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Housing stress and sex : effects on corticosterone and interleukin-6

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HOUSING STRESS AND SEX: EFFECTS ON CORTICOSTERONE AND
INTERLEUKIN-6

A Thesis

Presented to

The Faculty of the Department of Psychology

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Diane Carney

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ABSTRACT

HOUSING STRESS AND SEX: EFFECTS ON CORTICOSTERONE AND INTERLEUKIN-6

by Diane Carney

The combined effects of sex and housing population on neuroendocrine-immune measures of stress were examined in 20 male and 20 female C57BL/6J mice. It was hypothesized that acute crowded housing elicits a stress response in males and has an opposite effect in females, as measured by corticosterone and interleukin-6 (IL-6) concentrations. After four days of individual or crowded, single-sex housing, corticosterone concentrations were significantly higher in individually housed and crowded housed males than in controls. Conversely, corticosterone concentrations were significantly lower in crowded housed females compared with controls. Analysis of IL-6 concentrations revealed a main effect for sex, $F(1, 29) = 16.73$, $p < .001$ and a main effect for housing, $F(2, 29) = 5.0$, $p < .01$. The results suggest that acute crowded housing has an opposite effect in males and females.

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Introduction

Stress has been a topic of study for approximately the past 75 years. In 1932, neurologist, Walter Cannon, discovered the bio-behavioral response to stress and explained that emotional reactions to a stressor are accompanied by physiological and behavioral changes. When an organism is faced with a stressor, chemicals are released from the adrenal medulla, and at the same time, a fight or flight behavioral response is initiated (Cannon, 1932). Since that time, fight or flight behavior has become well established in the literature as the standard behavioral response to stress, while additional physiological components of the stress response have been identified and defined. However, it is important to note that the majority of research on the topic of stress has been performed with male species alone. That being the case, it seems reasonable to question the default assumption that the stress response is characteristically the same in both sexes (Troisi, 2001). Along these lines, it is interesting to note that it is commonly reported that men and women react differently to challenges in their environments throughout the lifespan. But the questions for science are, do these tendencies have meaning beyond preference, and if so, to what benefit do they exist?

In a fairly recent theoretical article, Taylor et al. (2000) made a compelling argument in which they suggested that males and females cope with stress by using different behavioral strategies. Based on anecdotal observation combined with an assumption of evolutionary advantage, it was suggested that rather than primarily responding to stress by fighting or fleeing, females rely on cohesive behaviors to moderate stress. These behaviors include tending to their young through increased focus of attention and/or

befriending select same-sex cohorts, with the intention of establishing, securing, and maintaining social support networks. From an evolutionary standpoint, it is plausible that these patterns of behavior are more likely than fight or flight behaviors to ensure the survival of both mother and progeny (to maturity). Given this argument, a thorough examination of the behavioral responses to stress as well as their physiological correlates must be evaluated in both males and females in order to develop a cogent sex specific model of the stress response.

Assessment of the Stress Response: the Rodent Model

The rationale for the present study is rooted in observations of human behavior, supported by arguments of evolutionary advantage. However, the investigation of neurobiological and behavioral factors associated with stress is greatly limited in human subjects. In a large part, this is due to the obvious limitations associated with human subjects. Among these limitations is the low probability of maintaining control over possible confounds and that of obtaining behavioral, physiological, and morphological data from the same individual.

The animal model presents a viable alternative with which to circumvent such limitations. Rodent models allow for the possibility of manipulations and interventions while implementing stringent controls. Rodents are also a suitable model with which to thoroughly investigate sex differences in the stress response because they are short-lived, making it possible to compare the same variables in males and females in great numbers and at various points in the lifespan. Additionally, rodent models allow for easy modification of the original paradigm as findings might suggest.

Of the rodent models, this laboratory found the C57BL/6J mouse to be a good match for this work. This mouse strain is a particularly suitable model with which to examine the effects of stress. The C57BL/6J mouse is sensitive to stress and consistently experiences alterations of immune function with stress.

