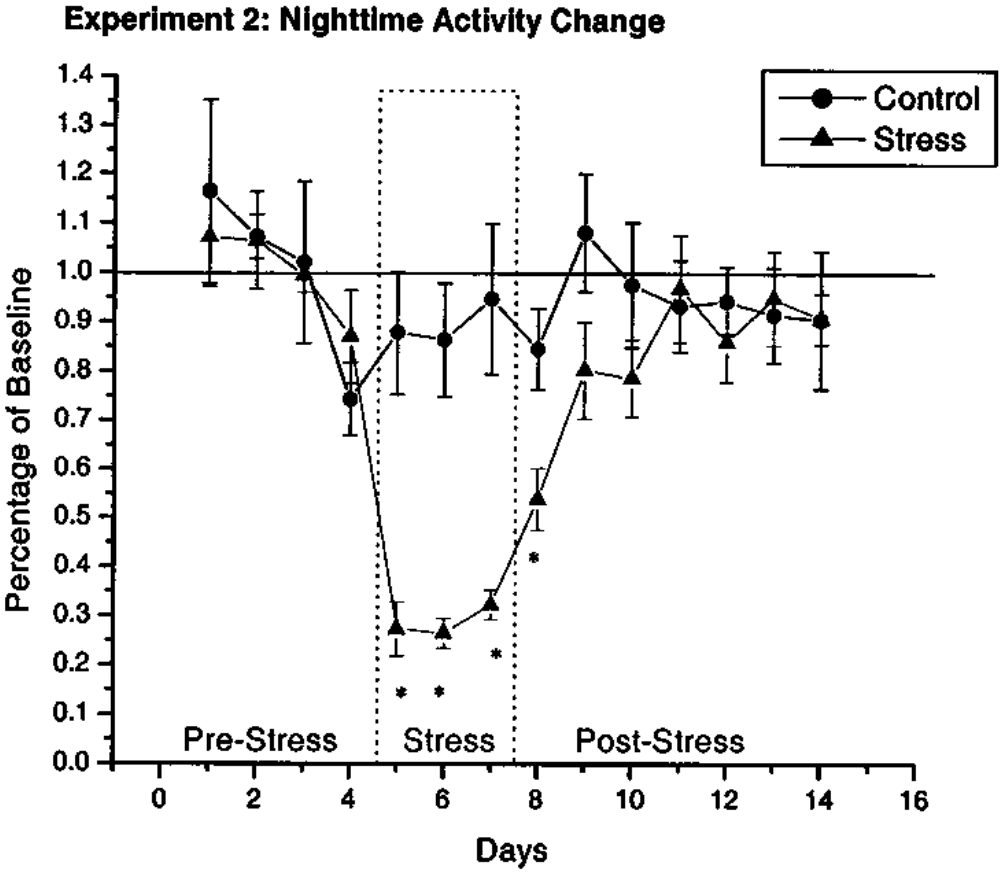

Figure 15: Nighttime activity changes for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$.

