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DEPARTMENT OF BIOLOGY

THE TEMPORAL EFFECTS OF STRESS ON WOUND HEALING PATTERNS

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A thesis
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ABSTRACT

Stress is the process through which environmental demands exceed an individual's perceived ability to cope, thereby resulting in affective, behavioral, and physiological changes. When approached with chronic social or nonsocial stressors one's immune system becomes impaired and its healing ability becomes compromised. This study aims to address the temporal effects of stress and compare the isolation model (social stressor) to the restraint model (nonsocial stressor). Female mice were subjected to stress before, after, or both (before and after) wounding. The results indicate that the temporal presentation of stress influences the degree to which wound healing rates are impaired. In conclusion, the results suggest the reduction of nonsocial stress post-operation and social stress before and after wounding/operation would improve wound healing patterns. Thus, reducing stress in these manners may lead to improvements in clinical outcomes, such as with patients that have experienced surgery, biopsies, and any skin abrasions.

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ACKNOWLEDGEMENTS

I would like to acknowledge Brooke C. Mattern, a Doctoral Candidate in the Engeland Stress and Immunity Lab, for helping on surgery days, helping to weigh and take photos of the mice, and blinding all studies prior to photo scoring and the Chandlee Lab, for allowing me to house my mice in their facility and always keeping our mice room clean and up to par. Lastly, I would like to acknowledge Dr. Engeland, my principal investigator and research mentor for always challenging me to think critically about problem-solving, supporting me throughout the process, and guiding me through the data analysis process. Everyone involved is greatly appreciated.

Chapter 1
Introduction

Stress is the process through which environmental demands exceed an individual's perceived ability to cope, thereby resulting in affective, behavioral, and physiological changes (Cohen, Kessler, & Gordon, 1997). There have been many indications that stress not only affects the psychological qualities of life but there has also been evidence of stress affecting the quality and rate of wound healing. Wound repair takes place in three phases: (1) inflammation, (2) re-epithelization and (3) tissue remodeling (Werner & Grose, 2003; Barrientos, et. al., 2008). During the inflammation phase, the recruitment of leukocytes such as macrophages and neutrophils takes place. These leukocytes are recruited by cytokines and growth factors such as IL-β and TNF-α. The cytokine and growth factors are used to initiate the formation of granulation tissue; which is the start of the re-epithelization phase. Along with the formation of granulation tissue, keratinocytes are recruited to the wound site. Keratinocytes differentiate allowing the production of collagen and elastin to replace the granulation tissue; which constitutes the tissue remodeling phase (Sanchis, et al. 2012).

Inflammation to some degree is beneficial in the wound healing process. Cortisol is often present in the presence of high stress. The overexpression of cortisol can cause significant impairment in the inflammatory phase due to its anti-inflammatory characteristics. Cortisol also decreases the expression of cytokines and growth factors, such as IL-β and keratinocytes. IL-β is one of the primary cytokines necessary in the inflammation phase of wound healing (Lusk & Lash, 2004; Guyre, Yeager, Munk, 2008). The impairment of the inflammatory and tissue remodeling phase

produced by cortisol can slow the process of wound healing and reduce the quality of wound repair (Sheridan, Padgett, Avitsur, & Marucha, 2004).

Specifically, stress is mediated through two major systems within the body (1) the hypothalamic-pituitary-adrenal (HPA) axis and (2) the sympathetic nervous system (SNS). The HPA axis starts with a signal from the integrative cortex which identifies the body is under stress and triggers the paraventricular nucleus of the hypothalamus to release corticotropin-releasing hormone (CRH). CRH then triggers the anterior pituitary to release adrenocorticotropic hormone (ACTH) which then causes glucocorticoids (GC) to be secreted by the adrenal cortex. In humans, the primary GC is cortisol and in rodents, like the SKH-1 mouse used in this study, the primary GC is corticosterone (Engeland & Marucha, 2009).

There are GC receptors on immune cells that have immunosuppressive effects that tend to cause anti-inflammatory and anti-mitotic properties (Beer, Fassler, Warmer, 2000). The overexpression of GC can cause a decrease in the expression of cytokines and growth factors, such as IL-β and keratinocytes, respectively (Lusk & Lash, 2004; Guyre, Yeager, Munk, 2008). These effects have been attributed to impairment in wound healing. In mice specifically, the increase in corticosterone has been attributed to a reduced expression of mRNA that codes for the keratinocyte growth factor (Brauchle, Fassler, Werner, 1995) and a reduction in pro-inflammatory cytokines, such as IL-β and TNF-α (Engeland & Marucha, 2009). IL-β is one of the primary cytokines necessary in the inflammation phase of wound healing because it is a chemoattractant for leukocytes such as neutrophils and macrophages. (Lusk & Lash, 2004; Guyre, Yeager, Munk, 2008). Thus, the decrease in IL-β leads to an impairment in the inflammatory phase. Due to the decrease of

keratinocytes, the appropriate amounts of collagen and elastin are not being produced which then causes insufficient tissue remodeling; phase three of wound repair.

Regarding the SNS, stress causes an increased amount of norepinephrine (NE) production which causes vasoconstriction. Vasoconstriction leads to a decrease in capillary permeability of neutrophils and macrophages (Lusk & Lash, 2004; Guyre, Yeager, Munk, 2008) which then restricts the access of neutrophils and macrophages to the wound site. Also, increased NE causes local tissue edema and increases endothelial cell wall adhesion (Koopman, 1995), and inhibits epidermal cell migrations (Donaldson & Mahan, 1984). In an unstressed SNS, the SNS should call for a moderate amount of NE which would limit the endothelial cell wall adhesion allowing for successful re-epithelization (Engeland & Marucha, 2009). In other words, NE expression plays a role in the re-epithelization phase of wound healing and contributes to the formation of granulation tissue.

It is important to note that evidence shows that both the HPA axis and the SNS can have significant effects on the rates of wound healing. However, evidence has also shown there are discrepancies in how and to what extent stress affects wound healing. These discrepancies could be found in the types of stressors used in each study. Thus far it is known that there are inconsistencies in the effects of stress when different stress models are used (Engeland & Marucha, 2009). Exactly how these different stress models affect healing patterns are a matter of question within this study.

In many instances rodents, such as mice, are used to gain a better understanding of how stress affects the rate and quality of wound healing. Compared to humans, the SKH-1 mice used in this

study have shown to have similar wound healing patterns when under comparable stress (Padgett, Marucha, & Sheridan, 1998). Since it is widely accepted that mice have similar cellular mechanisms for dealing with stress, they have been used to study how stress affects wound healing. For this study, SKH-1 mice were used as a model organism because they provide similar inflammatory responses compared to humans, clear visualization of the wound healing due to being hairless, and ways to produce various stress responses are known and well-studied.

The use of standard, well-developed and characterized models of stress-impaired healing were employed for this study to investigate how nonsocial vs social stress-induced mechanisms affect wound healing. The first model used in this current study was the restraint stress model created in 1998 by Padgett et al. In restraint models, it was seen that mice placed in restraint, 3 days pre-wounding and 5 days post-wounding, had slower healing rates and took longer to fully heal from cutaneous wounds. It was also seen that mice in restraint consistently and significantly showed higher corticosterone levels as compared to control groups (Padgett, Marucha, & Sheridan, 1998; Sheridan, Padgett, Avitsur, & Marucha, 2004; Sanchis, et al. 2012).

