Natural Stressors, Posttraumatic Stress Disorder, and Wound Healing, in a Murine Model

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This study investigated the use of “naturalistic stressors” such as physical restraint and animal pheromones on the etiology of Posttraumatic Stress Disorder in a murine model. Pilot data suggest that stress effects may lead to an increase in the amount of time needed for cutaneous wounds to heal. Pilot data to support the creation of this model are presented suggesting that a delayed stress response may inhibit healing rates. In the present study an animal model of PTSD was used to investigate the effect of stress on the immune system. Yehuda and Antelman’s (1993) nonhuman animal model of Posttraumatic Stress Disorder was tested with respect to the animals’ immune response to cutaneous wounding. Additionally, effects of stress on exploratory behavior and activity were examined. The findings support the hypothesis that restraint and pheromonal stress and housing arrangements influence the ability of mice to heal a 1.5 mm punch biopsy, and exploratory behavior. The findings also support a profile for the Post-Traumatic Mouse.
DEDICATION

To Belle and Deedee, it is through the sacrifice of research animals such as you, which allows us the opportunity for true scientific discovery.
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Natural Stressors, Posttraumatic Stress Disorder and Wound Healing, in a Murine Model

Posttraumatic Stress Disorder (PTSD) is a psychiatric disorder that can affect any person, regardless of ethnicity or socio-economic background (Davidson, Hughes, Blazer & George, 1991; Helzer, Robins & McEvoy, 1987; Kosten, Mason, Giller, Ostroff & Harkness, 1987; Pynoos, Frederick, Nadar, Arroyo, Steinberg, Eth, et al., 1987; Sonnenberg, 1988).

PTSD is a disorder of arousal (Everly and Benson, 1989) involving an approach and or avoidance behavioral response to trauma. This involves the chronic overactivation of Cannon’s “fight or flight” response (Cannon, 1935; Cannon, 1939; Kopin, 1995; Selye, 1956; Turkington, 1999). Individuals with PTSD do not so much remember the traumatic event, so much as relive it (Golier and Yehuda, 2002). The symptoms of PTSD may develop immediately following the trauma or the expression may be delayed for years (Kardiner, 1941; Thomas, 1997; Yehuda & Antelman, 1993).

According to Jordan, et al., (1991) more than one-third of combat veterans develop PTSD. Although the lifetime prevalence of PTSD among civilians is believed to be only 1% (Beers & Berkine, 1991; Helzer, Robins, McEvoy, 1987; Malloy, Fairbanks & Keane, 1983; Yehuda & Antelman, 1993), it has been reported that as much as 7% of the population may experience the effects

According to the DSM IV (1994), PTSD (diagnostic code 309.81) appears after exposure to an extremely traumatic event such as one involving actual threatened death or serious injury, or a threat to one’s physical integrity. This may include witnessing an event involving death, injury or a threat to one’s well-being (Saxe, Stoddard, Hall, Chawla, Lopez, Sheridan, et al., 2005). Interestingly, a stress response may also result from simply learning about an unexpected death (Kilpatrick & Resnick, 1993) or from the threat of death to a loved one (Golier, Harvey, Steiner, & Yehuda). The disorder is characterized by fear, helplessness, and horror.

Additionally, the DSM IV (1994) states that people who suffer from PTSD may perceive sensations while re-experiencing the trauma. They may exhibit persistent avoidance of stimuli associated with the trauma and display a “numbing” of general responsiveness. Symptoms of PTSD may also include hyperaroused behavioral outbursts and a state of persistent increased arousal. These symptoms may surface in social interactions, at home and at work. Symptoms must also persist for more than one month to be diagnosed as PTSD.
A Biological Basis for PTSD

Recent research indicates that PTSD assaults the neurobiological, Hypothalamic-Pituitary-Adrenal Axis (HPA) and the Sympathetic Nervous System (De Bellis, Baum, Birmaher, Keshavan, Eccard, Boring, Jenkins & Ryan, 1999; Everly, 1993; Golier & Yehuda, 1998; Pitman, 1989; Turkington, 1999; Yehuda, McFarlane & Shalev, 1998; Gordon & Wraith, 1993). Because of the activation of the HPA, the evaluation of glucocortical (cortisol) and catecholamine (epinephrine) levels become important tools in determining the impact of stress on the neurobiological system. During a response to stress the hypothalamus releases corticotropic hormones, activating the pituitary gland to release adrenocorticotropic hormones. The adrenocorticotropic hormones in turn stimulate the adrenal glands, facilitating the release of cortisol into the bloodstream.

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Insert Figure 1 here

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This neurobiological system is activated by stress and creates a negative feedback loop with cortisol on the hypothalamus (see Figure 1). The increase of catecholamines and cortisol levels produces a set of adaptive mechanisms that prepare an individual for the “fight or flight” response.
Characteristically, heart rate increases, blood flow to vital organs increases, and the skin vasculature constricts, preserving blood flow for the vital organs. Stress is a generalized adaptive response, so that catecholamine-mediated haemostatic changes also occur, including increases in platelet aggregation and other clotting factors (De Bellis, et al., 1999). This adaptive mechanism is thought to prevent blood loss in the event of an injury accompanying the “danger” signals perceived by the body. Once this neurobiological system is activated by the perception of a stressor, accurate cognitive processing may become impeded due to a decrease in blood flow to the brain (Brown, Rush & McEwen, 1999; De Bellis & Thomas, 2003; Ohl, Michaelis, Vollmann-Honsdorf, Kirschbaum & Fuchs, 2000; Yehuda, Golier, Halligan, & Harvey, 2004; Yehuda, Keefe, Harvey, Levengood, Gerber, Geni & Siever, 1995).

In PTSD there is a breakdown of the HPA system characterized by decreased levels of circulating cortisol and an increase in the number and sensitivity of glucocortical receptors (De Bellis, et al., 1999). There is an overall sensitization of the HPA system and an increase in its responsiveness accompanied by a breakdown of the system’s negative feedback inhibition (De Bellis, et al., 1993; Yehuda, Resnick, Kahana, & Giller, 1993). Yehuda (1993) reports an increase in the urinary output of norepinephrine (noradrenalin),
epinephrine, and dopamine in response to the constant stimulation of the HPA. This increased urinary output is believed to be influenced by the effect of stress on the sympathetic nervous system. This provides physiological evidence of a chronically hyper-aroused nervous system.

According to Pitman (1989), PTSD is accompanied by an increase in the level of catecholamine (specifically epinephrine) in the blood stream. Epinephrine impacts the nervous system by increasing arousal and is involved in the consolidation of memories. Pitman describes the result of increased epinephrine in individuals with PTSD as “over consolidation” of memories. Potentially, this may explain why survivors of trauma report reliving the memories, instead of just remembering the event.