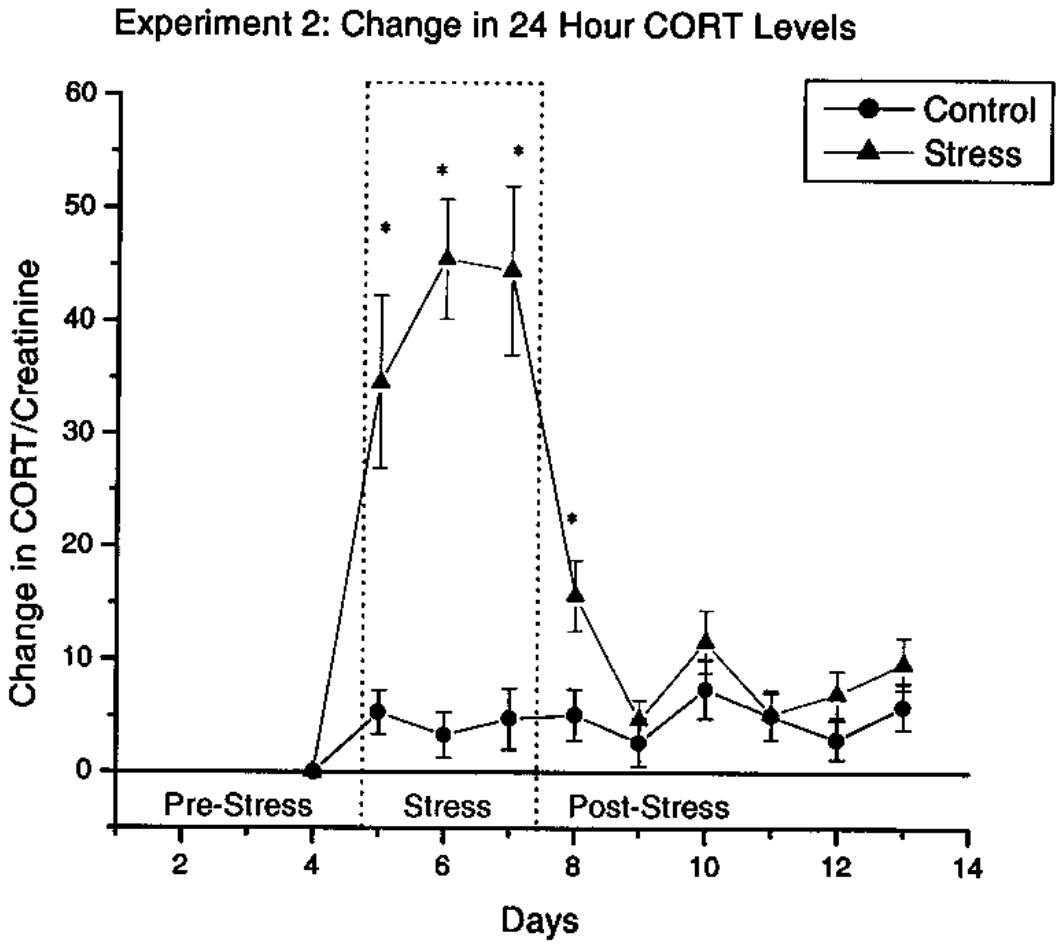

Figure 16: Changes in 24-Hour urinary cortecosterone levels for stress and control animals exposed to three days of stressors. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$.

Experiment 3:

In an attempt to minimize the stressor exposure and see if similar effects of stress persist after one stressor exposure, Experiment 3 compared the differences in temperature, activity and urinary corticosterone in rats exposed to one day of inescapable tail shock stress with those in non-stressed controls.

Temperature

The daytime temperature change can be seen in Figure 17. The data are represented as changes from a pre-stress mean as in both previous studies. The Pre-Stress mean for the control animals was 36.87 $\pm$ 0.07°C which was similar to 36.89 $\pm$ 0.04°C for the stress animals. There was no significant stress effect during the stressor exposure, $F(1,11) = 2.91$, $p>0.1$. However, there was a significant stress by day interaction during the recovery $F(2,92)=7.2$, $p<0.01$. The daytime temperature mean for the stress group was significantly higher than that for the control rats on the first four days post stressor exposure, $p<0.05$.

The nighttime temperature change can be seen in Figure 18. The pre-stress mean was the same in stress and control animals, 37.72 $\pm$ 0.05°C and 37.74 $\pm$ 0.05°C respectively. The ANOVA revealed a significant stress effect on the stress day, $F(1,11) = 31.41$, $p<0.001$. Unlike previous studies, there was a significant decrease in temperature on the evening after the stressor. However, similar to Experiments 1 and 2, nighttime temperatures are significanty higher for