The second stress model used was the isolation model. The isolation stress models address social stressors, unlike the restraint model that addresses nonsocial stress. The isolation model tends to lower the baseline corticosterone concentration which is unlike the restraint model stress response (Pyter, Yang, Rocha, Engeland, 2014). Despite the different wound healing patterns that the isolation verses the restraint model produces, both models produce sufficient amounts of stress in mice allowing the investigation of stress on wound healing patterns.

This study looks to address the limitations of the literature by addressing the temporal effects of stress and compare isolation (social stressors) to restraint (nonsocial stressors). The main focus of this study was to identify when stress relief is most important for wound healing and to determine if the changes in the wound healing patterns are stressor-type specific. To address this, the experimental groups were segregated into groups that were stressed at different times; BEFORE wounding, AFTER wounding, BOTH (before and after) wounding, or no stress (control group). Two sub-studies were conducted using these conditions in a female restraint model and female isolation model. It was hypothesized that the mice that experienced stressors (social or nonsocial) in the BOTH group would have the slowest rate of wound healing compared to the other conditions and the mice in the AFTER group would have slower wound healing than those in the BEFORE group (Slowest Rate of Healing: BOTH < AFTER < BEFORE < Control group/ No Stress: Fastest Rate of Wound Healing).

Significance

With money, work, and family responsibilities being amongst the top causes of stress affecting the vast majority of Americans (Clay, 2011), it is of utmost importance to investigate how stress is affecting the physical health and recovery from injury in humans. The results of this study would lend to a better understanding of pre/postoperative care in humans. By determining how different types of stressors (social vs nonsocial) affect healing patterns, a targeted plan of stress relief can be used when humans are about to embark on an operation. (i.e. When planning pre and post-operative care a physician can know how significant the effect of reducing social verse nonsocial stressors would affect their patients' healing pattern.) Understanding when stress relief is most

beneficial for healing is also a significant implication of this study. By knowing such information, a physician could better guide a patient in their recovery.

Chapter 2

Methods

SKH-1 hairless outbred mice aged 29-42 days with no immunological or physiological alterations were used. The mice were fed and provided tap water. All mice were housed in polypropylene cages under a 12:12 light:dark cycle with the lights coming on at 6 a.m. daily. All mice were housed in the vivarium for at least a week after arrival before any experiments started.

In the restraint and isolation model, the mice were divided into four conditions: control group/no stressor, stress before wounding, stress after wounding, or stress before and after wounding. A between-group study was conducted where the BEFORE group only experienced the stressor before being wounded; the AFTER group only experienced the stressor after wounding and the BOTH group experienced the stressor before and after being wounded.

Photos were taken daily using the vertical half of a 50mL conical tube with a $1cm^2$ cut out to allow the wound to be exposed. A red sticker was placed on the conical tube and incorporated in every picture acting as a standard (a consistent reference area that will later be used to find the ratios of the wounds and was used to ensure that all wounds were compared on the same scale) when determining the area of the wounds (Fig 2).

Body weights were taken daily to monitor the health of each mouse. If the mouse lost more than 20% of its start weight, it was removed from the study. If a mouse showed signs of unnecessary pain or distress, it was removed from the study (e.g. inactive, shivering, socially withdrawn,

unkempt, etc.). After each study, all mice were euthanized via carbon dioxide exposure followed by cervical dislocation. The conclusion of the study was determined when all wounds were fully healed.

Study 1: Female Restraint Model

Forty (40) female SKH-1 hairless mice were used in this study. Five mice were housed together per cage, with two cages per condition (control group, BEFORE, AFTER, BOTH). One (1) BEFORE group mouse was found dead in the tube the day after surgery, before the conclusion of the study. Data were analyzed for 39 mice.

Individual 50mL conical tubes, with ventilation holes drilled in them, were used for each mouse during its restraint period (Fig 1). Each hole was created via a drill bit and then smoothed out using hand sanding. Each conical tube needed to be free from debris, sharp edges, and any hanging plastic to ensure there would be no additional injuries to the mice. This step was critical to ensure the healing of the wounds were not agitated by the structure of the tube.

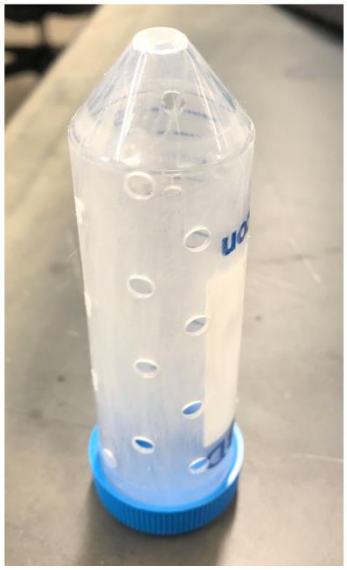

Figure 1: 50mL Conical Tube w/ Ventilation Holes used in the Restraint Study.

The mice were restrained in the conical tubes overnight for 13 hours (6 pm to 7 am) with no access to food or water to ensure that the access to food did not affect wound healing patterns. Thus, all food and water were removed once the mice were put in the restraint tubes. The control group was also food and water-deprived during the same times that the other mice were in the tubes. This was to ensure that the extra access to food overnight would not contribute to the wound healing patterns.

The control group was neither restrained before or after wounding. The BEFORE group (pre-wounding) was restrained for 4 nights and then freed after surgery. The AFTER group (post-wounding) was restrained for 4 nights post-wounding. The BOTH group was restrained for 4 nights pre-wounding and 4 nights post-wounding. Throughout the study, the mice were restrained by the same person to ensure consistent handling. During the restraining process, each mouse was held by its tail over the tube and allowed to crawl into the tube, with its posterior end closest to the cap. No force was used to insert the mice into the tube; this would add inconsistent stress. The tube was then capped off. Note: it was important to make sure the mouse's tail was not captured within the tube's cap.

Upon the release of each mouse, any feces found on the mouse was gently removed. If fecal matter was found in the wound, a dry Q-tip was used for removal. This step needed to be done gently and diligently. Any feces left on a wound could cause infection and alter the natural healing process. All tubes were cleaned using Alconox detergent, rinsed thoroughly, and left to air dry until the next restraint time.

Study2: Female Isolation Model

Thirty-six (36) mice were used, and data were analyzed for all 36 mice. Three mice were housed together per cage and three (3) cages per condition. The mice experiencing isolation were housed one (1) mouse per cage during their respective timeframes. Control groups were always housed with three (3) mice per cage. The BEFORE group mice were housed individually for 14 days before wounding and then group-housed for the remainder of the study (10 days). The AFTER group mice were group-housed, three (3) mice per cage, then, individually housed for 10 days after

wounding until the conclusion of the study. The BOTH group mice were housed individually for 14 days before wounding and individually for 10 days after wounding day until the conclusion of the study (Total 24 days). It was the intention of the study to continue isolation 14 days post wounding however all wounds healed before 14 days. The mice had continuous access to food and water.