Social Stress

A number of rodent studies have used social stress paradigms to identify and define sex specific norms in relation to stress (Kaiser, Kirtzeck, Hornschuh and Sachser, 2003; Weiss, Pryce, Jongen-Rêlo, Nanz-Bahr and Feldon, 2003). Social stressors are increasingly preferred in experimental research because these models more closely simulate situations that are encountered in the natural environment (Blanchard et al., 2001). These stress paradigms are associated with distinguishing behavioral and neuroendocrine outputs in male and female rodents, because, in part, it appears that the social environments that induce stress in one sex may not induce it in the other (Westenbroek, Den Boer and Ter Horst, 2003).

Social Housing Stress

One form of social stress involves the manipulation of housing population. Specifically, individual housing, group housing and, in some cases, crowded housing conditions are established to compare the effects on males and females after exposure (Brown and Grunberg, 1995, 1996). Currently, housing population stress is one of the most widely used social stressors used to study differences in behavioral and physiological measures of sex differences in rodents.

Most studies that have used housing population stress have measured behavioral outcomes from these exposures. Palanza, Gioiosa, and Parmigiani (2001) examined the effects of social context in the housing environment on exploration and anxiety in male and female mice as a possible source of stress. It was hypothesized that individually and single-sex group-housed male and female mice would exhibit differences in risk-taking behaviors in response to the social environment. Using a modified free-exploratory paradigm, animals were given the opportunity to choose between exploration of a novel environment or to stay in a familiar environment. These methods were chosen to specifically examine risk assessment behaviors and exploratory propensity. Upon removal of a barrier, the test animal had free access to the home cage as well as to a novel environment. Palanza et al. reported that, when given the opportunity to explore the new environment, individually housed females left their home cages for shorter periods of time, explored the unfamiliar area less frequently and displayed more risk assessment behaviors in comparison to group housed females.

Conversely, the behavioral profile of males in these conditions revealed opposite trends. Specifically, individually housed males were more likely to explore a novel environment than group housed males. They displayed a lower latency to enter the novel area, spent more time exploring, and demonstrated a higher level of rearing behavior as compared with the group housed males. It was concluded that acute isolation is a mild stressor in female mice, but it is not in males. Additionally, males were more affected by group housing, with a reduction of exploratory behaviors in a novel environment, suggesting that group housing is a mild stressor in males. In total, it was concluded that

housing conditions could be used as a model for social stress in male and female mice. Based on these findings, one must ask, if housing conditions differentially affect behavior in males and females, does it also result in sex specific changes in physiological correlates of the stress response?

Neuroendocrine Correlates of Stress

The stress response, as it has been defined, is dependent on the activation of the neuroendocrine system. In mammals, the neuroendocrine components of the stress response involves the limbic-hypothalamo-pituitary adrenal (LHPA) system, wherein stressful stimuli trigger a cascade of neuroendocrine and autonomic events leading to varied behavioral, emotional, and cognitive outputs. Adrenal glucocorticoids are reported to be key molecules in this cascade (McEwen and Norton, 2002).

Brown and Grunberg (1995) examined the effects of individual vs. crowded housing conditions on corticosterone, a glucocorticoid found in animal species. Corticosterone was measured as a key biochemical marker of the LHPA system's response to stress. It was hypothesized that individual and crowded housing conditions would differentially effect male and female rats on this measure.

The procedure involved 7 male and 7 female, 22-week-old Wistar rats. Animals were housed either individually or 4 per cage (which defined the crowded condition). They were maintained in these conditions for 18 hours per day for eight consecutive days. On the last day, animals were decapitated and trunk blood was collected. Plasma was assayed for corticosterone using a standard radioimmunoassay kit.

