Gender differences in stress appraisals and sympathetic nervous system responses to repeated psychosocial stress

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Spring 2015
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1 Abstract

Women are more likely to get autoimmune diseases than men. The immune system can be activated by acute stress via stimulation by the sympathetic nervous system. Many studies have documented gender differences in the SNS inflammatory stress responses, and finding a pattern of increased SNS activity in women would help to explain the differences in disease rates. In this study, we exposed healthy young and old men and women adults to a psychosocial stressor (TSST) on two consecutive days and measured a marker of the SNS, salivary alpha amylase, in order to study stress reactivity and habituation. At the same time, we also used pre-TSST stress appraisals to measure if different appraisals of the stressor would have impact on the SNS reactivity. We found that the psychosocial stressor induced an increase in amylase release on both study days, but this increase was attenuated in men only, indicating the presence of habituation in men but not women. While there were no differences in pre-TSST stress appraisals between men and women, stress appraisals such as threat and challenge were predictive of amylase increases on both study days in women only. Taken together, we found patterns of stress reactivity in women that could predispose them to autoimmune disease; furthermore, stress appraisals predict heightened reactivity in women only, suggesting a psychological link between stress and health that differs between the sexes.
2 Introduction

There are several discrepancies in health measures across the lifespan between men and women. For example, men have shorter lifespans than women, but women are more likely than men to get autoimmune diseases. During our daily life, we see our mothers and other female relatives reporting more physical illness, taking more prescribed medicines, visiting the doctors more, and suffering from more diseases. Most of these diseases are related with immune system dysfunction, including Sjogren’s syndrome, systemic lupus erythematosus (SLE), scleroderma, rheumatoid arthritis (RA) and multiple sclerosis (MS). Women are also more likely to suffer from depression than men. Stress can exacerbate these conditions, and could actually cause them to develop. Because of different gender roles, the same stressor could result in different amounts of stress for women and men. The feminine role could predispose women to depression by encouraging them to feel and act helpless (Lips, 2008), and these reactions could cause downstream physiological dysregulations that could result in negative health consequences. Both emotional and physiological responses to stress might be different between men and women, and these differences could result in different health outcomes between the sexes.

2.1 Prevalence of Autoimmune Diseases: Post-Menopausal Women

There are marked differences in the prevalence of autoimmune disorders between men and women (Ngo, Steyn, & McCombe, 2014), especially as people age. Differences in immune system functioning may underlie gender bias in autoimmune diseases (ADs). Women have a
higher potential risk to get autoimmune diseases: in fact, they are nine times as likely as men to develop an autoimmune disease. This class of disease, which includes diabetes, thyroid disease, and neurological degenerative conditions, has been described as “a leading cause of death” for women (Stephen & Laurie, 2000). More detailed data highlights the very high (9:1) gender bias toward women for systemic autoimmune diseases including Systemic lupus erythematosus (SLE) and Sjögren’s syndrome (SS) occurring independent of country of assessment. Although diseases can occur at any age, older women have a higher risk of developing autoimmune diseases, and they also tend to have poorer prognoses than their younger counterparts (Feghali & Wright, 1997). For example, older adults’ immune systems show increased levels of pro-inflammatory cytokine, which are key modulators of inflammation (Feghali & Wright, 1997), as well as increased antibody titers.

Differences in immune response could underlie gender bias in autoimmune diseases, but the higher risk of older women developing autoimmune diseases suggests older women get more diseases overall. However, women in general have higher risk of ADs.

2.2 Sex Steroids and the Immune System

Based on these gender differences in risk for ADs, gonadal hormones may have a role in this sex differential (Pennell, Galligan, & Fish, 2012). Ovaries produce three classes of sex steroids: estrogens, progestins, and androgens, which are the main factors affecting the immune system (Bouman, Heineman, & Faas, 2005). Throughout the menstrual cycle and pregnancy, the
levels of these hormone vary, and for instance, estrogen levels increase during the menstrual cycle as well as pregnancy (Pennell et al., 2012). On the other hand, women with systemic lupus erythematosus (SLE), tend to have lower androgen levels than healthy women, and SLE tends to worsen during pregnancy and to remit after menopause, because of higher level of estrogens and progestins. Therefore, sex hormones are crucial for SLE regulation (Quintero, Amador-Patarroyo, Montoya-Ortiz, Rojas-Villarraga, & Anaya, 2012). Changes in sex hormones often correlate with alterations in the immune system; therefore, factors associated with reproduction can regulate immune responses (Bouman et al., 2005).