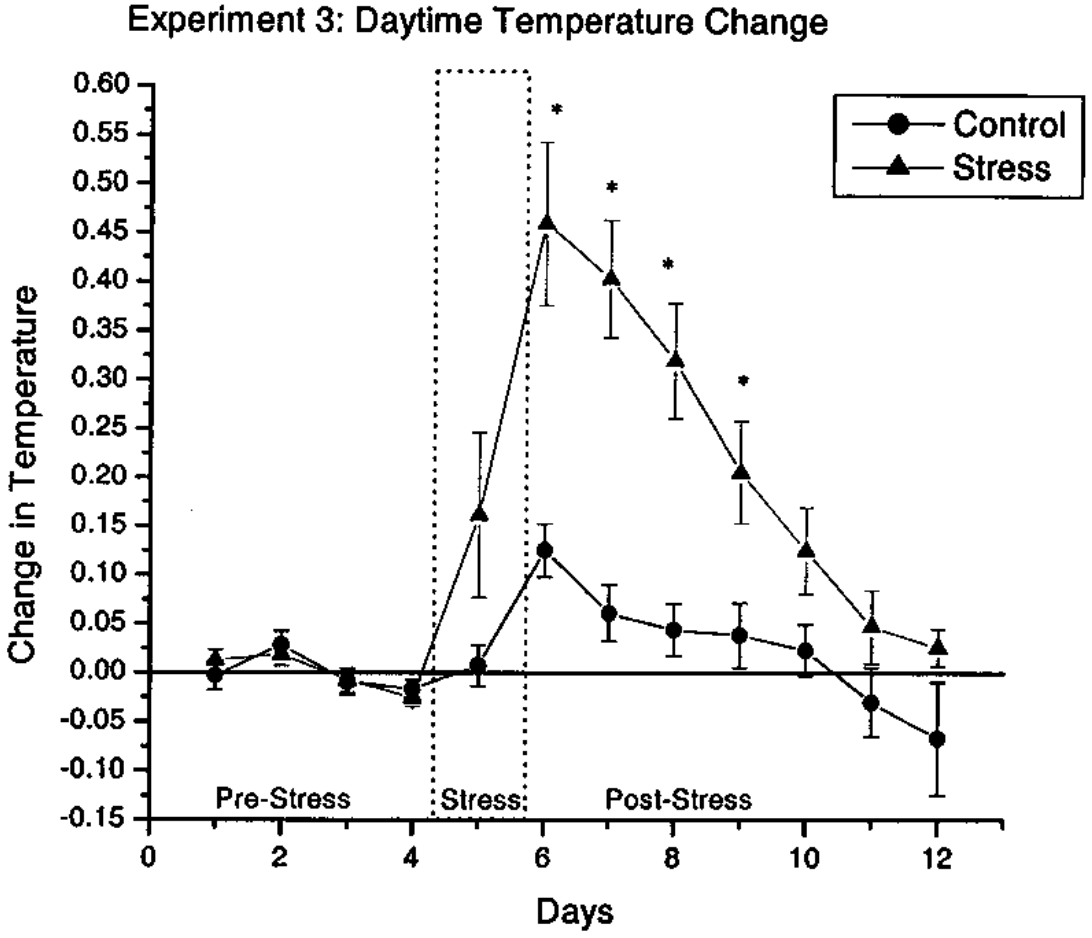

Figure 17: Daytime temperature changes for stress and control animals exposed to one day of stressor. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$.

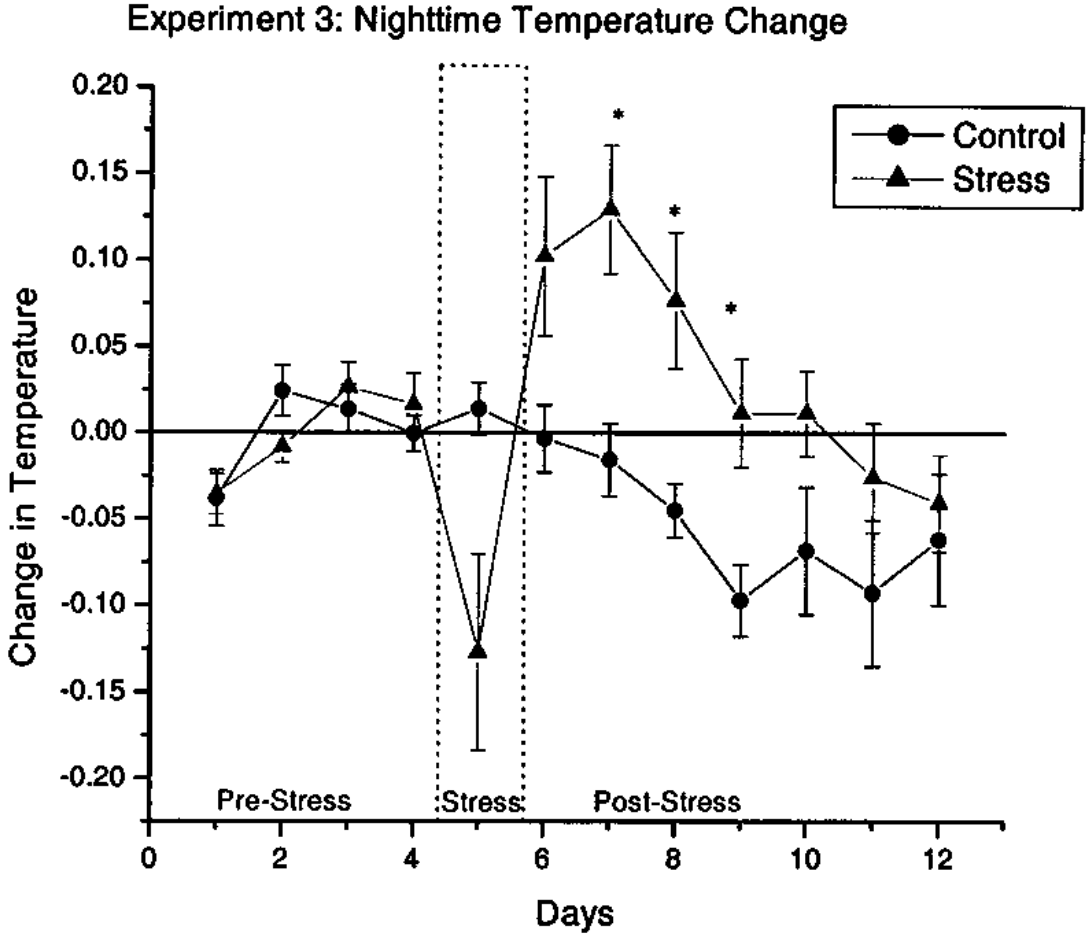

Figure 18: Nighttime temperature changes for stress and control animals exposed to one day of stressor. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$.

the stress rats than the controls on days 2, 3 and 4 after the stressor exposure, interaction $F(2,12) = 4.7$, $p<0.01$, and $p<0.05$ for each of these 3 days.

Activity

The daytime activity change for Experiment 3 can be seen in Figure 19. Activity data for Experiment 3 are represented as percentage of a Pre-Stress average as in previous studies. The daytime Pre-Stress mean for control animals was 257 $\pm$ 50 counts which was similar to 179 $\pm$ 33 counts for the stress animals. On the day when the animals were exposed to the stressor, there was no significant difference in activity counts for the stress group $F(1,7) = 2.20$, $p>0.1$. However, activity levels on the stress day rose over their baseline levels in both groups. Moreover, the stress animals' activity counts return to baseline the day following the stressor exposure and were less than those in the stressed animals, $F(2,92) = 6.24$, $p<0.01$, probably due to plasma sampling on this day.