The results showed that males had higher corticosterone levels after crowded housing when compared with males maintained in isolation. However, females had higher levels of corticosterone after individual housing when compared with females in crowded housing. The authors suggested that the ensuing stress may have resulted from the combination effect of spatial density and the number of social interactions, with each sex coping with crowding differently.

Overall, Brown and Grunberg (1995) concluded that when males and females are housed similarly, a clear biochemical difference exists. The authors further pointed out that these biochemical changes, conceivably, interact with additional physiological variables such as pharmacological agents, viral infections, and pathophysiological conditions. As such, housing stress could be used to investigate whether sex interacts with these variables to further distinguish the stress response in males and in females.

Neuroendocrine-Immune Response

It is well established that the immune system is linked to stress indirectly through the LHPA system and the actions of corticosterone and directly via corticotropin-releasing hormone receptors on immune cells (McEwen, 2000; Haddad, Saade, and Safieh-Garabedian, 2002). To the best of this author's knowledge, the effects of social housing stress on the neuroendocrine-immune interplay has not been examined. As such, the purpose of this project was to examine whether individual or same sex crowded housing provokes a stress response in male and female mice, and, if so, to further determine whether such a response would be associated with immune reactivity.

Psychological and physiological stress dysregulates the immune system, resulting in fluctuations of immune molecules within the organism. Interleukin-6 (IL-6), a proinflammatory immune molecule, is a pleiotropic cytokine and is involved in mediating cellular communication both in physiological and pathological states (Muñoz-Fernández and Fresno, 1998; Zhou, Kusnecov, Koolhaas, Van Zutphen, and Baumans, 1993). Previous work conducted in this laboratory, using C57BL/6J mice, found that acute cold-water stress is associated with an increase in proinflammatory immune molecules, such as IL-6 (Zhu et al., 1996). Additionally, IL-6 has recently been implicated in the regulation of emotionality. In open-field stress tests, IL-6 deficient mice displayed a higher degree of anxiety when compared with control mice (Butterweck, Prinz, Schwaninger, 2003). Therefore, changes in IL-6 levels might be expected to accompany the psychological stress associated with changes in housing population. It seems reasonable to test whether this molecule is produced in increasing amounts in response to social housing stress. It was hypothesized that crowded housing conditions will differentially affect male and female mice. Specifically crowding will cause a neuroendocrine-immune stress response in males, and it will have an opposite effect in females.

Method

Subjects

Twenty male and 20 female C57BL/6J mice were obtained from Jackson Laboratory, Bar Harbor, Me at 6-8 weeks of age. Upon arrival, same-sex mice were weighed and housed 5 per group in separate male and female chambers. Food and water were provided ad libitum. Animals were maintained in a controlled 12:12 light/dark cycle. They were given 2 weeks to recover from shipping stress.

Materials and Procedure

The stress paradigm for this experiment consisted of individual or crowded housing of same sex animals for 4 consecutive days (Brown and Grunberg, 1995; Palanza, 2001). Animals were randomly assigned and placed in either individual (n = 5, individually housed males; n = 5, individually housed females) or same-sex crowded housing conditions (n = 10, per cage). Individually housed animals were kept in 27 x 15 x 13 cm cages, and crowded animals were housed in 44 x 23 x 20 cm cages. Control animals remained in the same 44 x 23 x 20 cm cages they were placed in upon arrival (n = 5) (Van Loo, Mol, Koolhaas, Van Zutphen, and Baumans, 2001).

After 48 hours of housing manipulation, experimental and control mice were injected with Thioglycolate IP to elicit macrophages to the peritoneum, 1.5 ml per male and 1.0 ml per female.

At the beginning of the fourth and final day of the experiment, animals were weighed, and samples of vaginal cells were collected via vaginal swabbing from both experimental and control female mice. Microscope slides were prepared with the sample to determine

estrus cycle phase at expiration: Di-Estrus, Early Estrus, Estrus, Pro-Estrus, and Post Estrus.