Surgical Technique

On the day of surgery, the mice were anesthetized with a ketamine and xylazine mix (administered intraperitoneally; 100 mg/kg Ketamine; 10 mg/kg Xylazine). A tail and toe pinch determined if the mice were in the state of surgical anesthesia. An alternating Betadine, alcohol, Betadine scrub was used at the wounding site before administering Lidocaine (administered subcutaneously; 6 mg/kg). After cleansing and numbing the dorsal area, a sterile 3.5 mm punch biopsy (Miltes Instrument Company, York, PA) was used to create two small excisional wounds just below the dorsal side of the shoulder blades (Fig 2). The wounds were left opened and allowed to heal. If necessary, bleeding was stopped by pressing a gauze to the wounds. The mice were allowed to recover from anesthesia before being placed back in their permanent cages.

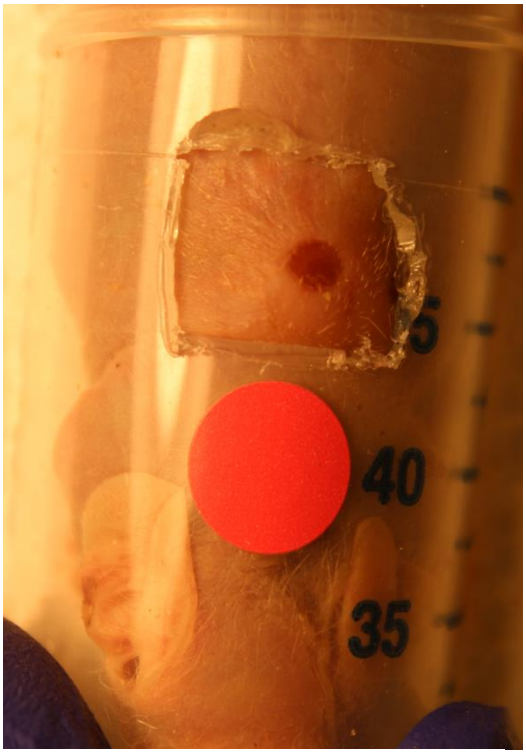

Figure 2: Right biopsy punched wound w. standard red dot-sticker.

Software

All photos were uploaded and stored on PSU (Penn State University) Box®, blind coded, by another investigator, and then transferred to ImageJ to score each photo. The oval, elliptical, and free-hand tool were used to trace the reference dot and wound. Note all scoring was done on a PC due to ImageJ's inability to run on the newer IOS. IBM® SPSS® (Statistical Package for Social Sciences) Version 26 was used to run statistical analysis.

Wound Photos & Scoring

Photos were taken immediately after surgery and daily for all studies. Pictures of the left and right wounds were taken respectively. After the randomization of each study, each photo was scored individually.

Scoring of the photos consisted of using the elliptical/oval tool of ImageJ to measure the area of the standard red circular sticker that acted as the standard to which the wound size was compared. This corrected for the photos taken at different distances away from the mouse. Then the wound was measured using the polygon tool by placing points all around the wound then connecting them and allowing the software to calculate the wound area.

Step1 - For each picture each day the wound-to-standard area ratio was created by dividing the wound area by the standard area of the red sticker from the same picture.

Step 2 - The standardized wound was created by dividing the standardized wound (found in step 1) for each day by the wound on day 0 for the left and right-wound each day. (e.g. left day 1

standardized wound ÷ left day 0 standardized wound; left day 2 standardized wound ÷ left day 0 standardized wound).

Step 3 - the average wound ratio for each day was determined using the average of the left and right-wound ratio from step 2 (Ex. (left day 1 + right day 1) ÷2).

Statistical Analysis

The day was a within-subject variable and the conditions (Control group, BEFORE, AFTER, & BOTH group) were a between-subject measure. Differences in wound closure were analyzed using repeated-measures analysis of variance (ANOVA). Post-hoc analyses were performed using univariate ANOVA. Data were determined to be statistically significant when $p<0.05$. Error bars represent standard error of the mean (SEM).

Chapter 3

Results

Female Restraint Model

The timing of stress, in the female restraint study, significantly altered the wound healing pattern ($F_{3,35}$= 13.82, p=0.000, Fig 3A). The BEFORE group compared to the control group had significantly quicker wound healing ($F_{1,17}$= 4.992, p=0.039, Fig 3B). The AFTER group compared to the control group had significantly slower wound healing patterns ($F_{1,18}$= 9.775, p=0.006, Fig 3C). The BOTH group compared to the control group had slower wound healing patterns ($F_{1,18}$= 14.701, p=0.001, Fig 3D). The AFTER group compared to the BOTH group did not have significantly different wound healing patterns ($F_{1,18}$= 0.097, p=0.759, Fig 3E).

Compared to the control group, post-hoc analyses revealed the BEFORE group had significantly faster rates of wound healing on days 6-8 post-wounding (Fig 3B). Compared to the control group, the AFTER group had significantly slower rates of wound healing on days 3-6 post-wounding and the BOTH group had significantly slower rates of wound healing on days 2-6 post-wounding (Fig 3C & Fig 3D).

Stress conditions, in the female restraint study, significantly altered the body weight ($F_{3,35}$= 23.195, p<0.001, Fig 4A). Compared to the control group, the BEFORE group and AFTER group had significantly lower body weight after the first day of restraint ($F_{1,17}$= 15.314, p=0.001, Fig 4B &

$F_{1,18}$= 32.360, p=0.000, Fig 4C, respectively). The BOTH group compared to the control group had extremely low body weights from 2 days pre-wound to wounding day and from days 1 to 4 post-wounding. ($F_{1,18}$= 64.233, p=0.000, Fig 4D). The AFTER group compared to the BOTH group did not have significantly different body weight patterns ($F_{1,18}$= 3.825, p=0.066, Fig 4E).

Female Isolation Model

Stress conditions, in the female isolation study, did not significantly alter healing patterns ($F_{3,32}$= 1.410, p=0.258, Fig 5A). The BEFORE group compared to the control group did not have significantly different wound healing patterns ($F_{1,16}$= 0.024, p=0.879, Fig 5B). The AFTER group compared to the control group did not have significantly different wound healing patterns ($F_{1,16}$= 1.221, p=0.286, Fig 5C). The BOTH group compared to the control group had a close to a significant difference in wound healing patterns with the BOTH group healing slower than the control group ($F_{1,16}$= 3.905, p=0.066, Fig 5D). There was no significant difference between the healing patterns of the AFTER group and BOTH group ($F_{1,16}$= 0.619, p=0.443, Fig 5E).

Compared to the control, the AFTER group and BOTH group had significantly larger wound sizes on day 7 post-wounding (Fig 5C & 5D, respectively). Compared to the BOTH group, the AFTER group had significantly larger wound sizes on day 2 post-wounding (Fig 5E).

