Spring 2013

Relationship between Cognitive Functions and Hormones

Daniel C. Miao
San Jose State University

RELATIONSHIP BETWEEN COGNITIVE FUNCTIONS AND HORMONES

A Thesis

Presented to the Faculty of the Department of Psychology

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Daniel Chi-Chun Miao

May 2013

The Designated Thesis Committee Approves the Thesis Titled

RELATIONSHIP BETWEEN COGNITIVE FUNCTIONS AND HORMONES

by

Daniel C. Miao

SAN JOSE STATÉ UNIVESITY

APPROVED FOR THE DEPARTMENT OF PSYCHOLOGY

May 2013

Dr. Cheryl Chancellor-Freeland	Department of Psychology
Dr. Sean Laraway	Department of Psychology
Dr. Mark Van Selst	Department of Psychology

ABSTRACT

RELATIONSHIP BETWEEN COGNITIVE FUNCTIONS AND HORMONES

by Daniel C. Miao

Stress has been implicated by recent research to significantly contribute towards many cognitive and physiological deficiencies. One of the most popular topics of study is the effect of stress on inhibition, the all-or-none decision about an action or inaction. However, only recently have scientists begun investigating neuroendocrine molecules that link stress and inhibitory processing. Participants included San José State University undergraduates (27 male, 63 female, 1 unstated) who were exposed to the Trier Social Stress Test, an established stress task, and who were assessed before and after stress exposure for cortisol levels. Participants were also given a pre- and post-test using a cued Go/No-Go Task (GNGT) with 75% cue validity. Performance on the task can be used to measure how well participants can inhibit a previously prepared (i.e., "prepotent") response. Participants were assigned to either the control group (n=47) or the stress-exposure (experimental) group (n=44). The stress-exposure group was later divided according to cortisol reactivity as being either stress responders (n = 28) or stress non-responders (n = 16). It was hypothesized that exposure to a social stressor would impair the stress responder group's performance on the cued GNGT, whereas the stress non-responders and the control group would have no impairments on the cued GNGT. Thus evidence for a differential impairment in the ability to inhibit responses was not found in the stress condition nor the control condition.

ACKNOWLEDGEMENTS

This thesis is the result of the efforts of many individuals. First, I would like to thank my advisor, Cheryl Chancellor-Freeland, who has spent so many hours at the desk to help see this project finish. This thesis would never have come to fruition if it were not for her enthusiasm and unwavering dedication. Second, I would like to thank Sean Laraway for his constant concern and advice. His expertise and critical analysis was much appreciated. Finally, I would like to thank Mark Van Selst for his insights and expertise on the Go/No-Go Task. This thesis would never have gone as smoothly without their input.

Also, I would like to thank the professionalism and efforts of the International Neuroeconomics Institute. I would especially like to thank Wei-Ju Chen, Nikki Labrovich, Maheem Akhtar, Webster Lincoln, and Atsuko Iwasaki for their especially large contributions to this work. I would like to thank my friends and family for their constant support. Finally, I would like to thank the students of SJSU for their contribution and participation in the study.

Table of Contents

Section Page

Introduction…….…….…..…….1

Cortisol…….…….....1

Stress on Executive Function…….…….....3

Cued Go/No-Go Task……..……..4

Hypothesis……..……..5

Method…….…..…..…..…....6

Research Participants…….…..…….……..6

Measures and Instruments…….……...….....7

Trier social stress test……7

Go/No-Go task……..8

Perceived stress scale……9

Brunel mood scale…….9

Procedure…….....10

Cortisol samples……...11

Results……..…….……...….....11

Preliminary Tests……..…….....11

Analysis of Variance……..…….…….…....13

Additional Analyses……..…….……...….17

Discussion……..…….…..…...……..19

Future Studies……...20

References……..……..…..…....23

Appendices……..…….…..…..…..26

Appendix A: IRB Approval Letter……..……..….....26

List of Tables

TABLE PAGE

Table 1. Independent *t*-tests between stress and control conditions for baseline measures ……..……...…...12

Table 2. Mean accuracy and reaction time across blocks and conditions ……..….15

List of Figures

FIGURE PAGE

Figure 1: Differences in pre-TSST and post-TSST Go-Trial reaction time across conditions. ……..……..…..…….14

Figure 2: Differences in pre-TSST and post-TSST Go-Trial reaction time across conditions……..……..16