The nighttime activity change is represented in Figure 20. The Pre-Stress means for the two groups were similar. Activity for the control rats was 946 $\pm$ 160 counts, and 702 $\pm$ 111 counts for the stress group. The nighttime activity was significantly lower for the stress group on the night following stressor exposure $F(1,12) = 5.98$, $p<0.05$. The stress group also had lower activity for two days after stressor exposure, $p<0.05$.

Urinary Corticosterone

The change in urinary corticosterone is represented in Figure 21. Data are presented as a change from a Pre-Stress average as in Experiment 2. The

Pre-Stress mean for the control rats was 16.32 $\pm$ 2.25 ng/mg creatinine which was similar to that for stress rats of 16.08 $\pm$ 1.39 ng/mg creatinine. The ANOVA revealed a significant increase in urinary corticosterone on the stress day, $F(1,14) = 23.4$, $p<0.01$. There was a significant stress by day interaction during recovery, $F(2,60) = 5.11$, $p<0.05$, due to elevated levels on the first two days after stressor exposure, $p<0.05$.

Plasma Corticosterone

Plasma was also taken for the animals in the morning the day following stressor exposure. Non-stress rats were found to have a mean corticosterone of 1.07 $\pm$ 0.24 ug/dl, whereas stress rats had a mean level of 11.51 + 3.21 ug/dl. T-test revealed a significant difference between the stress and non-stress rats $t(14) = 3.24$, $p<0.01$.

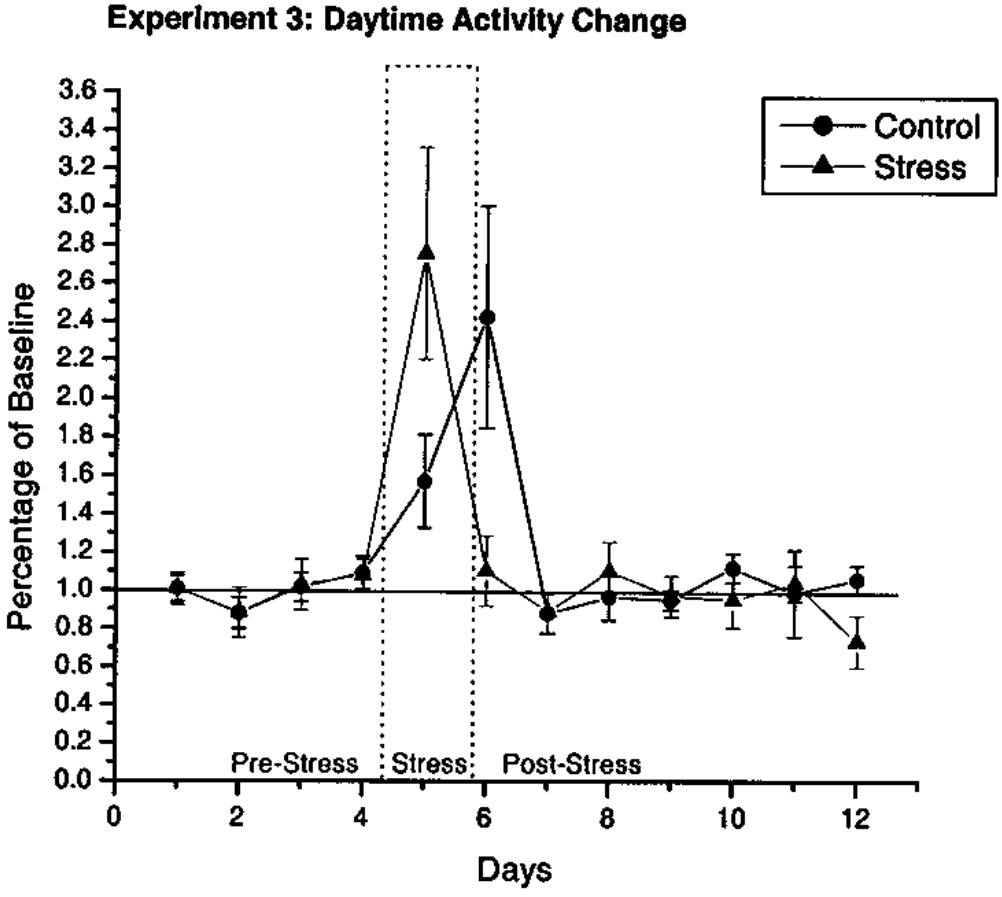

Figure 19: Daytime activity changes for stress and control animals exposed to one day of stressor. The dotted line indicates the point of stressor exposure.