Corticosterone Determination

Trunk blood was then collected via decapitation (immediately following cervical dislocation), and it was stored in heparin washed epindorf tubes. Specimens were centrifuged at 1200 rpm for 12 minutes. Serum was collected and stored at -80° C for future determination of corticosterone concentrations (Alpco Diagnostics, Windham, NH).

Serum concentrations of corticosterone were determined with an OCTEIA corticosterone competitive enzyme-immunoassay kit (Alpco Diagnostics, Windham, NH). This kit utilizes a polyclonal corticosterone antibody coated onto the inner surface of polystyrene microtitre wells. Calibrators, controls, and diluted samples were incubated with enzyme labeled corticosterone (horseradish peroxidase) overnight at 2-8°C. Subsequently, wells were washed and color developed using chromogenic substrate (TMB), which was followed by the addition of 1M phosphoric acid as a stop solution. The assay consisted of establishing a standard curve with two-fold serial dilutions of calibrators. Calibrators, controls, and diluted samples were tested in duplicate. Assays were read at a wavelength of 450 nm on a Finstruments (96-well) Microplate Reader, with color intensity development being inversely proportional to the concentration of corticosterone.

Cell Culture

Peritoneal exudate cells (PEC) were obtained by lavage with 10 ml of sterile RPMI. Following collection of the trunk blood, PEC were collected from individual mice, washed and centrifuged at 1200 rpm for 10 minutes at room temperature. Viability was determined and cells were resuspended in supplemented RPMI media (RPMI 1640 = 10% Fetal Bovine Serum, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, 2mM L-glutamine (Sigma Corporation, St. Louis, MO). Plates (96-well plates) were seeded with 10^5 cells per well. Cells were allowed to adhere for 2 hours at 37° C in a humidified atmosphere with 5% CO₂ and washed 3 times with RPMI to remove non-adherent cells and to establish a pure macrophage population. Peritoneal exudate cells were incubated (37° C, 5% CO₂) in supplemented RPMI 1640 medium overnight. Supernatant fluids were stored at -80° C for later determination of IL-6 production.

Interleukin-6 Determination

Interleukin-6 concentrations in supernatants were quantified using a sandwich type ELISA. IL-6 primary goat anti-murine and secondary biotinylated monoclonal antibodies were used according to manufacturer's specifications for concentrations and handling (Pharmingen, San Diego, CA). Wash solution, BSA blocking solution, and peroxidase substrate system were purchased from Kirkegaard and Perry Laboratories, Inc. (Gaithersburg, MD). Avidin-Peroxidase was purchased from Sigma Corporation (St. Louis, MO) and 1M phosphoric acid was added as a stop solution. A standard curve was

established with serial two-fold dilutions of recombinant interleukin-6 (Sigma Corporation, St. Louis, MO) beginning with 5ng/ml. Samples and standards were tested in duplicate. Light absorbance of the assay was read at a wavelength of 450 nm on a Finstruments (96-well) Microplate Reader, employing supplemented RPMI media as blanks tested in duplicate.

Results

It should first be mentioned that there were a number of potential confounding factors that may have affected this experiment. These include extreme temperature changes in the animal facility and non-adherence to light/dark maintenance. It is well established in the literature that corticosterone levels rise and fall within a diurnal cycle and are highly sensitive to environmental changes in temperature and light/ dark exposure (Seggie and Brown, 1974). With these events in mind, it was decided that extreme values would not be considered in the statistical analyses. These data were not included because they did not accurately represent the nature of the outcome caused by the housing manipulations.

Furthermore, a number of animal samples were not included in the analysis of the corticosterone assay. Of the excluded animals, one female control died prior to the experiment. One crowded housed male was excluded from the analysis because of an insufficient quantity of serum for testing. One female control and 2 crowded housed females were excluded because of concentrations below the acceptable range.