Figure 3: Differences in pre-TSST and post-TSST No-Trial accuracy percentage for control and stress responders…….……..…..….17

Figure 4. Mean accuracy (successful inhibitions) across blocks by cue validity and stress condition for the No-Go trials…..……...……....19

Introduction

The concept of inhibition emerged in the 19th century following the demonstration of reflex inhibition and central inhibition in the brain (Aron, 2007). Since its conception, inhibition has entered the general vocabulary to make commonplace such phrases as, “He became drunk and lost his inhibitions” or “I must suppress my urge to eat candy.” The scientific definition of inhibition has also expanded and is generally recognized as the stopping and overriding of a process. Researchers have applied varied terms to describe specific processes in which inhibition applies, such as “cognitive inhibition” as the stop and override of a mental process. From this body of research, one of the most controversial forms of inhibition is active inhibition, also known as e*xecutive function* (*EF*). The theory of EF is defined as a set of higher-level processes that optimize and schedule lower-order ones (Miller & Cohen, 2001). Examples of activities associated with EF include planning, memory, and emotional control (Miyake, Friedman, Emerson, Witzki, Howerter, 2000). All of these functions are known to be reduced following exposure to severe stress, as supported by a wealth of literature concerning post-traumatic stress disorder (Aupperle, Melrose, Stein, & Paulus, 2011). A major hormone known to increase drastically with stress and bind directly to receptors in the brain is cortisol (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007).

Cortisol

Stress is a function of a change or disruption in homeostasis as a result of the internal or external environment. Therefore, when an organism becomes exposed to a stressful object or situation, the organism responds physically with a change in the

regulation of hormones, neurotransmitters, and signal molecules in the brain. This physiological change, popularly termed the "fight or flight" response, prepares the body for action through subtle changes in physiology, including in the brain. Initiation of this response begins in the hippocampus which perceives a stressful stimulus and responds by signaling the release of adrenocorticotropin hormone (ACTH). The pituitary gland then releases ACTH into the blood stream. Once in, ACTH travels through the body and attaches to receptors in the adrenal glands to promote the release of cortisol, epinephrine, and norepinephrine. The glucocorticoid, cortisol, is especially notable because of its ability to stop the production of ACTH and therefore the entire response (Lenbury & Pornsawad, 2005). Cortisol's ability to pass through the blood-brain-barrier enables it to directly bind with glucocorticoid receptors in the brain. In this way, cortisol is a putative neurotransmitter to areas of the brain with the appropriate receptor. Furthermore, cortisol can be measured non-invasively and with no discomfort through the collection of saliva. The reliability and validity of cortisol as an indicator of stress is well supported in the literature (Kirschbaum, Pirke, & Hellhammer, 1993). For these reasons, a large body of literature has focused on cortisol and the study of EF.

There are two types of glucocorticoid receptors known in the brain (Lupien et al., 2007). Type I receptors are primarily located in the hippocampus and limbic system. These receptors possess a much higher affinity for cortisol than Type II receptors. Type II receptors are located in the prefrontal cortex, a brain region responsible for the majority of EF. Type I receptors are associated with impairments or improvements to working memory, declarative memory, and episodic memory. The Type II receptors are

thought to affect stress reactivity and startle responses (Rochford, Beaulieu, Rousse, Glowa, & Barden, 1997). However, the lower affinity of Type II receptors makes study of the effects of cortisol on EF difficult. Evidence remains inconclusive on how these glucocorticoid receptors may affect inhibition, a subset of EF. It is currently known however that these receptors are affected by increases in stress, and that exposure to stress does have an effect on executive function.

Stress on Executive Function

Sprague, Verona, Kalkhoff, & Kilmer (2011) recruited adult participants from a Midwestern semirural county. Participants answered questionnaires on perceived stress and reported stressors in the preceding month. This was followed by a battery of EF tests including the Aggression Questionnaire, the State Anger Scale, Wisconsin Card Sorting Test (WCST), Trail-Making Test (Trails B), Controlled Oral Word Association Test (COWAT), and Wechsler Adult Intelligence Scale (WAIS). The Aggression Questionnaire measured aggressive behavior, the State Anger Scale measured intensity of anger at the moment, the WCST measured changes in set shifting and planning, the Trails B test measured attention, the COWAT measured mental flexibility, and the WAIS measured differences in working memory. From Sprague's analyses, higher scores in perceived stress were correlated with higher scores in aggression and anger but not with any other specific test. Performance on the WCST, Trails B, COWAT, and WAIS were used to calculate an overall score of EF. A higher EF score was shown to have a mitigating effect on anger and aggression. Although enlightening, it is uncertain whether the increased stress had a greater effect on anger and aggression or on the EF construct.