Because the production of sex hormones fluctuates with ovarian activity (Bouman et al., 2005), young women would have more estrogens and progestins, compared to post-menopausal women. Because women’s sex hormones increase and stimulate autoimmune processes (Bouman et al., 2005), it would be expected that autoimmune diseases are less prevalent in post-menopausal women. On the contrary, the truth is that older women develop more diseases overall. Therefore, some other reasons should also be considered.

2.3 Stress and the Immune System

Apart from sex steroids, stress could also be an important factor that influences susceptibility to autoimmune diseases, because stressful life experiences can have significant effects on a variety of physiological systems, including the immune system. For instance,
immune cell products can act on the brain, altering mood and cognition, potentially contributing to depression (Kemeny, 2003).

Since stress is related to shame and other negative self-evaluation (Dickerson, Gruenewald, & Kemeny, 2004), women could actually face more stress than men and so could be more likely to be helpless or powerless. The social feminine role could teach women to respond to stress in powerless, helpless ways (Radloff, 1980; Ruble, Greulich, Pomerantz, & Gochberg, 1993); this finding is consistent from childhood onward. Gender differences first become noticeable in adolescence, when girls begin to show significantly more depressive symptoms than boys do (Cicchetti & Toth, 1998). Some empirical evidence indicates that parents are more responsive to boys’ disharmonious emotion than to girls’ submissive emotions, specifically, sadness and anxiety (Chaplin, Cole, & Zahn-Waxler, 2005). Parents use more anger-related words with boys than with girls and refer more to sadness and happiness with girls than with boys (Adams, Kuebli, Boyle, & Fivush, 1995; Fivush, 1989; Fivush, Brotman, Buckner, & Goodman, 2000). This suggests that during childhood, parents put more anger in boys’ consideration and this affects boys’ emerging ideas of male-appropriate behavior. Males could be more powerful in society because they have been encouraged to be angrier. Boys would assume that as male characters in society, they should be more aggressive, and girls are not able to cope with the anger-related words. People actually begin to be shaped in their childhoods, and they could take gender bias to their adulthoods in both teaching how their sons or daughters
should behave. This also applies to how adults are encouraged to behave in their specific gender roles.

However, women, on average, tend to report greater life satisfaction and happiness than men do – a seemingly paradoxical finding in view of women’s higher rates of depression (Wood, et al., 1989). However, recent theories have posited that rather than engage in fight or flight responses to stress, women may ‘tend-and-befriend’, which means they will seek social affiliation in response to stress (Taylor et al., 2000). Women responses to stress are not well characterized by fight-or-flight, which has been represented as an essential mechanism in the survival process, because usually women create, maintain, and utilized social groups, especially relations with other women, to reduce vulnerability and manage stressful conditions, but this behavior may build on attachment caregiving processes that down-regulate the sympathetic nervous system (SNS) and the hypothalamic-pituitary adrenal (HPA) axis response to stress (Taylor et al., 2000). For older women, poor regulation of the immune system could also contribute to gonadal hormone factors, because stress could stimulate gonadal hormone’s production (Ranabir & Reetu, 2011). Therefore, women have more possibilities to develop irregular response to stress than men do.

The relationship between stress and ADs can be explained as below. There are two main systems that regulate inflammation: the HPA axis, which produces cortisol and has a suppressive effect on inflammation (Tsigosa & Chrousos, 2002), and the SNS, which is a faster acting system
producing catecholamines such as norepinephrine (NE) that can stimulate inflammation (Tsigosa & Chrousos, 2002). Since autoimmune diseases could be caused or driven by over activity of the immune system, and catecholamines are immune system stimulants. Therefore, if women have more catecholamines they will be more likely to get autoimmune disease through overstimulation of inflammation. Meanwhile, if women have the same catecholamines, they will also be more likely to get ADs, because of lower cortisol release (Taylor et al., 2000).