As for the analysis of IL-6 concentrations, 1 female control was not included due to death prior to the experiment. Among the remaining animals, data from 1 male control, 2 individually housed males, and 1 individually housed female were not included because of inadequate sample volume.

Figure 1 shows the mean serum corticosterone concentrations for males and females: controls, individual and crowded housing conditions. Among the males, corticosterone levels were lowest among control animals and highest among those in the crowded condition. In the case of the females, corticosterone concentrations were highest among

Figure 1. Corticosterone Concentration: Mean (+/- SE). Corticosterone levels indicate a significant main effect for housing, ($p < .01$). There also exists a housing X sex effect, ($p < .001$). Crowded females and crowded and individual males were significantly different from controls. ** $p < .01$. *** $p < .001$.

control females and lowest among individually housed females. However, the mean concentration of the crowded females was only slightly greater than that of the individually housed females. Consideration should be given to the fact that the sample size of the individually housed females was less than half of that of the crowded females, which may account for this finding. Comparable sample size, in the future, may better distinguish the differences between the two conditions.

An analysis of variance (ANOVA) was conducted with housing configuration (control, individual or crowded housing) and sex (male and female) as the independent variables and with corticosterone concentration as the dependent measure. The overall analysis showed that there was a main effect for housing, $F(2, 29) = 6.75$, $p < .01$ and an interaction effect between housing and sex, $F(2, 29) = 21.90$, $p < .001$.

To determine the nature and source of the significant interaction effect, simple effects analyses were conducted examining the effect of housing condition on males and females separately. Simple effects for housing condition among males was significant, $F(2, 29) = 20.76$, $p < .001$. Simple comparisons revealed that corticosterone concentrations were higher in individually housed males ($M = 5.1$) and crowded housed males ($M = 6.7$) than in controls ($M = 2.8$). The simple effect for housing condition among females was significant, $F(2, 29) = 3.96$, $p < .05$. Simple comparisons revealed that corticosterone concentrations were lower among females in the crowded condition ($M = 3.1$) than controls ($M = 12.0$).

Figure 2 shows the mean IL-6 concentration for males and females: controls, individual and crowded housing conditions. Of the males, IL-6 concentrations were highest among controls and lowest in the crowded population. Of the females, the highest concentrations were found in the controls and the lowest were found in the individually housed females

An ANOVA was also conducted with housing population (control, individual or crowded housing) and sex (male and female) as the independent measures and IL-6 concentration as the dependent measure. The overall analysis revealed a significant main effect for sex, $F(1, 29) = 16.73$, $p < .001$, with IL-6 concentrations higher in females than in males. Overall analysis also showed a significant main effect for housing, $F(2, 29) = 5.0$, $p < .01$. Main comparisons were conducted to determine the nature of the main effects for housing. Analysis showed a significant difference between the control and individual housing conditions, $F(1, 29) = 7.08$, $p < .01$, with IL-6 concentrations lower in individually housed animals than in control housed animals. There was also a significant difference found between the control and crowded conditions, $F(1, 29) = 7.81$, $p < .01$, with IL-6 concentrations lower in crowded housed animals than in controls. The sex by housing interaction was non-significant.

Estrus phase at time of death was evaluated to determine if hormone cycles were synchronizing among females in any of the three housing conditions. The only possible trend observed was that more than 50% of the females in the crowded condition were in the estrus phase of their cycle.

Figure 2. IL-6 Concentration: Mean (+/- SE). IL-6 levels indicate a main effect for sex, $F(1, 29) = 16.73$, $p < .001$, with IL-6 levels significantly higher in females than in males. There was also a main effect for housing, $F(2, 29) = 5.0$, $p < .01$; IL-6 levels were significantly lower in individual and crowded housing than control.

* $p < .05$. *** $p < .001$.