Studies examining the relationship between cortisol and EF specifically have provided conflicting information. Lok and Bishop (1999) recruited 327 adults ranging from 18 to 60 years in age and administered the Emotion Control Questionnaire (ECQ), the Perceived Stress Scale, the Hassles Scale, and two physical health questionnaires. Of the five subscales used, physical health correlated the most to perceived stress. After accounting for physical health, only "benign control" (i.e., the ability to control impulsive behaviors) was found to contribute significantly using a Multiple Regression Correlation (MRC) analysis. These results indicate that inhibition is negatively correlated with perceived stress, suggesting that lower stress is associated with better control over impulsive behavior. Therefore, it is reasonable to postulate that participants suffering from stress will score lower on measurements of impulsive behavior and inhibition. In support of this hypothesis, was a longitudinal study correlating higher cortisol responses to impairments in verbal memory, attention, and inhibition (Li et al., 2006). In contrast, an increase in cortisol was correlated with an increased number of behavior inhibitions in children in the study of Gunnar, Kryzar, Ryzin, and Phillips (2010). In the present study, we attempted to clarify the effects of cortisol on inhibition by specifically testing a sub-measure of cognitive inhibition, motor inhibition.

Cued Go/No-Go Task

The Go/No-Go Task (GNGT) is based on work by Donders (1868) that laid the foundation for studying cognitive processes via analysis of reaction time. The task specifically measures a Donders Type C reaction, which requires the participant to suppress a response to one form of stimulus and activate a response to a second stimulus

(Vidal, Burle, Grapperon, & Hasbroucq, 2011). The cued GNGT follows this basic format but is modified so that participants are presented with a cue that indicates the type of response (i.e., either Go or No-Go) that is most likely to be seen. Examining differences across cue validity for reaction times on Go trials and the proportion of successful inhibitions on No-Go trials can be used to identify manipulations that selectively impair inhibitory processes (Marczinski & Fillmore, 2003; Marczinski, Abroms, Van Selst, & Fillmore, 2005). As such, a cued GNGT is a measure of motor inhibition insofar as the Go trial requires a motor response to be made. Motor inhibition is characterized by the processing of an all-or-none decision about an action or inaction (Rubia et al., 2000).

Hypothesis

In the current study, I addressed the question of whether a stress experience inherently produces a concomitant decline in the ability to inhibit a response. Kofman, Meiran, Greenberg, Balas, and Cohen (2006) reported a significant decrease in performance of a Stroop Task when participants were exposed to the stress of taking an exam. Forty-eight participants (7 male, 41 female) were divided evenly into control and stress conditions. The study recruited participants at two time intervals: the control group at the start of the semester and the exam stress group two weeks prior to finals. Both groups were instructed to participate in the Stroop Task and a perceived stress questionnaire. The Stroop Task involved the presentation of 72 word-color stimuli. The control group measured its Stroop performance early in the semester (when stress was

presumably low), whereas the stress group measured their performance two weeks before exams (when stress was presumably high).

From the preliminary pilot data for this study, the Stroop Test results indicated a possible ceiling effect. To address these limitations, the present study was modified to resolve this shortcoming and expand on the field by measuring inhibition using a cued GNGT and an experimentally induced form of social stress. The Acute Stress Response was measured in the present study using salivary cortisol. Based on Kofman et al.'s (2006) study, I hypothesized that exposure to an acute social stressor would result in decreased response inhibition, as indicated by poorer accuracy on the cued GNGT. This study furthered our understanding about the relationship between cortisol and inhibition and provided further insights into the mechanisms of cortisol on EF.

Methods

Research Participants

To test this hypothesis, we recruited 107 participants from the San José State University undergraduate psychology research pool. For their safety and to prevent the exploitation of special needs groups, participants were informed upon sign-up that they would be excluded from the study if they were pregnant, taking psychoactive medication, or diagnosed with an immunological disorder. Additionally, participants were reminded a total of three times of these conditions and to refrain from eating, smoking, exercising, or drinking anything except water 1 hr prior to their entry into the study. All of these aforementioned conditions are known to substantially affect the cortisol response and would significantly increase error within the experiment (Kirschbaum et al., 1993).