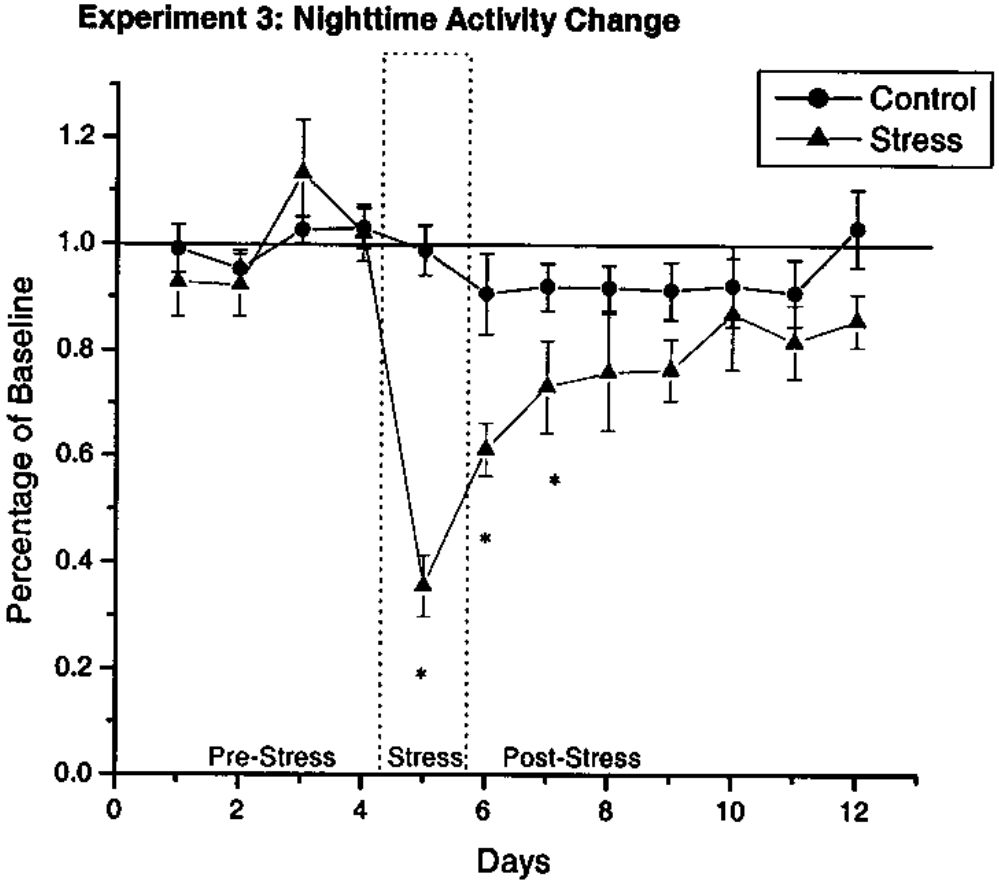

Figure 20: Nighttime activity changes for stress and control animals exposed to one day of stressor. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$.