The SNS comes online quickly after the introduction of a stressor, with NE concentrations peaking about 10 minutes after the onset of stress (Dimsdale & Moss, 1980). This system prepares the body for ‘flight or fight’ (Jansen, Van Nguyen, Karpitskiy, Mettenleiter, & Loewy, 1995); that is, in the event of a stressor, activation of the SNS allows more blood flow to muscles by increasing the heart rate, and triggering the release of glucose into the bloodstream to provide energy. This flight-or-fight response could be adaptive, because women’s “tend-and-befriend” (Taylor et al., 2000), but we do not know yet.

Under the same standardized conditions and more intense real-life stressor, gender roles and psychological factors are more important than biological factors in explaining differences in epinephrine response. For instance, men’s immune system increase epinephrine response significantly, but women do not respond at all or very little, and they perform as well or even better on the various tasks (Lundberg, 2005). However, women's stress levels tend to remain elevated also after work, and could be it because women have a greater unpaid workload due to
household chores and child care (Lundberg, 2005), which is to say, women have more possibility to have increased catecholamines and develop autoimmune disease. Therefore, assessing patterns of catecholamine responses in women compared to men across the lifespan could explain why women get more autoimmune diseases than men.

2.4 Allostatic Load

The term allostasis describes a process that our immune in which our stress systems protect the body by responding to internal and external stress by constantly changing in order to adapt to different circumstances (McEwen, 1998). Activation of allostatic response will command the SNS to release catecholamines, and also comment command the HPA axis to release cortisol, but returns catecholamine and cortisol secretion to base-line when inactivation happens, usually as the danger is past. If the inactivation is inefficient, constant or irregular exposure to stress hormones can eventually induce illnesses and weaken the body’s immune systems (McEwen, 1998).

Taken together, we can conclude that reducing allostatic load by proper activation and cessation of stress response systems, such as the HPA axis and the SNS can result in better health outcomes. Determining how to reduce allostatic load requires consideration of stress response patterns, rather than only studying first time stressful experiences. The HPA axis is known to habituate to repeated acute stress, and show an attenuated response to a second-time stressor (Wüst, Federenko, van Rossum, Koper, & Hellhammer, 2005). This would be an adaptive
pattern; when one encounters a first-time stressor, we expect a robust stress response. However, during a second encounter, when the person knows what to expect and should theoretically feel less threatened (because they survived the original stressor), they should have an attenuated response. However, not everyone does. We see non-habituation of the HPA axis in depression, high ruminators, and neurotic personalities (Gianferante et al., 2014; Kudielka et al., 2006; Morris & Rao, 2014; von Kanel, Kudielka, Preckel, Hanebuth, & Fischer, 2006). However, we do not generally see habituation of the norepinephrine response across multiple stressors (Schommer, Hellhammer, & Kirschbaum, 2003). However, habituation of both of these systems in older adults has not yet been studied. Because irregular or ‘unhealthy’ patterns of stress responses might cause later disease by having negative impacts on allostatic load, and older adults are more likely to develop disease, understanding stress response patterns in older adults may be key.

In summary, since we have already known that the HPA axis has adaptive patterns, it will be particularly interesting to know the SNS patterns of adaptation, as the SNS interacts with immune functions. Therefore, this study measures stress responses in middle aged adults and young controls across two in-lab stressors. Alpha-amylase is a marker of sympathetic nervous system activity that can be measured non-invasively, as it is found in saliva (Thoma, Kirschbaum, Wolf, & Rohleder, 2012). Salivary alpha-amylase (sAA) closely mirrors norepinephrine in plasma, and is stress responsive (Thoma et al., 2012). This measure of SNS activity will be examined by age and sex, and also in the context of subjective stress ratings, in order to better
understand both biological and psychological processes that could differ between men and women across multiple stressors.