Consequently, to control for these variations, participants who do not follow these instructions were excluded from the study. Out of the total 107 participants recruited, 90 respondents (27 male, 63 female) of a diverse ethnic background (20 Caucasian, 1 African American, 38 Asian, 18 Latino, and 13 other), with an average age of 20 years (52 freshman, 17 sophomore, 13 junior, 6 senior, 2 graduate) were retained. A total of 46 participants were retained in the control condition and 44 in the stress condition. This study was approved by the San José State University Institutional Review Board (see Appendix A for the approval letter).

Measures and Instruments

Trier social stress test (TSST). Following the screening questionnaire, participants were instructed to answer questionnaires and perform two cognitive tasks. One of these tasks, the Trier Social Stress Test (TSST), deserves special mention. The TSST is a brief 15 min procedure found to reliably induce a stress response and increase cortisol. The procedure induces a cortisol response through exposure to a combination of four social stressors (Kirschbaum et al., 1993). Participants in the TSST are first introduced to a panel of judges in white lab coats and informed the panel is especially trained in behavioral observations and will be analyzing their speech, establishing an audience stressor. Participants are exposed to an anticipation stressor when they are led to a separate room and given 3 min to prepare their speech. The topic of the speech was that of a mock-job-interview for the participant's ideal job. The public speaking task involves a 5 min speech and serves as a public speech stressor. The speech is followed by

a mental arithmetic stressor asking participants to count backwards from 2083, subtracting by 13 each time.

Go/No-Go task. The GNGT is designed to detect changes in EF, specifically planning and inhibition. This task involved the presentation of a vertical or horizontal rectangular cue followed by a green or blue colored stimulus. The rectangular cue was presented for 200, 300, 400, or 500 ms before providing the second stimulus. If presented with the green stimulus, participants were instructed to press a button. If presented with the blue stimulus, participants were instructed to not press a button. If presented with a vertical rectangle as a cue, there would be a 75% chance of receiving a green Go-trial. If presented with the horizontal rectangle, there would be a 75% chance of receiving a blue No-trial. Consequently, participants had to inhibit their cued response (rectangle orientation) to the actual color presented. Participant responses to the GNGT were aggregated and separated according to the type of cue and type of response to create four condition pairs. Data were categorized under these four pairs to better examine the changes in accuracy and RT for specific types of inhibitions. Of the eight subscales measured using the cued GNGT, only the Valid Go-Trial RT and Invalid Go-Trial RT are meaningful representations of inhibition (Marczinski et al., 2003).

The GNGT was presented to participants in 40-trial blocks comprised of four sets of 10 trials for each of the above listed cue durations. Blocks 1 and 2 were administered before the TSST and blocks 3 and 4 were administered after the TSST for a total of 160 trials. The GNGT was administered through E-Prime™ (Psychology Software Tools, Pittsburgh, PA) on a laptop PC using the Microsoft Windows XP™ operating system.

Perceived stress scale (PSQ). The PSQ is a measure of subjective stress, in which participants recount the number of incidences for 30 stressful events that have occurred in the past month on a 4-point Likert-type scale (Levenstein, Prantera, Varvo, Scribano, Berto, et al., 1993). The questionnaire was administered to measure participants' recent stressful experiences. Items describing recent events likely to reduce stress on the PSQ were reverse-scored and then analyzed to determine the overall long-term stress level of participants before exposure to the TSST. This score was used to determine if participants were abnormally stressed prior to their participation in the study because these experiences could impact performance on the GNGT.

Brunel mood scale. Previous research (Bunce, Handley, & Gaines, 2008; Eckhardt & Cohen,1997) reported that emotions such as anger, depression, and anxiety may affect the Stroop Task. To account for this potential confounding variable, mood traits were evaluated using the Brunel Mood Scale, a 24-item self-report questionnaire. Participants indicated their current perceived state on a series of mood dimensions, such as boredom, anger, or annoyance on a 5-point Likert-type scale that ranged from 1 (not at all) to 5 (extremely). The scores on the 24 items were combined to create six subscales that measured participants' perceived traits: anger, confusion, depression, fatigue, tension, and vigor. The Brunel Mood Scale used in the current study was modified to be an aggregate of 18 items across 3 constructs measuring momentary depression, anger, and tension.