Stress conditions, in the female isolation study, significantly altered body weight ($F_{3,32}$= 2.862, p=0.052, Fig 6A). Upon isolation, the BEFORE group compared to the control group did not have a significant difference in body weight patterns ($F_{1,16}$= 2.596, p=0.127, Fig 6B). The AFTER group

compared to the control group did not have a significant difference in body weight patterns ($F_{1,16}$= 0.065, p=0.802, Fig 6C). The BOTH group compared to the control group had significantly higher body weight ($F_{1,16}$= 4.083, p=0.060, Fig 6D). The BEFORE group compared to the BOTH group did not have a significant difference in body mass fluctuation patterns ($F_{1,16}$= 0.041, p=0.841, Fig 6E). The AFTER group compared to the BOTH group had significantly lower body weight ($F_{1,16}$= 7.345, p=0.015, Fig 6F).

Study 1: Female Restraint Model

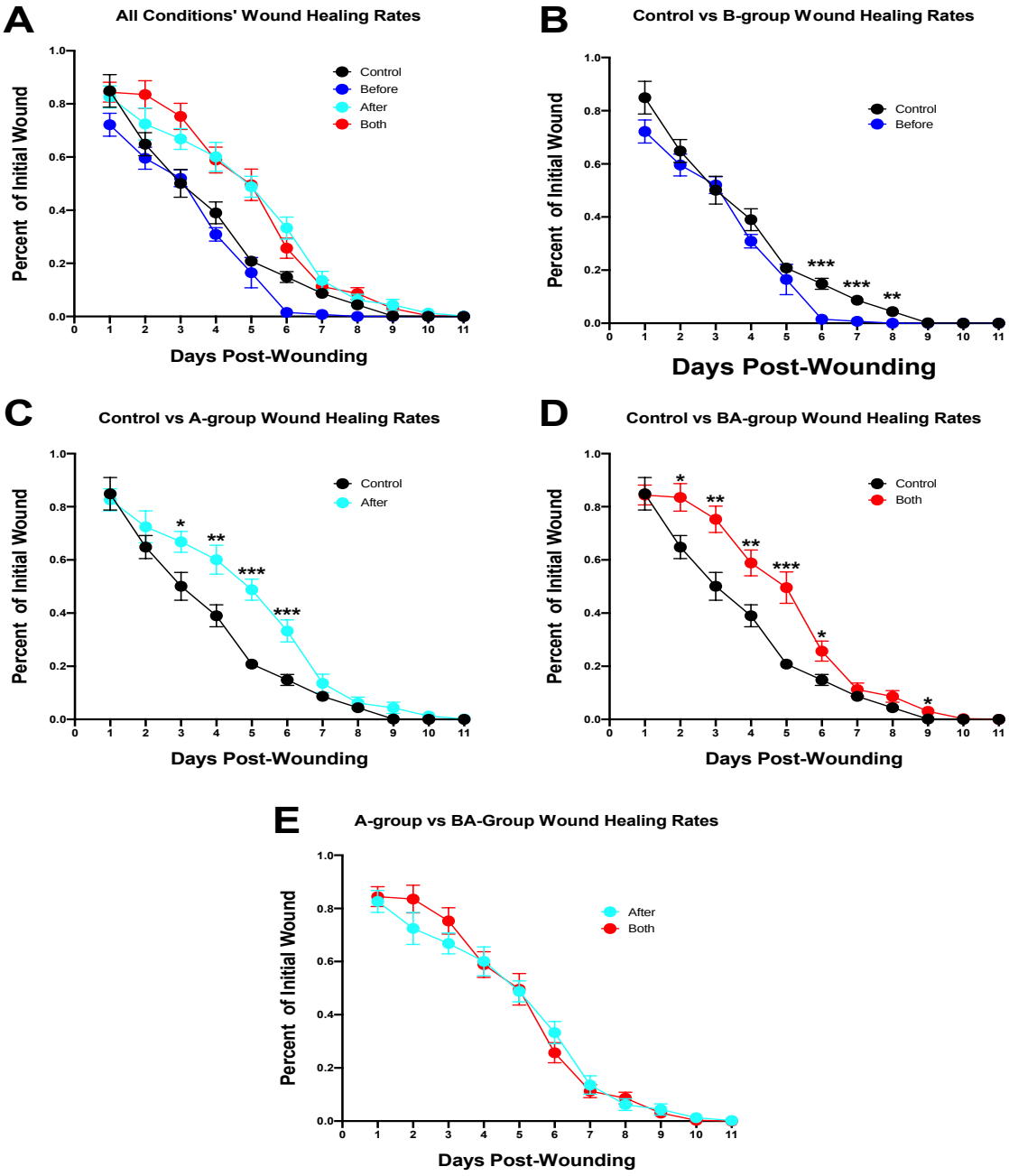

Figure 3: Female Restraint Study Wound Healing Patterns.

*(A)Nonsocial Stress (restraint) caused a change in wound healing patterns. (B) BEFORE group healed similarly compared to the control group. (C) AFTER group healed slower than the control group. (D) BOTH group healed slower than the control group. (E) The AFTER group and BOTH group healed similarly. Control group N=10; BEFORE group N=9; AFTER group N=10; BOTH group N=10. Error bars represent standard error of the mean (SEM). *p<0.05; **p<0.01; ***p <0.001.*