2.5 Specific Aims and Research Questions

We set out to examine patterns of SNS activity to repeated psychological stress in both men and women across the lifespan. We wanted to address the following questions:

1. Do women have higher SNS responses to novel and/or repeated stress compared to men?

2. Are there sex differences in psychological stress appraisals?

3. Is there an interaction of stress appraisals with SNS stress reactivity?

More specifically, we predict that women will show amplified amylase responses to a stressor and will not show a pattern of sympathetic nervous system habituation. Such a pattern could explain sex differences in autoimmune disease development. Furthermore, we specifically hypothesize that women will show greater negative subjective responses to an acute stressor, including increased feelings of threat. These findings would agree with literature regarding higher risk of depressive symptoms in women. Additionally, we hypothesize that stress appraisals will be correlated with increased SNS reactivity, and this relationship will be strongest in women.
3 Methods

3.1 Participants

Data for this research was collected over 2 years as one part of a large project on aging and stress responses. The pool of participants was comprised of young adults (age 18–35) and older adults (age 50–65) who were chosen from the Greater Boston area and the Brandeis University campus via newspaper, magazine, and Facebook advertisements and received monetary compensation.

All participant had to meet a specific selection criteria: (a) body mass index (BMI) within the reference range between 18 and 30 kg/m2; (b) luteal phase of menstrual cycle at time of participation, for women; (c) absence of psychiatric, endocrine, or cardiovascular diseases, or other specific chronic diseases; (d) no intake of psychoactive drugs, beta-blockers, gonadal steroids (hormonal contraceptives), GCs; (e) non-smoker, and (f) no previous experience with the stress protocol, collecting by telephone before testing. Four participants were excluded from analyses because their baseline catecholamine/norepinephrine scores fell at least 3 standard deviations above the mean on the first study day (n = 1) or the second study day (n = 3).

3.2 Procedure

All laboratory sessions were scheduled in the afternoon (starting between 1:30 and 6:30 pm) to control for circadian variation of stress hormones for eligible participants on two
consecutive days. Participants could not eat or drink anything but water for 1 hour before the laboratory sessions. Informed consent was obtained from the participants before participating in the research and ethical approval was granted by the Brandeis University Institutional Review Board. Participants were taking part in laboratory session which each lasted approximately three hours and included a resting period, and their saliva samples were collected at baseline and at the first 4 amylase time points (-1, +1, +10, +30 mins TSST) following exposure to a standardized laboratory stressor - Trier Social Stress Test (TSST) as described below. Depressive symptoms, and demographic factors were assessed using self-report paper-and-pencil questionnaires at the beginning of day 1.

3.3 Measures

3.3.1 State and Trait Measures

Depressive symptoms were assessed using the 20-item Center for Epidemiologic Studies Depression Scale (CES-D; (Radloff, 1977)), which had demonstrated reliability and validity (Radloff, 1977) and contained questions like how often respondents had felt or behaved in the stated manner over the past week, including statements such as “I felt depressed.” Using a 4-point scale (0 = Rarely or none of the time; 1 = Some or a little of the time; 2 = Much of the time; 3 = Most or all of the time). The total score was computed by summing scores on all items after reverse scoring four items. Stress appraisals were measured using the Primary and Secondary Appraisal Scale (PANAS; see Appendix A) which measures feelings of threat, challenge, self-competency, and control expectancy (Gaab, Rohleder,
Nater, & Ehlert, 2005). This measure is filled out in the 5-minute anticipatory period immediately before the TSST occurs.

3.3.2 Body Mass Index (BMI)

As a measure of adiposity, BMI was calculated using each person's weight and height by the following formula: weight in kg/ (height in m × height in m).

3.3.3 Stress induction paradigm

Because the Trier Social Stress Test (TSST; (von Dawans, Kirschbaum, & Heinrichs, 2011) showed reliability and validity and in producing strong biological responses to stress (Dickerson & Kemeny, 2004), this test was used to expose participants to acute psychosocial stress. Participants were coming to the laboratory and told to give a five-minute public speech which contained how one’s personality made one qualified for a dream job, and a five minutes mental arithmetic task, which involved counting backwards from 2043 by increments of 17 on the first study day and from 2011 by 13 on the second study day, in front of two judges, who were expert evaluators wearing lab coats and maintaining a neutral evaluative facial expression. In order to make sure participants got enough stress from the TSST, they were also informed that the judges would analyze their verbal and non-verbal behavior and their performance would be videotaped at the same time. After the TSST, participants rated how “distressed” they felt by the task on a 5-point scale (1 = Very slightly or not at all; 5 = Extremely).
3.3.4 Measurement of alpha-amylase

Saliva samples were frozen immediately after laboratory session and stored at -20°C until analysis. After thawing, salivates were centrifuged at 2000 rpm for 5 min, which resulted in a clear supernatant of low viscosity. Salivary alpha-amylase measurement was completed by using an enzyme kinetic method.