Another consequence of neurobiological changes in persons with PTSD is the interaction of catecholimines and glucocortoids resulting in increased levels of epinephrine but decreased levels of cortisol. This is attributed by researchers to the failure of cortisol to inhibit the negative feedback system (Pitmin, 1989; Yehuda et al., 1993). In theory, the failure to inhibit keeps the entire system in a constant state of arousal. In animal studies, De Bellis et al. (1998) found that a constant state of stress (elevated stress hormones) leads to a reduction in the volume of neurological structures. He reported that in
response to chronic stress there is a reduction in hippocampal size and an increase in abnormal synaptic pruning accompanied by an overall reduction in the size of the cerebrum.

**Glucocorticoids and the Hippocampus**

The effect of glucocorticoids (corticotropin, adrenocorticotropin, and cortisol) on the hippocampus may have a dramatic impact on the ability of an animal to assimilate new information. Damage to the hippocampus may explain some of the behavioral effects shown in PTSD. According to De Bellis et al. (1998) glucocorticoids target the hippocampus, leading to a reduction in memory processing and the destruction of the hippocampus.

Pribram (1991) reports that the hippocampus is responsible for providing a person with a sense of “context” and determining whether attention should be paid to particular stimuli. In his research, Pribram found that when the hippocampus was removed from monkeys, the animals displayed increased levels of caution. This may provide a neurobiological explanation of some of the symptoms of PTSD, specifically, the expression of avoidant behaviors and the inability to shift attention away from the relived trauma may be the result of the neurochemical assault on the hippocampus.

Given the evidence that there are altered levels of serum cortisol and epinephrine as the result of chronic stress, one
would be inclined to think of their presence as indicative of PTSD. On the contrary, as Resnick (Resnick, Yehuda, Pitman, & Foy, 1995) indicates, there is currently very little data available on these patients indicating a pre- to post-trauma blood serum change. Therefore, blood serum levels may be seen as a correlative and not a causative factor in PTSD. With this in mind, the measurement of blood serum levels provides valuable data in the investigation of chronic stress and illustrates the need for additional research.

Pavlovian Influences in PTSD

Kilpatrick (Kilpatrick & Resnick, 1993) theorized that PTSD is formed by association during observational learning. He utilizes influences identified by Social Learning Theory in which a powerful association is created through the observation of the traumatic event. Kilpatrick describes the association made as a “Vicarious Trauma”. Similarly, Van der Kolk (1996) states that PTSD may occur completely as the result of classical conditioning. The process involved allows the associated stimuli to produce constant stimulation of the person leading to a physiological response.

Pavlov (Grimsley & Windholtz, 2000) stated that one of the strengths of conditioning is its ability to activate and inhibit the nervous system. This demonstrates a possible mechanism for both the creation of PTSD and a possible mechanism for
treatment. Combined with findings of lower cortisol and increased epinephrine levels in children with PTSD, this ability provides possible applications of Pavlovian counter-conditioning techniques in the treatment of PTSD. If the associative cues can be identified, a successful counter-conditioning program might be established to train the hypothalamic pituitary axis (HPA) and the sympathetic nervous system (SNS) to respond at more normal levels.

Many behavioral symptoms resulting from dysfunction of the HPA and SNS may be explained by the assault of neurochemicals on a person's neurobiological system. Additionally, some findings show increased volume of the brains ventricles, which may contribute to the intrusive thoughts reported by sufferers of PTSD (De Bellis, et al., 1999). Damage to the hippocampus may also contribute to creating a "fixation on the event" described by sufferers of PTSD. Basically a person loses the ability to shift away from the traumatic memory (De Bellis, et al., 1999; Pribram, 1991; Yehuda, et al., 1993).

Influences of Learned Helplessness

The animal model of PTSD that has received the greatest acceptance has been derived from inescapable shock experiments or research on learned helplessness (van der Kolk, Greenberg, Boyd, & Krystal, 1985; Southwick, Krystal, Johnson, Charney, 1992; Yehuda & Antelman, 1993). Learned helplessness is a
learned inability to overcome obstacles in the environment or to avoid punishment. Symptoms of learned helplessness are similar to those of depression, including feelings of powerlessness and hopelessness, decreased activity, decreased aggression, loss of sexual drive and appetite, and (in humans) a tendency to see oneself as failing (Miller, Rossellini, and Seligman, 1977). This effect was initially described by Seligman (1974) who demonstrated this in his classic study on dogs.

When placed in one side of a divided box, dogs will quickly learn to leap to the other side when given a mild shock. If given a warning before the shock, most dogs will learn to avoid the shock by jumping to the other side. Seligman found that if the dog was restrained and prevented from escape then even when released from the restraint, the animal would not try to escape the shock (Overmier & Seligman, 1967; Seligman, 1974). The animals would helplessly accept their fate. Learned helplessness is now considered to be a major contributor to both depression and PTSD (Barlow & Durand, 2005; Nolen-Hoeksema, Girgus & Seligman, 1986; Tanaka & Huba, 1984).

Animal Models in PTSD

King, Abend, and Edwards (2001) looked at ingrained learned helplessness in an animal model. In their investigation, the authors looked at the effects of genetic disposition as a risk factor for the development of PTSD-like behaviors. “Congenital”
learned helplessness was described by these researchers as the result of breeding pairs (33 generations) of rats that had illustrated learned helplessness behavior in the presence of a fear stimulus (foot shock). The animals were observed for changes in pain tolerance, spatial memory and HPA Axis functioning following exposure to stress in the presence and absence of situational cues. Their findings showed an increase in pain tolerance, a decrease in spatial memory, and a decrease in corticosterone response for animals in the congenital learned helplessness group.

King, et al. (2001) theorized that learned helplessness is not only a major contributing factor in the development of PTSD but that this influence also contains a heritable component. In their congenital learned helplessness animal model, the stressed animals exhibited physiologic symptoms of analgesia, cognitive deficits and hypo-responsivity of the HPA axis similar to what is observed in humans with PTSD. Their findings suggest that the susceptibility to develop PTSD may be related to a gene-environment interaction. PTSD may very well be a disorder that some individuals are predisposed to develop.

Pynoos, Ritzmann, Steinberg, Goenjian and Prisecaru (1996) also looked at the role of situational reminders to fear provoking stimuli (foot shock). However, they looked at the influence of those stimuli on the animals’ startle reflex as it
relates to PTSD. In their animal model, the authors used repeated exposures to situational reminders of a traumatic experience (shock) and measured the effects of the trauma on the mouse’s exploratory behavior and startle response. Exploratory behavior was measured as the amount of time needed to cross a simple “plus” maze and the startle reflex was measured by a “startle box” (A startle box presents a loud noise and measures the movement of the animal within).

Pynoos, et al.’s (1996) study revealed an initial increase in locomotion activity in a neutral environment, but a persistent decrease in exploratory behavior and an increase in the startle response when animals were exposed to situational reminders of trauma. These changes continued to increase over time. They also found that situational reminders produced an increase in aggressive behavior, noting that the mice had developed behavior changes analogous to patients with PTSD. The animals showed increased aggression, increased startle response, and a decrease in exploratory behavior.