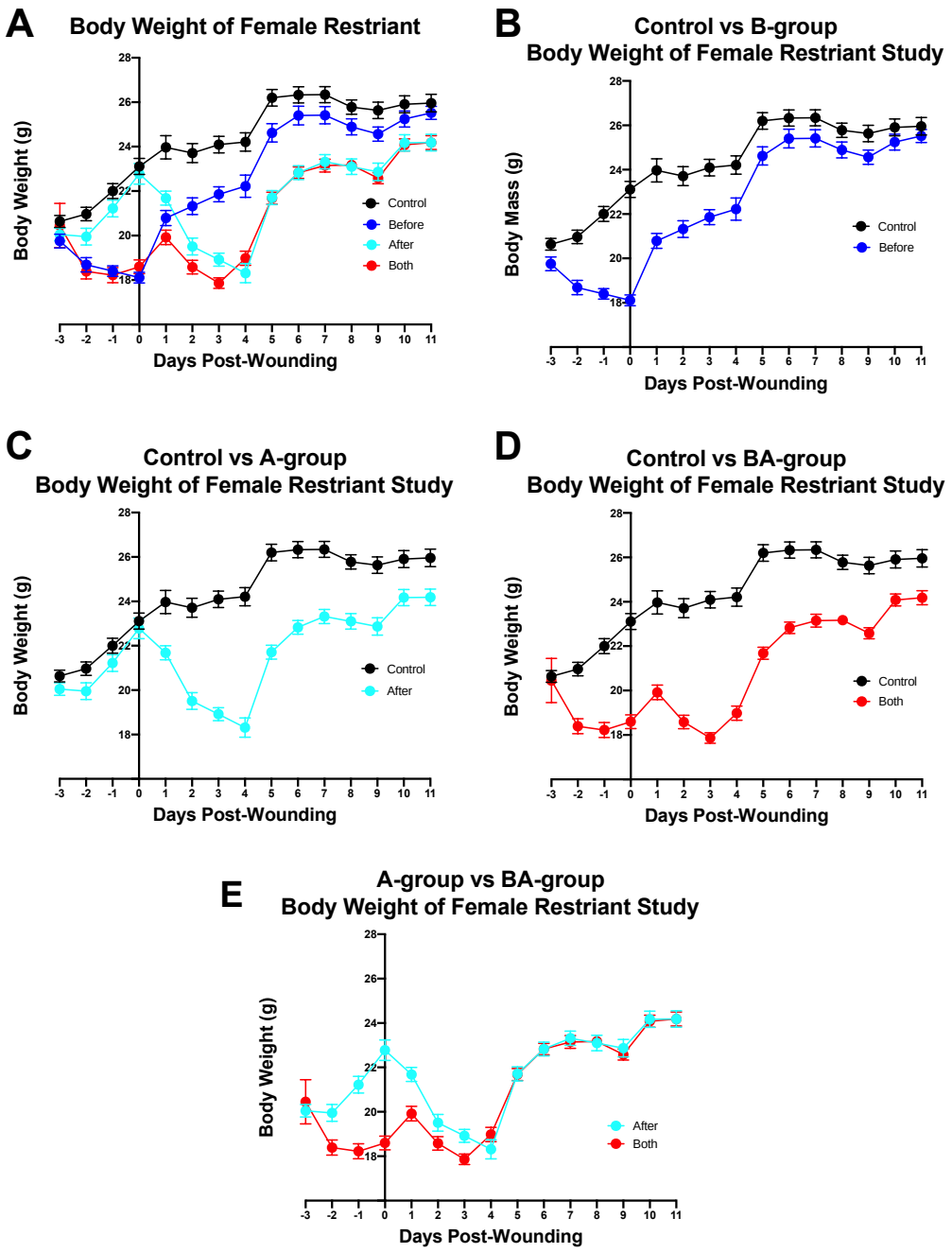

Figure 4: Female Restraint Study's Body Weight.

*(A)Nonsocial Stress (restraint) caused a decrease in body weight. (B) BEFORE group (C) AFTER group, and (D) BOTH group had lower body weights as a result of restraint when compared to the control group. (E) The AFTER group and BOTH group had similar weights post-wounding. Days -3 to -1 were pre-wounding days. Day 0 was wounding day. Days 1 to 11 were post-wounding. Control group N=10; BEFORE group N=9; AFTER group N=10; BOTH group N=10. Error bars represent standard error of the mean (SEM). *p<0.05; **p<0.01; ***p <0.001.*

Study 2: Female Isolation Model

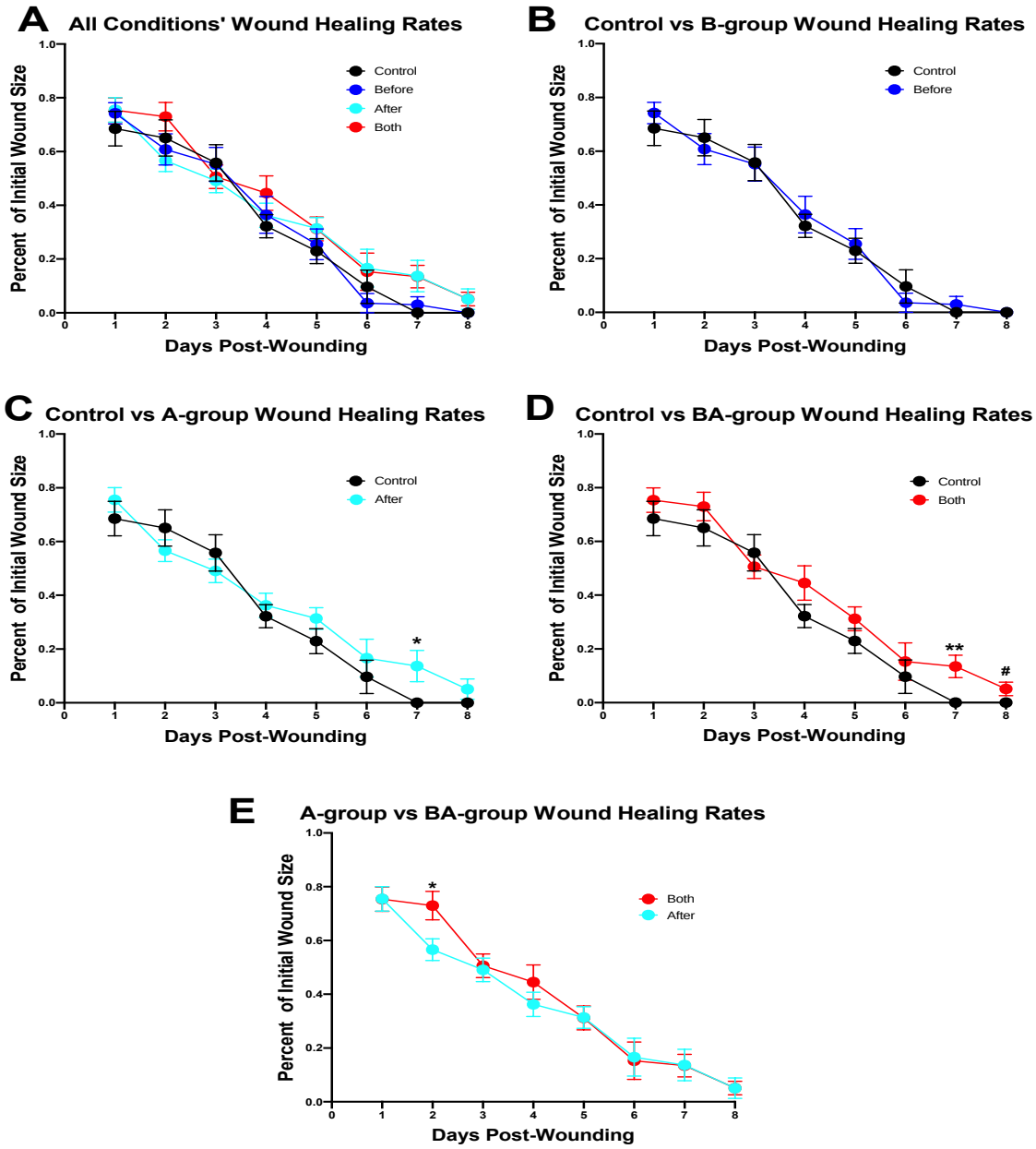

Figure 5:Female Isolation Study Wound Healing Patterns.