First, saliva was diluted 1:625 with double-distilled water. Twenty microliters of diluted saliva and standard were then transferred into standard transparent 96-well microplates. Standard was prepared from ‘‘Calibrator f.a.s.’’ solution (Roche Diagnostics, Mannheim, Germany) with concentrations of 326, 163, 81.5, 40.75, 20.38, 10.19, and 5.01 U/L alpha-amylase, respectively, and ultrapure water as zero standard. 50 µl of substrate reagent (alpha-amylase EPS Sys; Roche Diagnostics) were pipetted into each well using a multichannel pipette thereafter. The microplate containing sample and substrate was then warmed to 37°C using a thermomixer for 3 minutes. Immediately afterward, a first interference measurement was obtained at a wavelength of 405 nm using a standard ELISA reader (Anthos Labtech HT2, Anthos, Krefeld, Germany). The plate was then incubated for another 5 min at 37°C, before a second measurement at 405 nm was taken. Increases of absorbance in sample were transformed to alpha-amylase concentration using a linear regression calculated for each microplate. Inter- and intra-assay variation was below 10%.
3.3.5 Statistical analyses

All statistical analyses were performed using SPSS for Mac OSX (21.0) software packages (IBM, Chicago, IL, USA). Kolmogorov–Smirnov tests were computed prior statistical analyses to test for normal distribution as well as homogeneity of variance of all dependent variables. Student’s t-tests were computed for the assessment of differences in demographic characteristics between groups. For an estimation of a stress reactivity measure for sAA, we computed delta scores (peak values after stressor minus baseline values before stressor). For the analysis of a successful stress induction by the TSST in terms of sAA, we computed analysis of variance (ANOVAs) for repeated measures over all measurement points (−1 min, +1 min, +10 min, +30 min). In all ANOVAs, Greenhouse–Geisser corrections were applied if the sphericity assumption was violated (Greenhouse & Junker, 1992; Vasey & Thayer, 1987). Pearson correlations were computed to evaluate possible associations between indices of sAA and stress appraisal subscales. All reported results were considered to be significant at the p<0.05 level, and were considered a trend at the p<0.1 level. All tests were two-tailed. Unless otherwise indicated, all reported results shown are untransformed means± standard deviations (SD).
GENDER AND STRESS RESPONSES

4 Results

4.1 Preliminary data analyses

77 healthy adults (39 women) took part in two consecutive TSST’s (see table 1 for sample characteristics).

Depressive symptoms were measured to assess for any gender differences. The CES-D was internally consistent ($\alpha = 0.93$) and average scores fell below the clinical cut-off ($M = 12.82; SD = 0.55$). Men and women had no differences in depressive symptoms as measured by the CES-D ($t(73)=.06, p=.95$). Age and BMI are reported in the table below (Figure 1). We found no significant differences between age groups ($F(1,67)=.01, p=.91$).

<table>
<thead>
<tr>
<th>Participants</th>
<th>Young Men</th>
<th>Young Women</th>
<th>Middle-aged Men</th>
<th>Middle-aged Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>21</td>
<td>21</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.6 (+/- .87)</td>
<td>20.5 (.67)</td>
<td>55.88(1.3)</td>
<td>57 (1.06)</td>
</tr>
<tr>
<td>Age Range</td>
<td>18-33</td>
<td>18-29</td>
<td>47-65</td>
<td>51-65</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>24.86 (.71)</td>
<td>24.8 (.89)</td>
<td>27.87 (.91)</td>
<td>24.1 (.66)</td>
</tr>
</tbody>
</table>

Table 1. Sample Characteristics
4.2 Amylase Responses to Repeated TSST’s

To test whether initial and repeated stress induced increases in sAA, and whether these increases habituated, we computed a repeated measures ANOVA. Results revealed a significant day by time interaction (F=4.72; p=0.003) indicating different changes over time between the two days, which would be consistent with habituation. To further understand habituation, we tested if delta scores of sAA increases were different between day 1 and day 2. Results showed higher responses on day 1 of testing (mean amylase increase= 51.3 U/ml) and lower responses on day 2 (mean increase=33.88 U/ml) indicating habituation (t(71)=2.4, p<.05; see Figure 1).