Criteria for Animal Models

Animal studies have been and continue to be a valuable tool for the investigation of the nature of interactions between stress and the neurobiological systems involved in the development of PTSD (Rasmusson & Charney, 1997). Yehuda and Antleman (1993) noted in the animal literature that different
types of stress models can lead to different biobehavioral consequences and presented five criteria for the evaluation of animal models and PTSD. Yehuda and Antelman (1993) developed the criteria by paring down what they believed to be the most basic components of PTSD and identifying the relevant counterparts for these clinical characteristics based on animal studies.

First, according to Yehuda and Antelman (1993), even very brief stressors should be capable of inducing biological and behavioral symptoms of PTSD. The major defining characteristic of the stressor does not appear to be the actual duration of the stimuli, but the extent to which the traumatic event elicits the intrusive re-experiencing, avoidance, and hyper-arousal symptoms of PTSD. Yehuda and Antelman argue that animal models that depend exclusively on long-lasting stressors, training, or learning are not relevant to the induction of PTSD.

Second, the stressor should be capable of producing the PTSD-like symptoms and behaviors in a dose-dependant manner. Theoretically, any type of stressor of sufficient magnitude should be able to induce the biobehavioral aspects of PTSD. Therefore, the duration of the stressor does not seem to be as relevant as the dosage or the intensity of the stressor. For example in humans, PTSD is believed to be produced by a threshold dose of stress that is outside the area of normal experience (Yehuda & Antelman, 1993). In laboratory animals,
however, as the authors point out, any experimentally induced stressor is arguably outside the range of the animal’s usual experience.

Third, the stressor should produce biological alterations that persist over time or that become more pronounced with the passage of time. According to Yehuda and Antleman (1993) one would expect to see a gradual worsening of biological abnormalities with the passage of time, culminating in larger scale defects as the behavioral symptoms worsen. They recommend that when considering paradigms that might model the biological aspects of PTSD, the experimenter should choose those models which identify long term biological changes following the stressors. To demonstrate a relationship between immediate biological changes in response to stress and the subsequent development of PTSD, it is important that studies of acute biological responses be followed up by studies of long-term responses to stress.

Fourth, the stressor should induce bio-behavioral alterations that have the potential for bi-directional expression. Increasing or decreasing differing biological functions (changes in corticosterone, noepinepherine ect.). This would involve the possible activation and continued stimulation of the HPA axis. Therefore, the stressor should be sufficient enough to produce a response and elevate hormone levels leading
to an increased stress response behaviors (for example, a change in exploratory behavior). According to Yehuda and Antleman (1993) an ideal animal model will show cycling between excitatory and inhibitory behavioral changes. They refer to this criterion as a form of Occam’s razor to discriminate among potential animal models. An effect animal model should show an increase in some behaviors (i.e. startle, nervous activity) and a decrease in other behaviors (i.e. exploration of novel environments).

Finally, individual variability in response to a stressor should be present either as a function of experience, genetics, or an interaction of the two. They theorize that several factors other than the trauma may contribute to the induction of PTSD. Additionally, although exposure to trauma remains the most salient predictor of who will develop PTSD, not all people exposed to a trauma will develop symptoms. Yehuda and Antelman (1993) state that the major implication of interindividual variability in response to stress (for animal modeling in PTSD) is establishing the other variables that play a role in modulating the response to stressful traumas.

The Natural Stress Model

This project investigated the effect of “naturalistic stressors,” as opposed to stressors unlikely to occur in nature, such as an electrical foot shock. The stress model is based on
Padgett, Marucha, and Sheridan’s (1998) combined use of a Predator Pheromone (Red Fox urine) and Falcon tube restraint. The rationale for the restraint as a stressor is that the restraint tube (50 ml Falcon tube) mimics the collapse of the mouse burrow. This is believed to trigger coordinated neuroendocrine responses including an increase in glucocorticoids and activation of the sympathetic tone, responses evolved to facilitate digging out of the burrow and escaping. Additionally, isolation stress was investigated by this study. To accomplish this the mouse’s housing arrangement was manipulated. The mouse was either housed in isolation (single) or housed with co-habitants (group).

**Pilot Study – Part One: Stress and Wound Healing**

In the initial pilot research the impact of natural stressors on the immune system was investigated through a comparison of wound healing time in stressed (exposure to restraint + pheromone) versus unstressed animals, caged in or in single (isolated) living conditions. Twenty-eight SKH-1 (hairless) female mice were purchased from Charles River Laboratories. All “stressed” mice (single and group caged) received a small (1.5 millimeter) punch biopsy on the back between the shoulder blades. Mice in the single and group housing conditions were then placed in the combined restraint-
pheromone stress tube. The animals were in the stress apparatus for two hours.

The wounded mice were allowed to heal naturally. Differences in wound healing among the treatment groups were measured. Wound healing in each group was measured daily by reaction to hydrogen peroxide (presence of foaming) and measurement of the diameter of the wound until the wound was completely healed. The wound was considered healed once there was no reaction to the hydrogen peroxide. At the conclusion of the healing period, an additional behavioral measure of “escape time” was taken on all participants. Escape time was defined as the amount of time (in seconds) for the animal to escape from a clean restraint tube (an open-ended 50ml Falcon tube).

Wound healing was analyzed using a 4 x 14 mixed analysis of variance (ANOVA) with repeated measures. The between subject variable of group had four levels (single stressed, single control, group stressed, group control). The within-subject variable represented the number of days to heal. The ANOVA results show a significant main effect for the between variable of group, \( F(3,49) = 22.09, p < .05 \). There is a significant difference between the stressed and unstressed groups. For the within variable of “day” a main effect was also found \( F(13,623) = 431.42, p < .05 \). The animals' wounds showed significant healing changes on a day to day basis.
A significant interaction was also found between “group” and “day”, $F(39, 623) = 6.40, p < .05$. The rate at which each animal’s wounds healed was significantly influenced by the presence of natural stressors. Healing rates differed for all groups: single stressed, single control, group stressed, and group control (See Figure 2).

Insert Figure 2 here

Single housed animals exposed to natural stressors healed the slowest. This was followed by group housed animals exposed to stress. Animals housed singly with unstressed exposure healed at a faster rate and animals housed together without stress healed the fastest. Clearly the presence of natural stressors influences wound healing. Chronic stress slows the body’s ability to close a wound. But does this short term exposure lead to lasting effects on the immune system?

Pilot Study - Part Two: Extended Stress Effects

To investigate the extended effects of exposure to traumatic stressors this investigator allowed the female colony to recover for an additional two weeks in their original groups (four weeks total from initial wounding). The animals were again wounded using a 1.5 mm punch biopsy on the back between the shoulder blades. As in the first part of the pilot study, the animals were allowed to heal naturally and their progress was
checked by daily measurement and exposure to hydrogen peroxide. There were no additional “stress” exposures.