*(A)Social Stress (Isolation) did not cause a change in wound healing patterns. (B-D) All groups had similar healing patterns compared to the control group. (E) The AFTER group and BOTH group healed similarly. Control group N=9; BEFORE group N=9; AFTER group N=9; BOTH group N=9. Error bars represent standard error of the mean (SEM). *p<0.05; **p<0.01. # means near significance.*

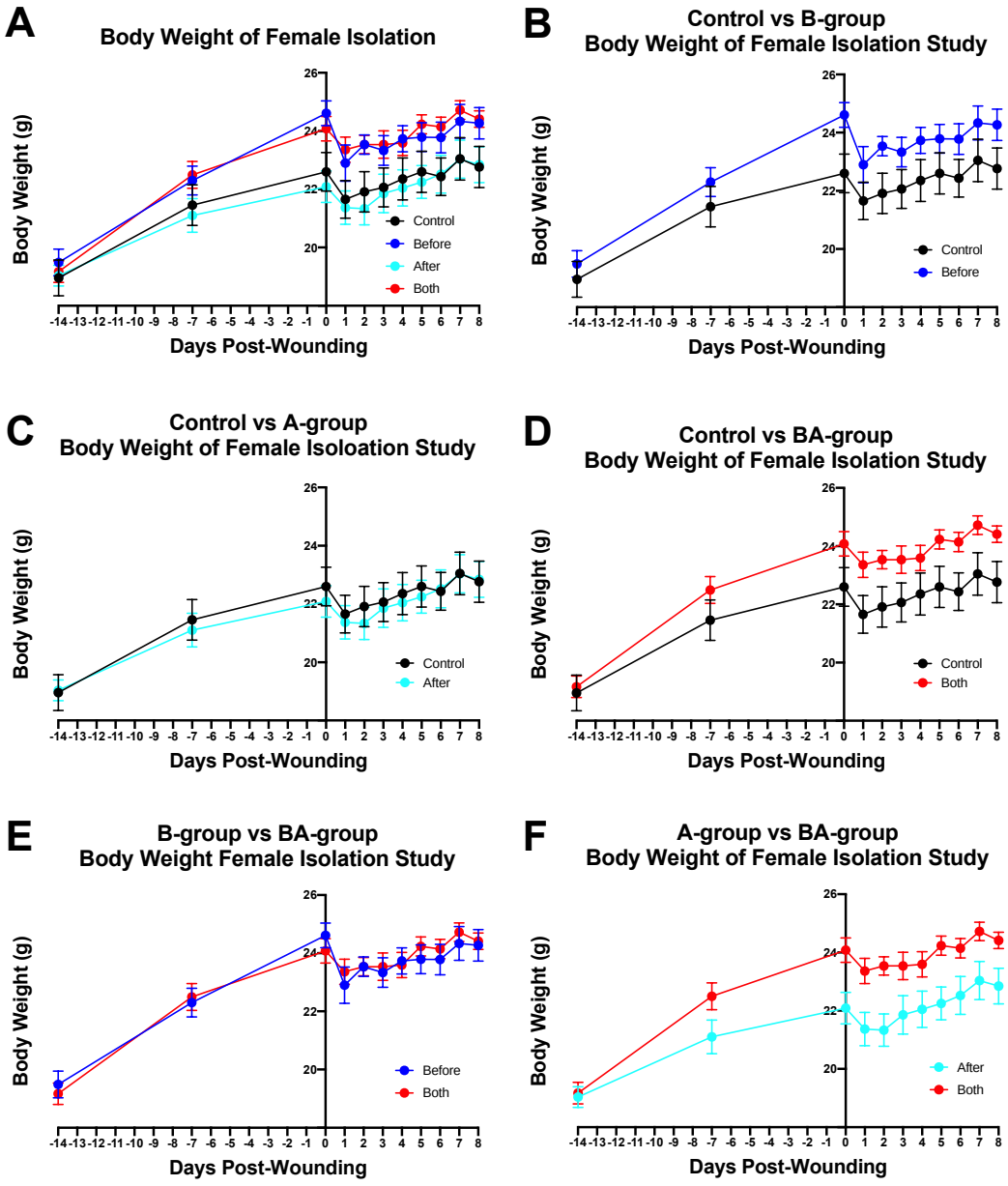

Figure 6: Female Isolations Study Body Weight.

(A) Social Stress (Isolation) did not cause any significant change body weight. (B) BEFORE group and (C) AFTER group's weight did not have a significant difference in body weight patterns. (D) BOTH group had higher body weights as a result of isolation when compared to the control group. (E) The BEFORE group and BOTH group had similar weights pre and post-wounding. (E) The BOTH group had larger body weight compared to the AFTER group. Days -14 to -1 were pre-wounding days. Day 0 was wounding day. Days 1 to 8 were post-wounding. Control group N=9; BEFROE group N=9; AFTER group N=9; BOTH group N=9. Error bars represent standard error of the mean (SEM).

Chapter 4

Discussion

The results of these experiments support that stress has an effect on wound healing and indicates that the timing of the stress influences the rate of wound healing. The female restraint model showed nonsocial stress post-wounding had the most impact on the wound healing pattern. The female isolation model showed social stress may only be impactful when presented before and after wounding. Thus, evidence supports that when given a choice one should limit the amount of nonsocial stress post-wounding and limit the exposure of social stress before and after wounding.

In the restraint model, post-wounding stress had the most effect on the rate of wound healing. This conclusion was evident in the similar healing patterns and body weight fluctuations of the AFTER group and BOTH group. There were no significant differences between the two groups healing patterns; while the BEFORE and control group healed with similar patterns. This indicated that the main influencer of delayed healing rates was the stress experienced after wounding. The practical implication of this result addresses the main question of this study, ‘when is stress relief most important for wound healing?’ In the case of a human, the wounding done in this study could be comparable to operations. Thus, the application of these findings would be used for determining pre and post-operative care. The results from this study suggest if a patient is scheduled for operation today, they should try to elevate all forms of nonsocial stressors, such as excessive worry about paying bills, from the time of operation forward.

There was some indication that restraint-stress presented before wounding had an advantageous outcome on the healing pattern. Past literature showed that when stress was present for a short

amount of time before wounding occurred the rate of wound healing increased compared to groups without stress (Graham, Song, & Engeland, 2012). A possible explanation for this phenomenon is when short-term stress is presented there is an elevation in the SNS which then causes an immune-related response by way of an increase in norepinephrine. Though the recruitment of too much norepinephrine may cause impairment in wound healing, the small amount recruited during the three days before wounding is thought to have caused the faster wound healing rate. In previous studies, it has been suggested that the expression of slightly elevated amounts of norepinephrine, not cortisol, has been correlated with faster wound healing rates (Eijkelkamp, Engeland, Gajendrareddy, & Marucha 2007). Though the evidence on this phenomenon is not clear, this may be an avenue to explore in later experiments.

To determine the type of effects different stressors have on wound healing patterns, isolation stress was used to model social stress. Compared to the restraint (nonsocial) stress model, the isolation (social) stress model results showed fewer effects on the wound healing rates. This could be contributed to when female rodents are in isolation, they tend to "stress-eat". This is seen in the increase in body weight in the mice in isolation. Thus, by "stress-eating" the female mice have ultimately found a coping mechanism that is otherwise not seen in the restraint model (Krolow, et al., 2013). This coping mechanism could be reducing the biological stress so much that the effects of stress that the mice are experiencing are no longer significant enough to cause impairment in wound healing. There were no significant differences in wound healing rates between the time conditions which indicated that social stress does not cause such a substantial impact on wound healing compared to nonsocial stress. There was a near significant difference between the control group and the BOTH group wound healing rates. No differences were seen between the BOTH

group and the AFTER group nor the BOTH group and BEFORE group which indicated that social stress must be inflicted both before and after wounding. This indicates that social stress tends to have less of an impact on the rate of wound healing since stress must be presented surrounding the time of wounding to see an impact in wound healing patterns. These findings would also be applied when determining pre and post-operative care. The results from this study suggest patients should reduce all forms of social stressors at all times before and after their surgery.