![Figure 1. Amylase responses in the group overall indicate the presence of amylase habituation to repeated stress.](image-url)
4.3 **Sex differences of amylase response among day 1, day 2 and habituation**

In order to test the effect of sex on amylase responses on each day, we ran a repeated measures ANOVA using within-subjects variables of day and time, and sex as a between subjects factor. There was no interaction effect of day*time*sex (F (3,201)=.58, p=n.s.). Because the main focus of this research is on sex differences, we further explored the data by analyzing men and women separately.

To test whether sAA stress responses and habituation were different between the two genders, repeated measures ANOVA were computed separately for men and women. Results revealed a significant day by time interaction for men (F (2.18, 72.16)=3.27, p <.05) but not for women (F(2,35, 79.95)=1.71, p =n.s.). To further understand sex differences in stress response and habituation, we tested if delta scores of sAA increases were different between day 1 and day 2 separately between men and women. We found that men had significantly higher responses on day 1 (mean increase= 53.78 U/ml) than on day 2 (mean increase= 27.55 U/ml: t(34)=2.57, p <.05). Women did not have significantly different decreases between day 1 and day 2 (t(9,36)=.93, p=n.s.). These results indicate that men had attenuated responses on day 2 of testing, while women did not (see Figure 2).
Figure 2. Amylase responses in the men/women group indicate the presence of amylase habituation to repeated stress.
4.4 Sex difference in Stress Appraisals

In order to assess possible sex and age differences in subjective ratings of the TSST, independent sample t-tests were used to compare men and women on different subscales of the PASA. We categorized PASA questions into four subscales: threat (items 1, 5, 9, 13), challenge (items 2, 6, 10, 14), self-concept (items 3, 7, 11, 15), control expectancy (items 4, 8, 12, 16). There were no significant differences between men and women in measures of pre-stress threat, challenge, self-concept of own competence, control expectancy, and total stress on either study day (lowest p=.27). When comparing subjective stress ratings between the two days of testing, there were no differences in how challenging participants rated day 1 vs. day 2, how much control they felt, or how much stress they felt. However, participants did report higher levels of threat on day 2 (t(76)=-1.9, p=.053) as well as higher feelings of self-competence (t(76)=-1.9, p=.057; see figure 3).

Figure 3. PASA responses in the group overall indicate the different perception of TSST.
4.5 Do stress appraisals relate to amylase?

In the group overall, there were no correlations between stress appraisal subscales and amylase responses on day 1 or day 2 (highest \( r = .12 \), all \( p = \text{n.s.} \)). When examining the sexes separately, there was also no correlation between stress appraisals and amylase responses on men (highest \( r = .21 \), all \( p = \text{n.s.} \)). In women, on the other hand, threat ratings on day 1 were positively correlated with amylase responses on that day (\( r = .39 \), \( p < .05 \)). Additionally, the total stress subscale of the PASA in women on day 1 was correlated with both day 1 and day 2 amylase responses (day 1 amylase: \( r = .37 \), \( p < .05 \); day 2 amylase \( r = .49 \), \( p < .01 \)). In women only, primary appraisals on day 1 (threat and challenge score combined) predicted both amylase responses on day 1 (\( r = .372 \), \( p < .05 \); see figure 4) and day 2 (\( r = .35 \), \( p < .05 \); see figure 5). Depressive symptoms, as measured by the CES-D were not related with amylase responses on either study day in men or in women (highest \( r = .25 \), lowest \( p = .13 \)).
Figure 4. Primary Appraisals after the first TSST predicted amylase responses to both the first and the second TSST.
4.6 Summary of findings

In this study, we wanted to investigate whether or not the post-menopausal women have higher SNS responses to novel and/or repeated stress, and whether or not there were sex differences in psychological responses to stress than men. If so, we also wanted to investigate the interaction between physical response and psychological response.