Wound healing was again analyzed using a 4 x 13 analysis of variance with repeated measures on the individual groups. As before, the between subject variable had four levels corresponding to the four groups. For the between variable, group number denoted in which stress/housing condition the animal resided (single stressed, single control, group stressed, group control). The within variable had 13 repeated measures, each measure representing the number of days involved in healing. The results of the ANOVA show a significant main effect for the variable of group, $F(3,50)= 3.99, p < .05$. There is a significant difference between the stressed and control groups. Stressed groups took more days to heal. For the variable of day, a main effect was also found, $F(12,600)= 771.52, p < .05$. The animals' wounds showed significant healing on a day to day basis, indicating a sufficient rate of wound healing.

A significant interaction was also found between “group” and “day,” $F(36,600)= 2.16, p < .05$. The rate at which the animal’s wounds healed was significantly influenced by the prior exposure to natural stressors. Healing rates differed for all groups: single stressed, single control, group stressed, and group control (See Figure 3).
Even though the animals received no further exposure to the “natural stressor,” there appears to be a continuing residual effect of exposure to the stress. However, can this be described as a “posttraumatic stress effect?” The difficulty is that although only the experimental groups were exposed to the “stressor,” all groups were previously wounded (a stressful trauma in itself). Additionally, a behavioral measure of “escape time” was recorded. Escape time was defined as the amount of time needed for the animal to exit all four limbs from the stress tube (50ml Falcon tube), without the presence of the fox pheromone. The animal was placed completely into the tube and the open tube was placed back into the animal’s cage. Escape time was measure from the release of the animal to exit. A one-way, between groups analysis of variance revealed a significant effect according to which group the animal was exposed, \( F(3,23) = 17.53, p < .05 \). The amount of stress and housing conditions of the mouse influenced escape (exploration?) behavior.

A Tukey post hoc analysis reveals that the single stress group (group 1) took more time (13.804 sec total) to exit the tubes than animals that were not stressed (group stressed = 4.84 sec, single control = 1.375, group control = 1.375). No other
groups were significantly different. Interestingly, there is a difference between grouped stressed and single stressed groups. The presence of other animals appears to insulate the participant from the stress. This finding may suggest the development of learned helplessness in high stress-single housed (no social support) animals may be a factor in the ability of the animal to recover from a physiological wound. This indicates a need for further research to establish the interaction of natural stressors on physiology and behavior.

Purpose of Current Study

With consideration to the criteria for animal models in PTSD by Yehuda and Antelman (1993), this study investigated the effect of (1) a short term (two week), (2) dose dependent (2 hours) exposure of a “naturalistic stressor” (Red Fox Urine). (3) Biological alterations were determined by corticosterone measurement and changes to the immune system were measured by wound healing. (4) Behavioral measures were gathered by measuring “escape time,” by observing exploratory behavior in a plus maze, and by changes in spontaneous motor activity (SMA). To address Yehuda and Antelman’s final point (5) of inter-individual variability, animals exhibiting increased escape times, decreasing exploration and increased corticosterone levels were identified and labeled as PTSD mice for comparison.
This created and additional comparison group of individual mice which met the new diagnostic criteria for PTSD.

**Hypotheses**

1. Stressed mice will exhibit slower wound healing, increased blood corticosterone, increased escape, and decreased exploration than unstressed control mice.

2. Single housed animals will show slower wound healing, increased blood corticosterone, increased escape, and decreased exploration than unstressed control mice.

3. Mice exhibiting the greatest corticosterone levels, greatest escape times, and slowest exploration will also show the slowest healing times.

**Methods**

**Subjects**

A power analysis was calculated using the standard deviation from pilot one \((\sigma=0.5642)\). Based on the findings of the analysis, a total sample size of 20 with 5 mice per cell was needed \((\alpha=0.05 \text{ and } \beta=0.50)\). To account for the possibility of mortality in shipping the n was increased to 6 mice per cell. Twenty-four SKH1 hairless male mice were purchased from Charles River Laboratories. While the previous pilots used female mice it was decided for the final study to switch to a male model. This will hopefully provide additional data for future research on gender comparisons. The mice were randomly separated into
single and group cages and designated “stress” or “control” (see Table 1). Charles River Laboratories policy is to ship an extra mouse incase of damage in shipping. This additional mouse was assigned to the “stress” group. The animals were allowed unlimited access to food and water and were on a regulated 12-hour light/dark cycle. Daily animal care was provided by the experimenter and two research assistants. Training in care and animal husbandry was received by the author at the Johns Hopkins Animal Services, with additional training from Old Dominion University and the Eastern Virginia Medical College. The project was approved by the Old Dominion University’s Animal Care and use Committee.

Apparatus

Combined Stressor

The “stressed” mice were placed in a modified 50 ml Falcon tube with a 5 ml cup of Red Fox urine placed at the opening of the tube (the scent of urine of a predator is a known trigger of the murine stress response). This stress apparatus was then placed in a sealed chamber to contain the pheremonal scent. The mice were exposed to the stressors for a total of 2 hours.

Plus Maze

The plus maze was constructed based on the design used by Pynoos et al. (1996). In their study, Pynoos constructed an
elevated plus maze with an elevation of 36 inches, considered the height at which fear is provoked.

Spontaneous Motor Activity

Spontaneous Motor Activity (SMA) was measured by means of an Opto-Varimax mini “A” activity meter (Columbus Instruments). The test enclosure measured 58 x 42 x 23 cm. Consistent with the behavioral observations in the Plus Maze, mice were again observed for 5 minutes, and total Ambulatory movement was recorded. Measures were taken at Baseline, 2 weeks, 4 weeks and 6 weeks.

Procedure

Mice were randomly separated into four groups: A = single (isolated housing) and stressed (combined pheromone and restraint), B = single and control (not stressed), C = group (housed with companions) stressed, and D = group (housed) and control (not stressed).

Insert Table 1 here

All animals were then placed randomly into the Spontaneous Motor Activity unit and ambulatory activity levels were measured. Mice in groups A and C were then placed in a modified 50 ml. Falcon tube with a caged insert containing a 1 inch square gauze pad dipped in the predator pheromone (Red Fox
urine). The animals remained in the unit for 2 hours. This procedure was repeated for a total of 14 days. The control mice remained undisturbed, although food and water were removed for the same period of time. On the 15th day of the study all mice were wounded at 1 pm. The animals each received a small 1.5 mm, bilateral punch biopsy between the shoulder blades. Mice were alternately rotated so that 50% of the mice were wounded through the left shoulder to the right and 50% of the mice wounded form the right shoulder to the left. This was done to control for any potential differences for entry versus exit wounds. The wounded mice were allowed to heal naturally. Differences in wound healing among the treatment groups was measured daily by a “blinded” research assistant, who observed the reaction to hydrogen peroxide ($H_2O_2$) and measured the diameter of the wound until the wound was completely healed. All measures were recorded between 2 and 3 pm.