On days 7 and 8 of the isolation study, the AFTER group, and the BOTH group showed evidence of slower wound healing rates. These results support the findings in the restraint study which indicates that stress impaired wound healing significantly in the AFTER group and BOTH group. Therefore, the significantly bigger wound size at days 7 and 8 in the AFTER and BOTH group, in the isolation study, supports the conclusion that stress after wounding can cause impairment in wound healing.

Though it is not the focus of this study, it is important to note that in the restraint model stress caused a decrease in body weight while in the isolation study significant weight gain was observed. Though further studies would be needed to better understand the correlation, in the restraint study, body weight fluctuation followed similar patterns as the wound healing patterns. As the mice lost weight, they also show slower rates of wound healing. In the isolation study, there were no significant differences in body weights. However, compared to the control group, the BEFORE and BOTH groups had higher body weights (near statistically significant). This could imply that the significant weight gained was before wounding and simply maintained through the duration of

the study. This is supported by the evidence of the AFTER group's weight being so similar to the control group's weight.

It is important to note that this study looks at how the timing of stress effects healing patterns in conjunction with how different stressors also affect healing patterns. In conclusion, no matter the form of stress, stress post-wounding/operation could lead to delays in healing. The present results suggest that nonsocial stressors are more important to elevate post-wounding/operation and may have some benefits if present before wounding/operation. Also, the results suggest social stress is most influential on wound/operation healing patterns if alleviated both before and after wounding/operations.

Limitations

(1) Due to ethical constraints, we could not induce stress in a human model to impair wound healing. Hence, mice were used. Thus, the conclusions made within this study must be taken with caution when predicting how humans may respond. (2) Only female mice were used in this study and studies have shown that females produce more robust inflammatory responses when compared to males (Rosenthal, Montag, & McClintock, 2006). The different physiology presented in females verses males could affect the way the body responds to stress. Thus, it would offer good insight to further investigate how the different stressors and timing of stress affects wound healing in males verse females. (3) Glucocorticoids (i.e. corticosterone), cytokines (i.e. IL-β), and growth factors (i.e. keratinocytes) were not measured directly. To make a more definitive connection between stress impairing the wound healing patterns these markers should be measured.

Future Directions

This study suggests nonsocial stress causes more significant impairment in wound healing. Also, the timing of the presentation of stress impacts the degree to which the wound healing rate will be stunted. It is suggested that nonsocial stressors are more influential post wounding and social stresses need to be present both pre and post-wounding to have a near significant effect on wound healing. These results suggest particular advisement for improving operational outcomes in humans.

The difference in male verse female immune systems is clearly stated throughout literature which makes conducting this project in males of great importance. For instance, female rodents produce more robust inflammatory responses which may, in turn, cause them to respond more severely to the stress which then can lead to exaggerated results. Thus, in the future finding a way to conduct the restraint and isolation model in male mice would further expand knowledge in the field. In addition, understanding the mechanisms of how and why the BEFORE group had faster healing patterns, in the isolation study, would be beneficial as well. This could be done by testing various glucocorticoid levels, such as corticosterone, IL-β and TNF-α, and using protein analysis, such as enzyme-linked immunosorbent assays (ELISA) to determine which markers are being recruited during various phases of wound healing. This would provide a more comprehensive understanding of the mechanism causing nonsocial stress to be beneficial for wound healing.

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ACADEMIC VITA
Marlisa Shaw

EDUCATION

The Pennsylvania State University, University Park, PA
B.S. Biology with the Vertebrate option, May 2020
B.S. Psychology with the Neuroscience option, May 2020

Schreyer Honors College
June 2016 to May 2020 **Pennsylvania State University** – State College, PA

Millennium Scholars Program – Cohort 4
June 2016 to May 2020 **Pennsylvania State University** – State College, PA
A merit-based scholarship program designed to prepare students for the pursuit of doctoral degrees in science, technology, engineering, and mathematics (STEM) disciplines.

RELEVANT COURSEWORK

Biological Courses
~Organic Chemistry (+Lab)
~Development Biology
~Statistics
~Mammalian Physiology (+Lab)
~Genetics
~General Chemistry (+Lab)
~HealthWorks Training
~Neurobiology
~Biochemistry

Psychological Courses
~Abnormal Psychology
~Research Methods
~Social Psychology
~Neuropsychology
~Clinical Neuropsychology

RESEARCH EXPERIENCE

Dr. Alfredo Quinones-Hinojosa - Summer Undergraduate Research Fellowship (SURF)
May 2019 to August 2019 **Mayo Clinic** – Jacksonville, FL
Under the direct supervision of Paula Valentina (Paula) Schiapparelli I studied Glioblastomas (GBM) which is the most aggressive primary brain tumor in adults. Specifically, I looked at how blocking the increased activity of chloride channels and chloride transport pathway using a novel inhibitor of SPAK kinase may decrease cell migration and proliferation. The synergistic effects of the SPAK inhibitor with temozolomide (TMZ) were also tested. Multiple combinations of the SPAK inhibitor with TMZ showed synergistic effects, resulting in decreased cell proliferation.

Dr. Jeremy Danes – Summer Undergraduate Research Program (SURP)
June 2018 to August 2018 **New York University** – New York, NY
Using *in situ* hybridization to identify and localize novel genes in Zebrafish spinal cord that control locomotion I sought to identify what genes are specific to motor neurons that regulate speed. I identified and investigated eight novel genes that were possibly linked to motor neuron development and maintains.

Dr. Christopher Engeland
Sept 2017 to May 2020 **Pennsylvania State University** – State College, PA
Using SKH-1 mice I am studying the effects of stress on wound healing. Using the two standard restraint models to implement stress on the mice I am looking at the effects on wound healing rates and quality. This project is currently being written to serve as my thesis projected and for publication in the near future.

Dr. Bruce Torbett's Lab – Summer Undergraduate Research Fellowship (SURF) Program
June 2017 to Aug 2017 **Scripps Research Institute** – San Diego, CA
The main focus of this project was to optimize library preparation methods for full-length sequencing of HIV-1 via optimizing reverse transcription (RT) conditions of HIV RNA to HIV cDNA and preparing cDNA sequences of HIV with barcodes for Next Generation Sequencing (NGS).

Dr. Suresh V. Kuchipudi's Lab
Dec 2016 to Nov 2017 **Pennsylvania State University** – State College, PA
Cultivated the skills of expanding several cell lines by cell culturing and media preparation. Preformed viral cell injections observed cytopathic effects in virus-infected cells, extracted viral RNA, and performed real-time PCR.

URISE
Sept 2016 to Dec 2016 **Pennsylvania State University** – State College, PA
Participated in a semester-long exposure and training program run by the Undergraduate Research Society that sought to directly improve undergraduate engagement in research. I was exposed to basic lab techniques in the form of interconnected modules, while also being taught how to operate in a group lab environment through firsthand experience.

CLINICAL EXPERIENCE

Dr. Alfredo Quinones-Hinojosa
May 2019 to July 2019 **Mayo Clinic** – Jacksonville, FL
This opportunity was connected to the Dr. Alfredo Quinones-Hinojosa research experience. As a part of using patient-derived samples, the samples I used were gathered from patients with Glioblastomas. To gather the samples neurosurgery was performed on multiple patients. During the surgery, I observed and was lectured on various techniques used.