Discussion

In their seminal article, Taylor, Cousino-Klein, Lewis, Gruenewald, Gurung, and Updegraff (2000) proposed that females generally rely on alternative behavioral strategies when confronted with a stressor, such as tending to young or affiliating with select same-sex cohorts. It was suggested that these behaviors may be biologically intertwined with the biochemical pathways associated with the mother-infant caregiver-attachment system. Behavioral and physiological normality of a distinct female stress response may, in fact, be rooted in the reproductive and childrearing roles of the mother. In other words, the most successful strategy of coping with a stressor may be the implementation of those behaviors that most likely secure the survival of the female and her offspring. Arguably, a successful strategy for coping, with all but the most threatening stressors, may not include direct confrontation of a stressor with fight behavior or securing safety through flight. If unsuccessful, either option leaves the offspring isolated and vulnerable. In order to investigate the possibility of alternative coping strategies, a distinction of the stress response in males and females must first be demonstrated.

As such, the overall purpose of this study was to examine and distinguish the effects of stress in male and female mice. Specifically, the relationships were evaluated among social housing population stress in male and female mice, neuroendocrine and immune reactivity. It was hypothesized that crowded housing conditions differentially affect male and female mice, with crowding causing a neuroendocrine-immune stress response in males and having an opposite effect in females.

Corticosterone

The data show that corticosterone concentrations increase in male mice that have been housed in either same-sex crowded housing or individual housing for an acute period of time relative to male controls. However, corticosterone concentrations are highest among males after crowded housing. This result supports the hypothesis that crowded housing is a stressor in male mice. Additional analysis revealed that corticosterone concentrations were lower in crowded housed females than in female controls. This supports the hypothesis that crowded housing may have an opposite effect in females. These results are consistent with prior studies that found acute crowded housing is a stressor in male rats and mice, and it has an opposite effect in females (Brown and Grunberg, 1995; Palanza, 2001).

Curiously, the data from the current study indicate that individual housing in both males and females causes different outcomes than those reported by Brown and Grunberg (1995) and Palanza et al. (2001). Both report that individual housing is not stressful in males, while it is in females. The data for the current study show increases in corticosterone concentrations in males in both individual and group housing and decreases in corticosterone concentrations in females in both conditions. While the statistical findings support the research hypothesis, it is interesting to consider that the results may indicate differences in stress reactions due to *change* in housing population rather than reactions to housing population density. Alternatively, the differences among the experiments may be explained by differences in protocols. Rats were used in the Brown and Grunberg study, while Palanza et al. tested group housing, with four mice per

group, as opposed to the 10 mice per group in the current study. Lastly, consideration should be given to the effect of cage size in the current study. Crowded animals were housed in 44 x 23 x 20 cm cages, while individually housed animals were kept in 27 x 15 x 13 cm cages. It may be the case that the current protocol tested the effects of group housing rather than that of crowded housing, considering the differences in cage size. If that were the case, it would be reasonable to expect less robust differences in corticosterone and IL-6 concentrations between housing conditions.

Interleukin-6

The data did not reveal significant relationships between housing and sex on this immune measure. However, it is interesting to note that mean IL-6 concentrations decreased in both crowded and individually housed males and females when compared with controls. The direction of these changes is not consistent with the previous findings of this laboratory (that IL-6 concentrations increase with acute coldwater stress in male mice). However, it has been suggested in the literature that not all stressors have the same effect on immune processes (Sheridan, Stark, Avitsur and Padgett, 2003). It may be the case that IL-6 concentrations are suppressed with housing population changes in both sexes. Overall, further investigation is warranted to determine the meaning of the directional changes of this immune molecule relative to that of corticosterone.

Limitations of the Study

The results of this study are called into question because of concerns about the impact of environmental conditions in the vivarium during the experiment. Temperatures in the housing facility fluctuated greatly during the experimental period (ranging from 70

degrees to 88 degrees, Fahrenheit), and rather than adhering to the standard 12:12 light/dark cycle throughout the experiment, the lights were left on for a 24 hour period. Explanations of the relationships among stress, corticosterone and IL-6 will detail the potential impact of these environmental events on this experiment.