Once all groups had completely healed, (as measured by $H_2O_2$ reaction) the animals were again placed in the Spontaneous Motor Activity chamber and activity was recorded. All animals were then placed in random order into the Elevated Plus maze and individual exploration times were recorded. Mice were placed into the closed end of the maze and number of entries into open and closed arms were counted for a total of 5 minutes (Walf & Frye, 2005; Walf & Frye, 2007).
At the conclusion of the maze running, the additional behavioral measure of “escape time” was taken on all participants. Escape time was defined as the amount of time for the animal to escape from a clean restraint tube (an open ended 50 ml Falcon tube). All behavioral measures were taken at Baseline, 2 weeks, 4 weeks and 6 weeks.

At the conclusion of the study the blood was drawn by cardiac puncture by the experimenter and Dr. Linda Hargrove of Old Dominion University’s Biology Department. The procedure began the day following the final mouse healing at 1 pm. Mice were anesthetized through Halothane inhalation. The blood was placed on ice and shipped overnight to Antech Diagnostics for Radio Immuno Assay (RIA) to determine corticosterone levels. Blood was picked up by Antech Diagnostics Technician at 4:30 pm sealed in a 12 x 12 inch syrofoam cube, packed in ice.

Results

Wound Healing - $H_2O_2$ Reactivity

A foaming reaction to $H_2O_2$ provided a measure to determine whether the subject had completed healing. A healed wound was defined as the absence of $H_2O_2$ foaming on the wound. The number of days needed to heal was measured up to the last day of $H_2O_2$ reaction or the last day wounded. For this comparison an equal n was desired, so one mouse from the single stressed group was randomly withdrawn. A 2 stress x 2 housing, factorial design
analysis of variance (ANOVA) was used to analyze the rate of wound healing. The variable of Housing consisted of two levels (single housing vs. group housing). The variable of Stress contained two levels (stressed vs. unstressed).

As depicted in Table 2, significant Main effects were found for Stress, $F(1,44) = 7.10, p = 0.0107$ and Housing, $F(1,44) = 4.08, p = 0.0496$. The interaction effect of Stress x Housing was not significant ($F(1,44) = 3.45, p = n.s$).

Single housed, Stressed mice healed the slowest ($\bar{X} = 10.5$ days), followed by the group housed, Stressed mice ($\bar{X} = 8.5$ days). Unstressed mice healed the fastest. Single unstressed mice healed somewhat faster ($\bar{X} = 8.1$ days) than did the group unstressed mice ($\bar{X} = 8.08$ days). Overall, stressed mice required 9.5 days to heal in comparison to 8.1 days for unstressed mice. Further comparisons among the means revealed that single housed mice required an average of 9.3 days to heal, whereas group housed mice met the criterion for healing in only 8.2 days.
Wound Healing - Wound Size

Following the bilateral 1.5mm punch biopsy, wound size was measured daily within a one-hour time window (2:00 - 3:00 p.m.) using Vortech Digital Calipers. Wound size diameter was measured in millimeters, following a foaming H$_2$O$_2$ reaction.

Insert Table 3 here

A 2 Stress (stressed, unstressed) x 2 Housing (single, group) x Day (1 through 13) mixed design ANOVA was conducted with Day as a repeated measure. There was no significant effect for Stress, F(1,46) = 3.11, p < 0.0847, Housing, F(1,46) = .79, p < 0.3791 or Stress x Housing, F(1,46) = .50, p < 0.4817.

A significant main effect was found for Day, F(12,552) = 537.24, p < 0.001, corresponding to a daily reduction in wound size. Furthermore, the Stress x Day and the Housing x Day interactions were also significant using wound size as the dependent variable.

Insert Figure 5 here

Inspection of the Stress x Day interaction effect (F(12,552) = 1.93, p = 0.0290) revealed differences in wound healing rate as a function of prior exposure to the stressor.
(see Figure 5). More specifically, stress resulted in a reduction in the rate of healing in comparisons with the unstressed group. Inspection of the Housing x Day interaction ($\overline{F}(12,552) = 1.91$, $p = 0.0307$) revealed evidence for the ameliorative effect of group housing on the healing process. Single housed mice healed significantly slower than group housed mice. The Stress x Housing x Day interaction was not significant, $\overline{F}(12,552) = .65$, $p = n.s.$

**Plus Maze**

Exploratory behavior was examined by placing the mice into an elevated plus maze for 5 minutes (Walf & Frye, 2005; Walf & Frye, 2007). Data collection was initiated upon the release of the animal into the maze and continued during arm crossings (open or walled). Time was monitored with a handheld stop watch. When all four limbs of the mouse entered an arm of the maze, then an “entry” was counted. Total entries for each arm, open or closed, were analyzed using a 2 Stress (stress, unstressed) x 2 Housing (single, group) x 2 Arm (open, closed) factorial ANOVA.

Overall, mice entered the closed arms significantly more often than the open arms, $\overline{F}(1,21) = 38.44$, $p = 0.0001$. Post hoc comparisons among the means using Tukey’s HSD demonstrated that overall, the mice preferred to stay in the closed environment (compare a mean of 12.9 entries into closed arms to 7.6 entries into the open arms). There was also a significant housing x arm
interaction ($F(1,21) = 16.40, \ p = 0.0006$), housing x week interaction ($F(3,21) = 6.67, \ p = 0.006$), arm x week interaction ($F(3,21) = 3.44, \ p = 0.0219$) and housing x arm x week interaction ($F(3,6) = 5.81, \ p = 0.0014$). Although this suggests that mice preferred to enter different arms according to Housing, post hoc comparisons did not support this.

Non significant ($p > 0.5$) results were found for stress x arm ($F(1,21) = 3.46$), stress x housing x arm ($F(1,21) = 0.04$), week ($F(3,21) = 0.50$), stress x week ($F(3,21) = 1.63$), stress x housing x week ($F(3,21) = 0.18$), and stress x arm x week ($F(3,63) = 0.67$) interactions. Based on the significant Housing effect and the lack of Stress effects, the Housing environment of the mice appears to have a greater influence on exploratory behavior than the pheromone-restraint stress experience.

**Ambulatory Movement**

Spontaneous Motor Activity (SMA) was measured by placing the mice into an Opto-Varimax mini “A” activity meter (Columbus Instruments) for five minutes. The test box consisted of a box enclosure measuring 58 x 42 cm. Ambulatory movements were recorded using optical laser. The Opto-Varimax “Ambulatory” setting allows for the complete movement of the mouse to be monitored. Total ambulatory movement was subjected to statistical analysis. A 2 Stress (stress, unstressed) x 2
A significant main effect for Week (of study) was revealed ($F(3,63) = 16.99, p = 0.0001$). Post hoc analysis demonstrated that all mice were significantly more active at week one ($\bar{X} = 1113.80$ movements) than at week two ($\bar{X} = 813.64$), four ($\bar{X} = 724.16$) or six ($\bar{X} = 697.60$). Additional Housing effects emerged, with a large Housing x Week interaction effect ($F(3,63) = 19.43, p < .0001$). As with the Elevated Plus maze, Housing condition influenced the exploratory movement, with single housed mice showing more activity than group housed mice.