HIV Counseling
Aug 2018 to May 2020 **Pennsylvania State University** – State College, PA
Counsel students through receiving free HIV/AIDS testing through Health Promotion and Wellness; funded by the Pennsylvania Department of Health. Practice conducting sensitive conversations about undergraduate and graduate students' sexual activates. Processes reports of sexual assault and/or sexual harassment. Educate students about HIV prevention and transmission. Reduce the anxiety of the student and create a personalized risk reduction plan.

Atlantis Fellowship
May 2018 to June 2018 **Rizzoli Orthopedic Institute** – Bologna, IT
A competitive physician shadowing program with placement in Bologna's orthopedic medical center with the focus of AAMC-compliant doctor observation and reflection. Along with a select group of pre-med students from universities around the United States and Canada, I completed 90 shadowing hours in pediatrics, oncology, trauma, surgery, and rehabilitation. Reviewed and edited a research article that was later submitted for peer-reviewed publication.

Clinical Research Shadowing
June 2017 **St. Michael's Medical Center** – Newark, NJ
Learned the daily schedule of an infectious disease chief, registered nurse, and medical student in a liver clinic and HIV clinic. Shadowed a clinical psychologist during counseling sessions dealing with substance and physical abuse.

Emergency Department Volunteering
Dec 2016 to August 2017 **Mount Nittany Medical Center** – State College, PA
Provided help to medical professionals by transporting patients, cleaning patient rooms, discharging patients, providing superior customer service, and creating positive experiences for patients and their families.

TLCC Ghana Medical Mission
June 2014 to July 2014 **Adopt one Village** – Ghana, West Africa
Traveled to Accra, Ghana to serve a small village in the town of Yaw Tenkorang. Provided bikes for all children in the village, participated in the construction of the village school, and provided medical assistance by acting as a medical scribe.

PROFESSIONAL ACTIVITIES

Envisions
Jan 2019 **Pennsylvania State University –** State College, PA
Mentored 12 young women, in 6th—12th grade, interested in the STEM fields. Guided mentees through exploring science concepts through hands-on activities in Penn State facilities. Facilitated informal conversations between academic advisors, students, and professional scientists.

Parents Program Student Ambassador Program
Feb 2018 to May 2020 **Pennsylvania State University –** State College, PA
Serve to (1) enhance communication between parents and family and the University, (2) provide a positive Penn State experience for parents and family of undergraduate students, (3) assist in disseminating information to parents and family at various University events, and (4) encourage involvement and participation from parents and families that reflect Penn State's diverse undergraduate student population. Plan, lead, and host a cultural performing arts event for ~300 families during Parents & Families Weekend in October.

NOBCChE
Sept 2017 to Jan 2019 **Pennsylvania State University** – State College, PA
Served as the vice-president and founding executive board member. Work to (1) encourage the professional development of students of color in fields including, but not limited to science, technology, and engineering, (2) provide academic and personal support to its members, and (3) promote diversity among future professionals within these fields.

HealthWorks
Aug *2017 to May 2020* **Pennsylvania State University** – State College, PA
Assumed the leadership role of a peer educator in University Health Services that aims to promote health among Penn State students. Provide outreach and promotional services through workshops and initiatives. Created the Minority Health Initiative to create more inclusive healthcare provisions for students and faculty on campus.

COMMITTEES & FOCUS GROUPS

Student Affairs Facilities Master Plan Focus Group
Oct 2019 to May 2020 **Pennsylvania State University** – State College, PA
Act as Healthworks's reprehensive. Offer student insight to Sasaki, an international interdisciplinary planning and design firm, Student Affairs, and the Office of Physical Plant about the necessary improvements of student space across campus.

Homecoming Court – Guide State Forward Award
Sept 2019 to current **Pennsylvania State University** – State College, PA
Award the gender-neutral title of the Guide State Forward recipient, formally known as "Homecoming Queen." Elected by faculty, staff and students to best represent Penn State's six core values: Integrity, Respect, Responsibility, Discovery, Excellence, and Community.

Minority Health Initiative
Jan 2018 to May 2020 **Pennsylvania State University** – State College, PA
Founded and lead the Minority Health Initiative to meet the health needs specific to minority students on campus while increasing their overall well-being. (1) Increase awareness of minority specific disparities by facilitating insightful conversations. (2) Improve marketing to provide relatable and relevant resources for minorities. (3) Educate all races on minority differences and how to approach them. (4) Enhance the environment/culture of Penn state via promoting more inclusivity. (5) Recruit more minorities in HealthWorks to provide a more inclusive environment for those who seek healthy guidance.

SAFE & AWARE
April 2018 to May 2019 **Pennsylvania State University** – State College, PA
Worked with a team of student affairs staff and Penn State's Media Services (WPSU) to complete a redesign of the Student Alcohol Feedback and Education (SAFE) and Sexual Assault Awareness (AWARE) programs. Contributed to the production of two highly engaging and effective modules for students.

INVITED CONFERENCES PRESENTATIONS

Annual Biomedical Research Conference for Minority Students (ABRCMS)
Nov 2019 **ABRCMS** – Anaheim, California

SACNAS - The National Diversity in STEM Conference
Nov 2019 **SACNAS** – Honolulu, HI

Mayo Clinic Summer Fellows Oral Symposium
Aug 2019 **Mayo Clinic** – Jacksonville, FL

Black History Month Research Poster and Symposium
Feb 2019 **Pennsylvania State University** – State College, PA

Millennium Scholars Interview Weekend Poster Session
Jan 2019 **Pennsylvania State University** – State College, PA

SURP Poster Session
August 2018 **New York University** – New York, New York

Leadership Alliance National Symposium (LANs)
July 2018 **LANS** – Hartford, Connecticut

Annual Biomedical Research Conference for Minority Students (ABRCMS)
Nov 2017 **ABRCMS** – Phoenix, Arizona

Benefactor & Distinguished Faculty Recognition Dinner Student Research Poster Presentations
Oct 2017 **Pennsylvania State University** – State College, PA

TSRI Summer Undergraduate Research Fellowship Symposium
Aug 2017 **Scripps Research Institute** – San Diego, CA

SCIENTIFIC SKILL HIGHLIGHTS

~Gel Electrophoresis
~PCR (troubleshooting)
~DNA Cleaning/ Gel Extraction
~Multiplex Eliza
~*In Situ* Hybridization
~Euthanization
~CyQUANT Proliferation Assay
~Culture/Split cell lines
~Harvest & Freeze cell lines
~SYBR Green I qPCR
~Primer Making
~Mice Maintenance, Care, & Handling
~Flow cytometry

TRAVEL AWARDS/GRANTS

Millennium Travel Grant
Nov 2019 **$3,000.00**

SACNAS Travel Grant
Aug 2019 **$ 500.00**

Millennium Travel Grant
Aug 2019 **$1,000.00**

Millennium Travel Grant
May 2018 **$2,000.00**

Schreyer's International Studies Grant
May 2018 **$ 300.00**

FASEB Dream Mentored Travel Award
Aug 2017 **$1,800.00**

ABRCMS Travel Grant
Aug 2017 **$ 125.00**