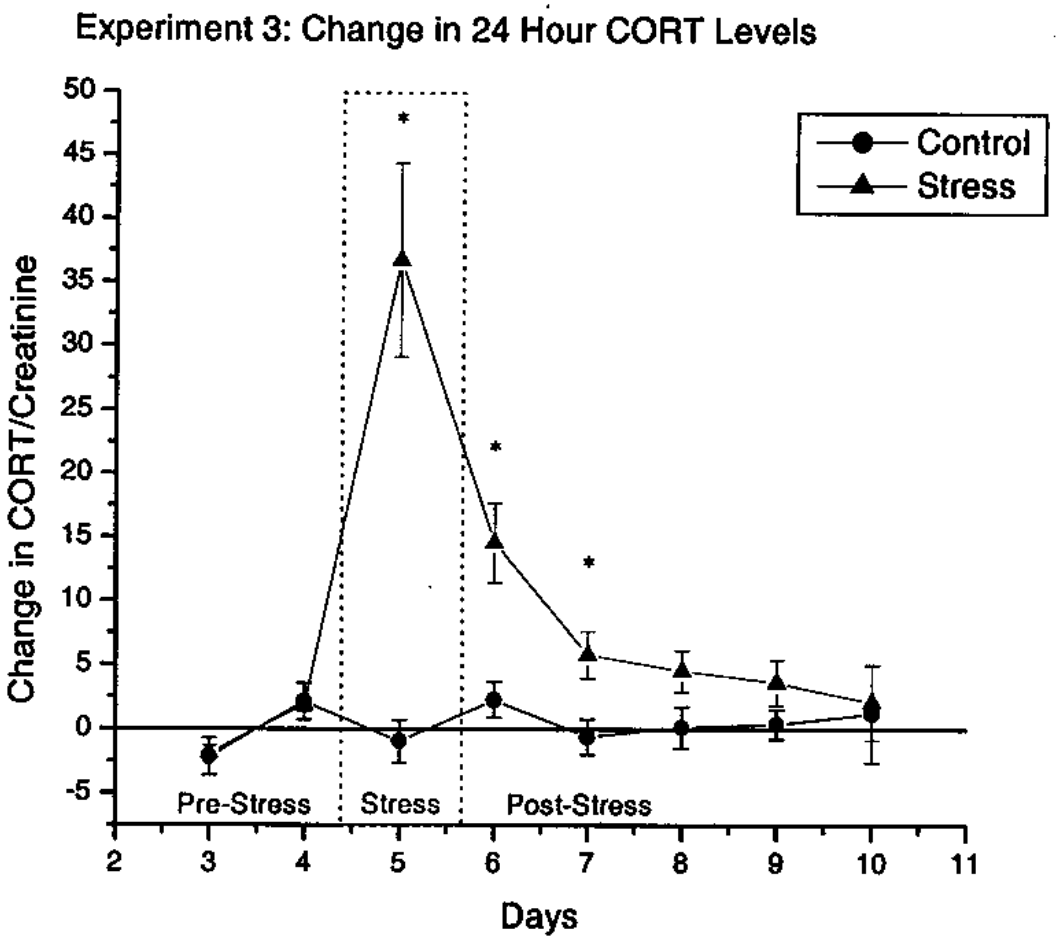

Figure 21: Changes in 24-Hour urinary cortecosterone levels for stress and control animals exposed to one day of stressor. The dotted line indicates the point of stressor exposure. Asterisks represent significant differences from control means, $p<0.05$

Discussion

Experiment 1:

Experiment 1 compared the responses of temperature and activity to three days of stressor exposure in stressed and non-stressed rats. We wanted to see if our chronic stress model (Pitman, et al., 1988; Ottenweller, et al., 1989; Ottenweller, et al., 1992;) could effectively be measured by non-invasive means.

Our results demonstrate that with three days of inescapable tail shock stress daytime temperature (Figure 3) is elevated on the second and third day of stress as well as the three days post stressor exposure. Other literature did not show such persistent effects. This may be due to the extreme stressor that was used. Other studies used variable forms of stress such as cage switch stress (Long, et al., 1990; Briese and Cabanac, 1991), social defeat stress (Harper, et al., 1996; Meerlo, et al., 1996; Meerlo, et al., 1996; Meerlo, et al., 1997;), sham intraperitoneal injection (Dymond and Fewell, 1998), surgery (Harper, et al., 1996), open field (Kluger, et al., 1987) escapable stress (Kant, et al., 1991) and inescapable shock at 1.6 mAmp (Deak, et al., 1997). Our stressor was 2mAmps. Deak, et al. (1997) noted elevations for two days post stressor exposure.

The nighttime temperature for the rats did show significant elevations on the nights following the second and third stressor exposure, as well as persistent effects up to three evenings post stressor (Figure 4). Most of the literature did not take into account the nighttime temperature changes. Nighttime temperature elevations occur naturally due to a circadian rhythm in the nocturnal rat (Gordon,

1990; Franken, et al., 1992; Refinetti and Menaker, 1992). However, our results demonstrate that stress leads to significant increases in the nighttime temperature above those due to the normal circadian rise.

For activity we found no significant effects due to stressor exposure on daytime activity (Figure 5). This is consistent with other literature (Meerlo, et al., 1996). However, we did find significant decreases in the nighttime activity (Figure 6). These decreases were not persistent and remained approximately forty percent below baseline only for the evenings following stressor exposure. Other literature has reported similar results (Meerlo, et al., 1997).

With these findings, we feel that temperature is a good way to assess the chronic effects of stress. Deak, et al. (1997) mention that temperature is also a good way to assess the acute effects of stress. Therefore, we can concur suggest that temperature is a good way to measure the persistent effects of stress. Activity on the other hand, returned to baseline the evening following stressor exposure. Therefore, it may not be as sensitive of a marker for the chronic stress response.

Experiment 2:

Experiment 2 compared the responses of temperature, activity and urinary corticosterone for three days of stressor exposure in stressed and non-stressed rats. We also attempted to attenuate or block the persistent effects of stress through the non-peptide CRH antagonist CP-154,526 or Antalarmin.

Previous research by Deak, et al., (1999) demonstrated that treatment with CP-154,526 prior to inescapable foot shock stress blocked the rise in ACTH levels. Research by Schulz, et al. (1996) further suggested that CP-154,526 demonstrates anxiolytic activity. With these results in mind, we attempted to see if this drug would block the chronic effects of our stress. Figures 7-11 demonstrate the results. We saw no differences between animals treated with the CRH antagonist versus the vehicle. However, we did see significant results due to stressor exposure. Our lack of significant differences for the vehicle versus drug animals may be due to a number of factors. On one hand, our stress model may be too severe. Deak, et al. (1999) only used 2, 1.6 mAmp foot shocks. We used 40, 2 mAmp tailshocks. In this same regard, Schulz, et al. (1996) tested this drug in rats exposed to acoustic startle. Secondly, the drug's effective concentration is 20mg/kg with a half-life of approximately one hour. We administered the drug approximately one half hour prior to stressor exposure. Therefore, the effective concentration may have been dramatically decreased by the end of the stressor session. Selye (1946) suggested that the General Alarm Reaction occurs 6-48 hours post stressor exposure. Hence, most of the stress response may occur well after the stressor exposure and after the drug had worn off.

Since data did not show any effect due to the drug or vehicle condition, data were collapsed and analyzed for stress versus non-stress rats. Experiment

2 was novel in the fact that it measured activity, temperature and urinary corticosterone all at the same time.