Stress is broadly defined as a physiological or psychological departure from homeostasis and is commonly measured by changes in circulating corticosterone levels (McEwen, 2000). It is well established in the literature that glucocorticoid levels rise quickly with stress. Specific stressors shown to effect corticosterone concentrations include: temperature change, experimental stimuli and departure from usual routine. In addition, corticosterone levels are known to fluctuate within a diurnal cycle, reaching highest and lowest levels two hours before the switch to dark and light, respectively (ALPCO diagnostic, 2001; Seggie and Brown, 1974).

Normal rhythms are dependent on predictable, consistent light/dark exposure and consistent temperatures in the housing environment. As such, corticosterone levels can easily be disrupted by temperature and or light/ dark cycle inconsistencies. Finally, since the immune system has been linked, in part, to stress through the actions of corticosterone (McEwen, 2000), a concern arises about the effect of the environmental inconsistencies on IL-6 concentrations as well as on corticosterone concentrations.

This study was designed to reveal normal relationships among stress, sex, and neuroendocrine-immune responses. As such, additional study of IL-6 responses with this stress paradigm can only reveal or confirm normal alterations associated with this

stressor. A future study of these variables would include strict adherence to standard temperature requirements and light/dark cycle maintenance in the housing environment.

An additional limitation of this study was the small and uneven number of animals in each condition. The original number of animals in the control groups and individual housing groups was so small that mortality and experimenter error resulted in a small number of animals per group for analysis. A greater number of groups of animals could increase confidence in the statistical analyses.

Conclusions

The data suggest that crowded housing may cause a stress response in male mice and may have an opposite effect in females. More specifically, housing population differentially affects corticosterone levels (a reliable measure of the stress response) in male and female mice. However, additional examination is warranted to understand the effect of housing population stress on immune processes. Future research should reexamine the relationships among housing population, corticosterone, and IL-6, with strict control over temperature and light/dark cycles in the animal facility.

The overall importance of these findings is two-fold. First, the value of using a social stress model, such as comparing differences in the effects of housing population in males and females, may be that this model more accurately represents stress as it is encountered in the natural environment. Furthermore, this stressor may be more appropriate than traditional stressors for examining the relationship between stress and emotional and physiological responses in males and females. In fact, these findings indicate that environmental conditions may not be perceived equally by male and female mice. The

importance of this is that from an evolutionary standpoint, males and females may negotiate their environments differently, such that males primarily respond to a stressor by assuming a posture of fighting or fleeing. As such, it would make sense that crowding would be a stressor in males, as a great number of males in the immediate environment could be perceived as potential adversaries. Conversely, it seems plausible that females, as the primary parent, in many species, would be driven to ensure their own survival and that of their young, which could be secured through interdependence or affiliation. By readily affiliating with others in the immediate environment through cohesive interactions, the female would establish and reinforce a reciprocating relationship that would secure an investment in the safety of cohorts.

While the argument for a female specific stress response is compelling, the scope of the current study examined a component of what is likely a larger model. Study of the systems involved in the caregiver-attachment system is warranted and may reveal additional physiological components of the female stress response. A comprehensive female specific model of the stress response will consider the involvement of additional psychoneurophysiological systems, such as neuroendocrine, immune and neuropeptide systems, and will include examination of behavioral correlates.

Determining whether sex differences in the stress response exist is an important step in establishing a more thorough understanding of the impact of stress on health and disease states in males and females. With a comprehensive definition of the stress response, future research may lead to a better understanding of differences in the occurrence rates of maladaptive coping and its effects on psychological and physiological

health. A clear understanding of a gender specific stress response can serve as a foundation for health and/or treatment models that consider the contribution of sex differences on those aspects of behavior that lend to maladaptive and adaptive coping.

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Appendix A

Letter of Official Protocol Review