No Stress effect was observed ($F(1,21) = 0.35$). The main effect for Housing ($F(1,21) = 1.64$) was not reliable. The interaction of Stress x Housing was also not significant ($F(1,21) = 0.85$). Finally, there was no interaction effect for Stress x Week ($F(3,63) = 0.34$) or Stress x Housing x Week ($F(3.63) = 1.01$).

**Escape Time**

Escape time was measured from the point of release of the animal from a modified 50ml Falcon tube. The amount of time needed for the animal to fully exit (all four limbs, tail excluded) was monitored by stopwatch. Escape time was recorded over a 6 week period. Single Stressed Subject One exited the tube 100 seconds slower than all other mice, creating outlying
data. Therefore, escape data related to this subject were excluded from the analysis.

Total Escape Time was subjected to statistical analysis. A factorial ANOVA with Stress and Housing as between subjects factors and with Measurement (Baseline, 2, 4, and 6 weeks) as a repeated factor was conducted. A significant main effect for Housing was found, $F(1,20) = 8.11, p = 0.0099$. A Tukey post hoc analysis showed an average escape time of 5.39 seconds for Single Housed mice as opposed to 0.57 seconds for group housed mice. No other significant main effects were found, based on Stress ($F(1,20)= 1.00$) or Week ($F(3,60) = 2.30$). Additionally, no interaction effects were observed among Stress x Housing ($F(1,20) = 0.86$), Stress x Week ($F(3,60) = 1.01$), Housing x Week ($F(3,60) = 2.16$), or Stress x Housing x Week ($F(3,60) = 0.73$).

**Corticosterone**

At the conclusion of the study a terminal heart puncture was used to harvest blood for analysis. Blood was packed on ice and shipped to Antech Diagnostics for corticosterone analysis. The lab mistakenly ran an analysis of cortisol measured by Radioimmunoassay (RIA) of blood serum cortisol. The returned cortisol measures were analyzed by a 2 group x 2 condition, factorial ANOVA. A main effect for Housing was observed with $F(1,21) = 6.02, p = 0.0230$. A Tukey post hoc analysis showed that single housed mice had significantly lower cortisol ($\overline{X} =$
than group housed mice (\(\bar{X} = 0.2250\)). There was no Stress effect (\(F(1,21) = 1.95\)) or Stress x Housing interaction (\(F(1,21) = 0.68\)).

The Post-Traumatic Mouse

To diagnose a murine model of PTSD, mice that exhibited the greatest cortisol levels, greatest escape times, and slowest exploration were used to create a separate grouping. The differential diagnosis was based on both behavioral and blood factors with the subject scoring above the mean on two out of three behavioral measures and high cortisol.

This diagnostic produced a grouping of four subjects. Two were stress and single housed, one was not stressed and single housed and one was stressed and group housed. This new PTSD group was then compared back to the sample population using two groups ANOVA. The result show a significant difference in the number of days needed to heal for PTSD mice (\(\bar{X} = 9.7853\)) compare to non-PTSD (\(\bar{X} = 8.4722\)) mice with \(F(1,48) = 4.76, p < 0.0340\).

Discussion

Pitman (1997) discussed that much of the research on the biology of PTSD is correlative. The beauty of the current project is the direct cause and effect relationship that can be drawn from the findings. This project tested the application of Yehuda and Antelman (1993) animal model of PTSD on wound healing. It can now be argued that (1) a short term (two week),
(2) dose dependent (2 hours) exposure of a “naturalistic stressor” (Red Fox Urine), causes (3) biological alterations (increase in cortisol) and changes to the immune system (wound healing). (4) Behavioral measures were gathered by measuring “escape time,” and by observing exploratory behavior in a plus maze, and observed changes in spontaneous motor activity (SMA). Illustrating changes in behavior as a result of experimental living conditions. Additionally, (5) inter-individual variability in animals as exhibited by an increased escape times; decreasing exploration and increased corticosterone were identified and labeled as PTSD mice for comparison. The findings support the theory that PTSD affects the body’s ability to heal.

Clearly this form of natural stressor (restraint + pheromone) leads to an increase in healing time. This increase (or disruption of the body’s ability to heal) due to stress is further exasperated by housing conditions; solitary (isolated) housing combined with restraint + pheromone stress, resulted in the further inhibition of the body’s ability to heal.

Stress Effects

Mice were stressed by mimicking the presence of a natural predator, without means of escape, resulting in a significant deceleration in the rate of wound healing.
The findings illustrate that hypothesis one was supported. Stressed mice exhibited slower wound healing, higher blood cortisol levels, longer escape times, and less exploration behavior (as measured by elevated plus maze) than unstressed control mice.

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Insert Figure 6 here

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Single housed, stressed mice healed the slowest, followed by the group housed, stressed mice. Whereas, single unstressed mice healed somewhat faster than did the group unstressed mice with unstressed mice healing the fastest. Overall, stressed mice required required and additional 25 hours to heal than unstressed mice. Further comparisons among the means revealed that single housed mice required an average of 9.3 days to heal, whereas group housed mice met the criterion for healing in only 8.2 days.

These findings are consistent with the findings of both previous pilot studies. The presence of a “naturalistic” stressor combined with housing conditions affects the rate at which wounds heal. Hypothesis one is also consistent with the findings of Padgett et al. (1998), in that restraint stress slows healing. Padgett et al. observed a 3.1 day difference in healing time between stressed and unstressed female animals.
Additionally, this finding was observed when mice were continually stressed in a similar model (50 ml falcon tube, no pheromone).

Detillion, Craft, Glasper, Prendergast, and DeVries (2004) used Siberian hamsters with similar results. They adapted Padgett’s et al. (1998) mouse model of stress and wound healing to Siberian hamsters, which they believed have stronger social bonds than mice (Crawley, 1984). In their findings, Detillion et al. (2004) reports that from a 3.5 mm punch biopsy, the wound diameters showed significant decrease in healing rate as demonstrated by measurement of wound diameters. This finding was greatest in socially isolated stressed hamsters. Although they did not report any findings for number of days needed to heal for individual animals, healing times for the entire group were reported.

It is important to note that the current study shows very similar finding in rate of healing with only a 1.5 mm punch. The findings with the current study show that this effect can be observed “Post-Stress”, even with a wound that is half the size.

Housing Effects

Hypothesis two was also supported as the housing condition had an effect on wound healing. Single housed, stressed animals showed slower wound healing, higher blood corticosterone (although cortisol was actually measured) levels, longer escape
times, and less exploration than unstressed control mice (group housed). Single housed mice required an average of 9.3 days to heal, whereas group housed mice met the criterion for full healing in only 8.2 days. Housing also successfully predicted differences on behavioral measures. As single housed mice took an average of 5.39 seconds to escape (a 50 ml Falcon tube) to 0.57 seconds for group housed mice. Interestingly, this finding has yet to be observed in prior research on wound healing and stress models. This finding is also consistent with both Pilot studies of the current investigation.