We confirmed that the TSST induced an increase in sAA on both study days. This increase was attenuated in men only, indicating the presence of habituation. While there were no differences in pre-TSST stress appraisals between men and women, both men and women who found the TSST more threatening would also find the TSST more self-competency. Stress appraisals such as threat and challenge were predictive of amylase increases on both study days in women only. Women who found the TSST only threatening had higher amylase release on day one.
5 Discussion

5.1 Summary and Interpretation of Findings

5.1.1 Research Question 1

*Do women have higher SNS responses to novel and/or repeated stress compared to men?*

We investigated if participants as a whole group showed habituation, but when we slipped down into different genders only men had habituation, because they had higher increase of amylase on day one. Women did not have higher amylase at baseline, but had higher response on day two. Unlike men, women did not have habituation. The results did not fully fit our expectation, because we expected women to have higher amylase responses at baseline and in response to stress.

Because amylase is a marker of the SNS, which stimulates inflammation, and women are more likely to get diseases related to inflammation, finding a pattern of increased SNS activity would help to explain the differences in disease rates. From the physiological perspective, the result suggested two possible explanations of the prevalence of autoimmune disorders. First, because the SNS of women were more sensitive to stress, it is easier for the SNS to stimulate inflammation, and women were more likely to develop autoimmune disorders. Second, there must be some internal relationships between the immune system reaction and participants’ feelings about the stress. Men did not have habituation probably because men were more likely
to convince themselves that they had the ability to control the situation better than before, and they took the threatening feeling as a positive feeling to encourage themselves. This might also be connected to men’s social feature that they were more likely to take a risk and enjoyed doing so (Lips, 2008).

5.1.2 Research Questions 2

*Are there sex differences in psychological stress appraisals?*

We confirmed that both men and women did not report the different feelings about pre-TSST stress appraisal. At the same time, both men and women who found the TSST more threatening on day two would also find themselves having more self-competency on day two. The results did not fit our expectation, because we expected women to feel TSST more threatening than men.

Because both men and women felt the same before the TSST on day one, there is no gender difference in feeling or predicting the unknown threat. It was also possible that participants did not fully know about the TSST on the day one, but after day one’s TSST, they knew how badly they did, and they knew what the TSST exactly was. Therefore, they found the TSST more threatening and felt they have more self-competency on day two, because they could predict how bad they would do on day two, and they already know the tests they were facing. The co-operation between threatening and self-competency could be explained as when participants fully understand the TSST after day one, they had higher expectation on how they
would do on day two. Therefore, while they found the TSST more threatening, they were hoping they could control more on the day two.

**5.1.3 Research Question 3**

*Is there an interaction of stress appraisals with SNS stress reactivity?*

Stress appraisals such as threat and challenge were predictive of amylase increases on both study days in women only. Therefore, the data fit our prediction and showed that there was an interaction of stress appraisals with SNS stress reactivity, but only for women.

The most interesting result was to relate the physiological and psychological data together. The interaction of stress appraisal with SNS stress reactivity suggested that the perception of how women deal with stress appraisal had stronger influences on SNS stress reactivity. Women had higher amylase release on both study days probably because on day one, they considered the unknown test more negatively. For day two, although they believed they could handle the situation better than the first time, they still considered the threatening and challenging feelings negatively. The data suggested that greater negative subjective responses to an acute stressor, including increased feelings of threat could affect women’s autoimmune response. These findings agree with literature regarding higher risk of depressive symptoms in women. The stress appraisals correlated with increased SNS reactivity, and this relationship was stronger in women.
5.2 Limitations

First, although amylase is a marker of the SNS, we are not sure about if amylase can one hundred percentage represent the SNS responses. Other variables, such as heart rate and SNS related catecholamines, could be measured to further describe the SNS response. Although amylase is usually correlated with plasma levels of norepinephrine, these catecholamines could also be measured directly for further validation.

While our study focused on acute in-lab stress procedures and found no differences in subjective stress responses, it could be that the TSST is not reflective of real life stress differences. For example, women may experience more day-to-day stress than men. This daily stress could stimulate the SNS and repeated stimulation over time could result in increased rates of autoimmune disease, as we see in the general population. However, this should also be narrowed down into different subjects, because different stressful life events have different impact on different gender roles. Some life events are more severe for women. For example, when respondents were asked to imagine how much stress they would be experiencing at the present time if specific events (e.g., deaths, separations, arguments, threats to self-image, financial setbacks, general changes) had occurred one week ago, one month ago, and so on up to three years ago, women rated all the sets of events as more stressful than men did (Horowits, Schaefer, & Cooney, 1974). It is possible that actually in the real life, such events are more stressful for women than for men, because women are more likely than men to suffer from
financial loss, and women are more likely than men to have more workload, such as a full-time job in a company and also a mother who has to take care of her family (Lips, 2008). Also women are less like to remarry, and are more likely to have childcare as their burden (Makosky, 1982).