For daytime temperature (Figure 12), the data demonstrated similar elevations as those in Experiment 1. However, the elevations above control values persisted for seven days post stressor exposure. For the stress animals the temperature elevations seem to return to baseline by 5 days post stressor exposure. The temperature in non-stress rats seems to decrease below baseline from days 3 to 7 post stressor. This change may have been due to a decrease in ambient temperature for the non-stress room or an effect due to the DMSO vehicle. Further study will be necessary to determine the reason for this effect.

The nighttime temperature change (Figure 13) does not demonstrate a significant elevation in temperature for the stressed animals versus the control animals. However, the stressed rats did have marginally significant increases on days 2 to 6 post stressor. This is different from Experiment 1, perhaps due to the smaller number of animals in this experiment. Both stress and non-stress animals demonstrate a trough in temperature on the first evening post stressor exposure. This trough returns to baseline for the stress animals gradually. However, the non-stress rats' temperatures remain lower. This may be another effect of the DMSO vehicle or a possible change in room temperature.

For the daytime activity (Figure 14) there appears to be no significant changes in activity counts between stressed and non-stressed rats. This is consistent with previous literature (Meerlo, et al., 1996). However, nighttime

activity (Figure 15) shows significant decreases post stressor exposure. This is consistent with the findings in Experiment 1, as well as previous literature (Meerlo, et al., 1997). Moreover, there is a persistent decrease in activity that remains for one day post stressor exposure. This finding when compared to Experiment 1 may suggest a difference in the way the rat deals with stress when housed in a metabolic cage versus a shoebox cage. Perhaps there is less movement in a rat housed in a metabolic cage than in a shoebox cage. This conclusion needs further study.

The final portion of the experiment involved a metabolic marker of stress such as urinary corticosterone levels. Former studies demonstrated that inescapable stress leads to persistent elevations of plasma corticosterone levels (Ottenweller, et al., 1989; Ottenweller, et al., 1992; Ottenweller, et al., 1994; Servatius, et al., 1994; Servatius, et al., 1995). Using urinary corticosterone to measure the stress response has been used previously (Bitter and Nielsen, 1972; Kley, et al., 1978; Brennan, et al., 2000), but obviously not as often as plasma CORT. There may be two reasons for this. First of all, the apparatus necessary to collect urine is expensive and more time consuming. Secondly, fewer time intervals can be sampled in a given period.

Urinary CORT assay of Experiment 2 (Figure 16) demonstrates a dramatic increase in CORT for stressed rats versus non-stress rats. Moreover, urinary CORT remains elevated for one day post stressor exposure. Therefore, 24-hour urinary CORT does demonstrate the persistent effects of inescapable stress that

are similar to findings by Brennan, et al., 2000). However, Brennan, et al. (2000) also demonstrated that urinary CORT remained elevated for 3 days post stressor exposure. Experiment 2 does not demonstrate such persistent effects. This finding may be due to the exposure to the CRH antagonist in some rats or the smaller number of rats in the groups.

Experiment 2 allowed us to measure temperature, activity and urinary corticosterone relative to stressor exposure, all at the same time. From our findings, we know that temperature is an effective measure for the chronic stress response. We know that activity may not be as sensitive for the same function. Finally, urinary corticosterone for this study does not demonstrate as long term changes as it has in previous literature.

Experiment 3:

Experiment 3 was an attempt to minimize stressor exposure and to see if one exposure to a traumatic stressor could lead to persistent results. Daytime temperature change (Figure 17) demonstrates persistent elevations in temperature for up to four days post stressor. Again, temperature is effective in measuring the long-term effects of stress as was demonstrated in both Experiments 1 and 2. One exposure to stress also demonstrates relatively cleaner data with reduced error. These data also show that one exposure to the stress model is sufficient to lead to long-term effects. Previous research by Ottenweller, et al. (1992) suggests that one day of stress also leads to significant metabolic and behavioral changes.

The nighttime temperature changes (Figure 18) demonstrate similar results as found in Experiment 1. There is an elevation in nighttime temperature on the second, third and fourth nights post stressor exposure. There is also a profound decrease in temperature for the stressed rats on the evening following stressor exposure. This study would need to be replicated to determine if this is a consistent effect. Unfortunately, previous literature has not provided information regarding nighttime temperature; therefore, there is nothing to compare with. However, this finding is not consistent with our previous experiments because we see a decrease in temperature for both stress and non-stress rats on the first night post stressor exposure.

Daytime activity for rats exposed to one day of stress (Figure 19) shows very interesting results. These findings may have been hidden by the three stressor exposures in the previous studies. On the stressor exposure day, daytime activity is elevated for the stress animals. On the day after stressor exposure, the daytime activity is elevated for the non-stress rats whereas, the activity of stress rats has returned to baseline. This may be due to the fact that a plasma sample was taken 24 hours after stressor exposure for both non-stress and stress rats. This disturbance may have lead to unnecessary stress on the rats. This finding does show that there can be pronounced exaggerated responses to the stress of blood collection. This fuels the argument that previous measurements of the stress response, actually increase stress. Looking back at daytime temperature (Figure 17), there is also a slight increase in temperature for

the non-stress rats 24 hours post stressor. This finding may be due to the blood sampling.