The Housing effect may also be interpreted in terms of social isolation. Detillion, Craft, Glasper, Prendergast and DeVries (2004) used Siberian hamsters to demonstrate the effect of social isolation on wound healing. Applying a similar model as Padgett et al. (1998), the Detillion et al (2004) wounded the hamsters cutaneously and immobilized the animals. To stress the hamsters, the researchers inserted them into small Plexiglas tubes (3 cm diameter, with 10 cm in length) for 2 hours per day for 14 consecutive days (without any additional pheromones). The restraint tubes allowed for minimal, confined movement. Following the stress, the hamsters received a 3.5 mm punch. Detillion, et al.’s findings confirmed that stress increased cortisol concentrations and impaired wound healing in isolated, but not socially housed, hamsters.
Additionally, Detillion et al. (2004), reported that treating the isolated hamsters with oxytocin (OT), a hormone released during social contact and associated with social bonding. The presence of this hormone blocked stress-induced increases in cortisol concentrations and facilitated wound healing. Recently, Vitalo, Fricchione, Casali, Berdichevsky, Hoge, et al. (2009) also demonstrated this model can be applied to burns in rats. In their study they found that when there was an enriched environment or oxytocin was given to rats (reared in isolation), the peripheral stress response as measured by burn injury healing was decreased. The findings indicate an association between the effects of nest making and the hormone oxytocin interacting to promote healing, while social isolation impedes healing.

While Detillion et al., (2004) and Vitalo, et al., (2009) found an effect for housing they did not show the same overall effect of stress as found in the current study. Additionally, although their studies demonstrate the positive effects of reducing stress hormones they do not capitalize on the robust effect of elevated stress hormones. This difference, though, may be attributed to differences in subjects’ breed, gender, and pheromone effects.
A Murine Model of PTSD

Hypothesis three predicted that subjects with the greatest corticosterone levels, greatest escape times, and slowest exploration will also show the slowest healing times. In essence, this defines the Post-Traumatic Mouse. This hypothesis was partially supported. By classifying mice with high cortisol and 2/3 behavioral measures the profile of the Post-traumatic mouse emerges. The liberal diagnosis model of PTSD employed in this study is based partially on Saxe et al.’s (2005) position that animal models need only hold plausible or face validity. Further research is necessarily to refine this diagnostic.

The Post-traumatic mouse shows reduced exploratory behavior as measured by elevated plus maze and a tendency to stay in covered areas. The PTSD mouse also exhibits elements of learned helplessness as demonstrated by an increase in the time needed to exit a 50 ml falcon tube. These elements, combined with elevated cortisol, may be used as predictors of a damaged immune system. The Post-traumatic mouse needed an average of 1.31 more days, or 31.50 more hours to heal a 1.5 mm wound. Although this difference in healing time may seem small, if this model is applicable to the human with PTSD and with a larger surgical or combat wound, then the difference may be equivalent to weeks added to recovery.
Future Directions

PTSD and Blood Platelets

At the conclusion of the study mice were sacrificed through cardiac puncture. While drawing blood for cortisol analysis it was observe that the blood of the stressed mice was separating into plasma and platelets in the syringe. This is a process that usually requires a 20 minute centrifuge at 3000 rpm. It was decided by the experimenter to add in a CBC to the blood analysis to investigate these phenomena.

Blood platelets play a primary role in hemostasis (the arrest of bleeding from a site) and when activated, promote thrombus or clot formation. When any kind of tissue containing vasculature is injured, platelets adhere to one another and to the edges of the injury, providing a seal to prevent blood loss. When subjects are faced with injury or even the potential for injury such as might occur in the perception of a threat or a mental stressor, the normal platelet cascade includes activation, adhesion to one another, and aggregation (Camacho and Dimsdale, 2000; Ware and Coller, 1995; Patterson and Krantz, 1973; Markovitz & Matthews, 1991).

Upon return of the sample from Anteck Diagnostics the results were submitted to SAS for analysis. Blood platelet functioning was analyzed by a 2 group x 2 condition, factorial ANOVA. A main effect for Stress was observed with $F(1,24) =$
11.65, \( p < 0.0026 \) and for Housing \( F(1,24) = 4.29, \ p < 0.050 \). The was no Stress X Housing interaction observed \( F(1,24) = 0.61, \ p = \text{n.s.} \). A Tukey post hoc analysis showed that stressed mice had significantly lower blood platelet count (\( \bar{X} = 245.2 \text{ n/µL} \)) than unstressed mice (\( \bar{X} = 601.9 \text{ n/µL} \)). Normal blood platelet count for a mouse ranges from 670 n/µL to 1701 n/µL (Peters, Cheever, Ellis, Magnani, Svenson, Von Smith et al., 2002). No additional Housing effects were detected by the Tukey separation.

For each individual grouping, group stressed mice produced the least amount of platelets (\( \bar{X} = 131.63 \text{ n/µL} \)), followed by the Single stressed mice (\( \bar{X} = 342.42 \text{ n/µL} \)). This produced an interesting reversal from the wound healing findings, where the group Stress mice healed faster that the single stressed mice. Their may be additional stressors about being housed in a group such as fighting behaviors. Even though the platelet count was not statistically significant by housing, the group no-stress mice produced and average of 476.00 n/µL blood platelets (200 n/µL below the normal range). Only the No-Stress mice were in a healthy normal range (\( \bar{X} = 727.83 \text{ n/µL} \)).

This finding appears to be unique, and certainly needs replication. If similar results appear during replication, the
platelet effect is the future of stress research. There is a literature base on PTSD apparently having no effect on Blood Platelet Aggregation (Vidović, Vilibić, Markotić, Sabioncello, Gotovac, Foliniegović-Šmalc, & Dekaris, 2007), so this finding of decreased platelets is unsupported. This finding also leads to exciting possibilities for future research.

Additionally, the “HPA Paradox” (Pitman, 1997) may play a role in the PTSD mouse diagnosis. Tradition (Seyle, 1946) holds that a chronic stress disorder should be characterized by hypercortisolism, however, Boscarino, (1996) found that cortisol may actually be reduced in stress victims. This is supported by the finding of the current study and the cortisol findings of both Pilot one and Pilot two. While mice with high cortisol were more likely to be slow healers, mice that displayed the behavioral symptoms of PTSD were also affected. In future research if the blood platelet finding holds true, this could help explain the “HPA Paradox” may exist because the biological basis of PTSD is in the blood. However, the effects may be found in the number of blood platelets and the relationship of platelet count to other hormonal factors. Furthermore, the importance of this finding is in the extrapolation of this research to a human population.
Stress and Platelet Dysfunction

The impact of intensive physical stress on platelet functioning is well documented (Cambria-Kiely & Gandhi, 2002; Frimerman et al., 1997; Larsson, Wallen, & Hjemdahl, 1994). Although mental stress has long been shown to be associated with an array of pathophysiologic cardiovascular factors, such as hypertension, the role of mental stress in pro-aggregatory function of platelets is not as well understood. There is evidence that intense mental stress may be responsible for increased platelet aggregation in vivo. (Cambria-Kiely & Gandhi, 2002; Frimerman, Mille, Laniado, & Keren, 1997; Larsson, Wallen, & Hjemdahl, 1994). If the current finding hold true than the mechanism for the mental stress to immune function may be established.