A limitation might be that TSST is a stress test for men, and women show non-habituation because maybe it continues to be stressful for them, while men get used to the stress faster, because we were not sure about whether public speaking is more male-kind or not.

5.3 **Broader Impact**

This study should be conducted because it helps us understand how men and women respond differently to stress, both on a physiological level and a psychological level. At the same time, this study helps to build a better understanding of regarding how gender affects emotionally experiences in health. For example, as described earlier, the perception of stress of women have stronger influence on their physiological reaction. Finally, this study also raises questions about how external factors which are still unknown will possibly affect people’s health.

5.4 **Future Direction**

In order to understand the correlation between physiological and psychological impacts in different genders, we could also add trait variables, such as confidence, mood, anxiety, or feelings of inferiority, which reflect how participants generally feel in their life over all. For instance, people who overall feel less confident might feel more threatened by the TSST, and
have higher amylase release on both day one and day two. However, this might suggest that whether people have habituation or not can be affected by the overall feelings of life stress, because it is not about a specific feeling, such as threatening, to the stress, but a broader perception of life events.

According to the limitation, it might make more sense to design and use different stress tests for different genders, because different life stress may have different impact on different genders. If use a stress test for women, such as baby-sit, the possible result could be that women show habituation, because they are more good at this test, and they are easier to get used to the stress, while men would show non-habituation. However, if the result shows non-habituation of women but still habituation of men, this study further suggest that generally women are more sensitive to repeated stress stimulus.

Also trying out a male posture before the TSST would also be worthy to consider. Previous study suggested that a power posing before giving a public speaking would decrease cortisol about 25 percent and increase testosterone by about 19 percent for both men and women, and in contrast, low-power poses increased cortisol about 17 percent and decreased testosterone about 10 percent (Carney, Cuddy, & Yap, 2010). We could investigate if a high-power pose would through increase in testosterone make women to have significant habituation in TSST, which is a male-like test, because changes in sex hormones affect the immune system (Bouman et al., 2005). On the contrary, we could also investigate if a low-power pose would through decrease in
testosterone make men to have non-habituation in TSST. If the outcomes fit our expectation, this study would give us a better understanding of how our body languages affect our emotion, and furthermore to have influence on our health. Also this study could propose that how much we could use outside factors to control our stress responses, which have impacts on our health.

Furthermore, while the psychological impact has stronger influence on women, we could also make women read a short article about how badly women do under stress compared to men, in order to incite anxiety of women. At the same time, we could also make men read a short article about how badly they do under stress compared to women, in order to see if more anxiety will affect men’s perception of stress and how much negative feelings would cause men to have non-habituation. This experiment would more directly to study the effect of perceived gender roles on stress responses, and also gives us a more clear understanding of the relationship between emotion and our physiological responses.

Finally, while our study did not show significant differences in different age groups, we could also focus on this question, because more experiences of life stress may actually make post-menopausal women more easily to get used to the stress stimulus. However, we were not sure about if this was true or not.
5.5  *In summary*

This study not only suggested women did not have habituation under repeated stress test while men did, but also gave us a possible explanation of habituation might be caused by different interpretation of stress from different genders.
6 Appendix A

PASA items

1. I do not feel threatened by the situation
2. The situation is important to me.
3. In this situation I know what I can do.
4. It mainly depends on me whether the experts judge me positively.
5. I find this situation very unpleasant.
6. I do not care about this situation.
7. I have no idea what I should do now.
8. I can best protect myself against failure in this interview through my behavior.
9. I do not feel worried because the situation does not represent any threat for me.
10. The situation is not a challenge for me.
11. In this situation I can think of lots of action alternatives.
12. I am able to determine a great deal of what happens in this interview myself.
13. This situation scares me.
14. This task challenges me.
15. I can think of lots of solutions for solving this task.
16. If the experts judge me positively it will be a consequence of my effort and personal commitment.
7 References