Nighttime activity change (Figure 20) demonstrates a decrease on the evening following stressor exposure that persists 48 hours later. This is a more pronounced effect than seen in the previous experiments. Meerlo, et al. (1997) suggest that stress-induced changes in activity return to normal in about three days. Our findings are consistent with this. Moreover, the more persistent changes to nighttime activity can be seen when the stressor is only one traumatic exposure. These findings also suggest validity to the observation in Experiment 2 -- animals housed in metabolic cages demonstrate more persistent decreases in activity than those housed in shoebox cages.

The measurement of 24 hour urinary CORT demonstrated significant elevations that persisted for two days post stressor exposure. This finding is close to the findings by Brennan, et al. (2000) which demonstrated elevations for three days post exposure after three consecutive stressors. Therefore, less exposures to traumatic events can lead to similar changes in urinary CORT.

Finally, 24-hour plasma samples assayed for corticosterone demonstrated elevations for the stress rats as compared to the nonstress rats. This finding was consistent with previous findings in our lab (Ottenweller, et al., 1989; Ottenweller, et al., 1992; Ottenweller, et al., 1994; Servatius, et al., 1994; Servatius, et al., 1995). This sample also demonstrated added stress to the animals which was evident through changes in behavior and temperature.

Experiment 3 enabled us to measure the effects of one stressor exposure on the non-invasive markers. We found consistent results with our previous experiments. We again found temperature to be a good marker of the chronic effects of stress. We also found activity demonstrated some persistent effects, but they did last as long as the temperature changes. Finally, we found that urinary corticosterone is persistently elevated.

General Discussion:

From the preceding findings we can conclude that stressor exposure leads to pervasive effects on the organism. These effects can be assessed through numerous methods. Unfortunately, some of these measurements add unneeded stress to the organism as was evident in Experiment 3 with blood collection.

Experiment 1 attempted to measure the persistent effects of stress through temperature and activity. It accomplished its purpose and demonstrated that Deak, et al. (1997) correctly concluded that temperture is a good measure for the acute response to stress. Our findings demonstrate that it is also a good measurement for the persistent effects of stress.

Experiment 2 extended the previous research and attempted to measure urinary corticosterone, core body temperature and behavioral activity all at the same time. Though we were unsuccessful at blocking the persistent stress response with the CRH antagonist CP-154,526, we were able to track the persistent effects of stress with our measure.

Experiment 3 was an extension of Experiment 2 in that core body temperature, urinary corticosterone and behavioral activity were measure at the same time. However, in this experiment only one stressor exposure was used. Ottenweller, et al. (1992) demonstrated that both single and triple exposures to stressors lead to pervasive changes in plasma corticosterone, behavior and activity. This study demonstrated that the more exposures, the more extreme the effects. Experiment 3, in regards to temperature, demonstrates a strong persistent effect after one stressor.

These experiments have set the stage for further exploration. The next step would be to try the CRH antagonist in a higher dose. Being that the effective dose is 20 mg/kg, perhaps increasing the dosage may help block the CRH induced adenylate cyclase action which leads to ACTH increases. Since the previous literature (Deak, et al., 1999; Schulz, et al., 1996) demonstrates the effectiveness of the antagonist, perhaps a higher dose may lead to a significant effect. Moreover, perhaps giving a higher dose to the rats exposed to only one day of stressor may help in blocking the stress response. Finally, perhaps administering the CRH antagonist after the stressor session may help block the persistent stress response. This is a possibility because the chronic stress response occurs over a period of days.

Having established this model of a persistent chronic stress state, we can use this knowledge to our advantage. We can look at the mechanisms that maintain the stress state. We can explore behavioral and pharmacological

interventions. Finally, this is only the beginning of all the possibilities that we can explore now that we have established a model and a way to measure it. Perhaps, we can bring help to those that suffer from illnesses such as Post Traumatic Stress Disorder.

Summary

Stress is pervasive in today's society. Recently, chronic stress exposure has been implicated as the cause for numerous illnesses. The preceding studies explored the persistent physiological and behavioral effects of stress in the rat. The stress response was evaluated in terms of its effects on core body temperature, activity and urinary corticosterone. The persistent effects that are caused by single or multiple stressor exposures was explored. Moreover, a CRH antagonist that is being marketed as an anxiolytic was tested to see if it blocks the persistent changes of stress.

The results of these studies suggest that persistent responses to stress last from 1 to 7 days post stressor exposure. Secondly, both daytime and nighttime core body temperature are elevated in response to stressor exposure. Whereas, nighttime activity is suppressed and daytime activity remains unaffected. Urinary corticosterone, is elevated during stressor exposure as well as 1 to 4 days after stress exposure. However, urinary CORT generally recovers before body temperature does. Finally, a CRH antagonist that was effective in blocking stress effects in other experimental paradigms was ineffective at blocking any of the factors for our severe stressor.

What these studies have done is open the door to understanding some of the physiology behind the stress response. By understanding the physiology, perhaps in the future, therapeutic interventions may be developed. This will help

those that are suffering from illnesses such as PTSD. Since exposure to an extreme stressor can be devastating, understanding what underlies the persistent responses is critical to helping these patients.

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