Studies have demonstrated that laboratory-induced mental stress produces a significant sympathetic response that differs from the response to physical stress. When compared to physical stress responses, mental stress produces a relatively greater increase in plasma epinephrine, a greater rise in blood pressure and a smaller increase in heart rate than that found with physical stress (Becker, Pepine, Bonsall, Cohen, Goldberg, Coghlan, et al., 1996). Relative to platelet activation, it is still unknown whether physical stress and mental stress produce similar responses in platelet aggregation. Theoretically, both
physical and mental stresses can cause an increase in catecholamines.

Limitations

This study is limited by multiple factors. Initially the data collected for Pilot I and Pilot II were designed to test the effectiveness of the "naturalistic combined stressor" and its relationship to housing conditions (Parker, et al., 2004), so the actual mechanism that inhibits the wound healing is difficult to tease apart. Research by Detillion, et al., (2004), Padgette et al., (1998), and Vitalo et al. (2009) would suggest that restraint alone may be sufficient to produce the reduction in wound healing observed in this study. Recent research by Levine, Leeder, Parekkadan, Berdicevsky, Rauch, Smoller, et al., (2008) has also demonstrated that isolated living conditions alone slows wound healing. It is therefore important to understand that both "Stress" (presence of a predator) and "Housing" (isolation) variables in this current investigation may be operationally defined as physiological stressors.

The impact of the error by Antech Diagnostics can not be overlooked. Although cortisol and corticosterone are both considered stress hormones and are both found in the murine animal, corticosterone is the more common marker of stress in the mouse (Detillion, et al., 2004; Padgette et al., 1998; Parker et al., 2004, & Vitalo et al., 2009). The impact here is
that we can not know for sure if there were stress effects beyond the housing effect observed.

The number of mice utilized also limited the study. The number of mice for the study was calculated as the bare minimum needed based on a “Natural Stress” model (Parker, et al., 2004), rather than the minimum thought necessary to produce a sufficient number of “PTSD” mice. With a larger pool of subjects to draw from, there would be a greater probability of finding those animals whose response to stress is more clinical in nature.

An additional limitation was the animal facility itself. This investigation was interested in the effect of pheromones, and in ideal conditions groups would have been kept in separate rooms. In reality, animals were kept in separate cages and groups were housed along two adjacent shelves. There is certainly a possibility of pheromonal exchange that could influence the animals’ immune systems. Additionally, the presence of mice involved in non-related experiments, but housed in the same room may have had unforeseen effects.

Finally, the wounding process itself limits the study. Although the wound quality of all subjects was held constant, we cannot enter the mind of the mouse to decide what it finds stressful. The nature of a skin piercing can only be assumed to create some level of pain to the animal.
Delimitations

The use of male mice in the final study also produces a potential confound. Pilot studies I & II utilized female mice, however, this investigator chose to alter the selection to male for several reasons. A significant number of men return from combat suffering from PTSD, so using a male model was preferable in order to more closely reflect human PTSD conditions. Additionally, males show increased fighting behaviors. The result of the fighting between cage mates in both the Stressed and Non-Stressed groups certainly was expected to add stress to the animals' immune systems. This is potentially a contributor to the housing effects observed and deserves further investigation.

A second delimitation is that only one predator pheromone was selected. Although mice have many predators, fox urine was selected based on its use as a "natural" mouse repellant and from previous research by this author.

This author also chose the size of the wound (1.5 mm) based on a literature search. The wound size varies among previous researchers from 1.5 to 3.0 mm (Detillion, et al., 2004; Padgette et al., 1998; Parker et al., 2004, & Vitalo et al., 2009). Selecting a larger wound diameter would have put greater pressure on the animals' immune systems and potentially allowed for more sensitive findings to emerge. But, considering
the surface area of an SKH1 mouse a a larger wound could be potentially lethal.

Finally, although Yehuda and Antelman’s (1993) theory on animal models proposes a dose-dependent stressor, there is also variability in the amount of exposure to the stressor. This varies by study and ranges from 90 minutes to 24 hours (Detillion, et al., 2004; Padgette et al., 1998; Parker et al., 2004, & Vitalo et al., 2009). Based on this and previous research by this author, a 2 hour stressing period was selected.

Closing Thoughts

Clinical implications from this study suggest the Post-Traumatic patient my potentially need additional time for healing. If the platelet finding holds up under replication this might suggest the underlying physiological condition slowing the healing process. If this finding appears in a human model, this would suggest a greater susceptibility to infection. This as a result of psychological stress.

Psychosocial stressors play a significant role in the function of the skin through effects on circulating (Dhabhar & McEwen, 1999) and local (Dhabhar, Satoskar, Bluethmann, David, & McEwen, 2000) inflammatory cells and antigen-presenting cells (Hosoi, Tsuchiya, Denda, Ashida, Takashima, Granstein, et al., 1998), transepidermal water loss (Garg, Chren, Sands, Matsui, Marenus, Feingold, et al., 2001),

Studies in humans have found that chronic stressors correlate with altered immunity and increased susceptibility to infections (Tausk & Nousari, 2001), progression of HIV disease (Kopnisky, Stoff, Rausch, 2004), development of cancer, and inhibition of wound healing (Kiecolt-Glaser, Marucha, Malarkey, Mercado, & Glaser, 1995). The findings of this current experiment suggest that similar phenomena may exist in the post-traumatic patient.
References


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Yehuda, R., McFarlane, A. C., & Shalev, A. Y. (1998) Predicting the development of Posttraumatic stress disorder from the
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Table 1: Table illustrating Housing by Stress Grouping

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<th>Group C</th>
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Table 2: Number of Days Needed to Heal (H$_2$O$_2$ Reactivity)

Summary Table of Factorial ANOVA.

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Note: * = significant value.  
\[ p < .05 \]
Table 3: Wound Diameter – Number of Days Needed to Heal

Summary Table of Factorial ANOVA

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Note: * = significant value. $p < .05$
Diagram of the Hypothalamic Pituitary Axis

Corticotropic → Hypothalamus → Pituitary → Adrenocorticotrophic → Adrenal → Cortisol
Diameter of Wound per Day of Study - Delayed Wound Healing - Pilot II

Days to Heal

Biopsy Diameter

- Family No-Stress
- Family Stress
- Single No-Stress
- Single Stress

Diameter of Wound per Day of Study - Delayed Wound Healing - Pilot II
Diameter of Wound per Day of Study - Post Trauma Wounding

Days to Heal

Biopsy Diameter

Family No-Stress

Family Stress

Single No-Stress

Single Stress

Diameter of Wound per Day of Study - Post Trauma Wounding
Blood Platelet Count (CBC)

Stress - Housing Grouping

- Single Stress
- Single No-Stress
- Family Stress
- Family No-Stress