Procedure

Upon arrival at the designated room and time, participants were given a consent form and a screening questionnaire. The questionnaire was designed to select out participants who, because of certain behaviors or preexisting conditions, have invalidated their cortisol responses. If participants reported having a chronic inflammatory condition, having smoked, being pregnant, or having ingested anything besides water one hr before the beginning of the study, they were excluded.

The experimenter then administered the PSQ to capture the participant's stress level before they were exposed to the acute stressor (i.e., the TSST). Participants were given approximately 3 min to complete the PSQ. Following the PSQ, the participant was asked to complete the GNGT on a computer. This session had the participants respond to two blocks of 40 trials of the computerized inhibition task as described in the previous section. This was then followed by the first salivary cortisol sample. A total of three salivary cortisol samples were taken during the course of the study. At each collection point, participants were instructed to chew vigorously on a cotton swab for 1 min. Immediately following the first saliva sample, participants were exposed to either the TSST or control condition.

Participants in the TSST condition were exposed to the TSST, a procedure known to reliably elevate cortisol through social stress (Kirschbaum et al., 1993). In contrast, the control group was not exposed to social stress, but instead watched a 15 minute travel video. There was no videotaping and no interacting with any other person during this time. Upon completing the TSST or control session, a second saliva sample was taken

from the participants. This sample was followed by blocks 3 and 4 of the GNGT in a separate room using a computer and program for 10 min. A third saliva sample was collected immediately after. This allowed for the determination of peak cortisol. That is, whether peak cortisol levels occur at sample 2 or sample 3, the difference between peak cortisol and baseline provided the $\Delta_{cortisol}$ value that was used throughout the analyses. After the third saliva sample was provided, the mood and demographic questionnaires were administered. Twenty minutes were given for participants' cortisol to return to normal before they were debriefed and released from the experiment.

Cortisol samples. Cortisol samples were taken at three time intervals: a baseline immediately before the TSST, immediately after the end of the TSST, and 10 min after the TSST. The three salivary cortisol samples were stored in an ice bucket until they could be placed in a laboratory freezer at -5ºC at the end of the day. At that temperature, the saliva can be stored for up to one year. Before assay for the cortisol was performed, the cortisol was thawed and brought to room temperature. Once thawed, the samples were assayed with a Salimetrics cortisol assay kit and the concentration of cortisol determined with spectroscopy. To standardize the cortisol samples and reduce individual differences, the raw data were turned into difference scores to find the total change in cortisol concentration ($\Delta_{cortisol}$). In this way, individual differences in baseline and peak cortisol were mitigated.

Results

Preliminary Tests

Participants were not explicitly told the direction or the significance of the cue in

the experiment and were thus learning the validity cuing in the first block. Because participants were still learning the task, the first block (i.e., the first 40 trials) of the GNGT was discarded. Consequently, the total number of trials was reduced from 160 to 120 for the analysis.

To evaluate the effectiveness of the randomized control procedures, independent *t*-tests compared stress and control group means across the characteristics of perceived stress, age, gender, and baseline cortisol level (all as measured prior to the TSST). Each of these characteristics could affect cortisol response or inhibition. For perceived stress, the control group mean PSQ (M = 68.10, SD = 12.52) did not differ from the stress group mean PSQ (M = 66.18, SD = 13.75), $t(89) = 0.70$ (p = .57); for age, the stress group (M = 19.45, SD = 3.10) did not differ from the control group (M = 20.23, SD = 3.83), $t(89) = -1.06$ (p = .32); for baseline cortisol, the control group (M = 4.91, SD = 2.25), did not differ from the stress group (M = 5.82, SD = 2.98) $t(89) = -1.64$ (p = .105). Thus, as seen in Table 1, there were no significant differences between control and stress groups on the pretest measures. These results support the assumption that the control and stress groups did not differ in key characteristics that might affect cortisol response prior to the TSST.

Table 1

Independent t-tests between stress and control conditions for baseline measures

Variable	**Mean Difference**	***SE***
Perceived Stress Questionnaire	1.92	2.76
Age	-.78	.73
Baseline Cortisol	-.911	.56

After confirming that baseline perceived stress and cortisol scores did not differ between the stress and control groups prior to the TSST, a second independent *t*-test was

conducted examining differences in $\Delta_{cortisol}$ to determine if cortisol was significantly different 10 minutes after exposure to the TSST. Stress and control conditions differed significantly in their $\Delta_{cortisol}$ scores, with mean $\Delta_{cortisol}$ for the stress group *(M* = 3.33, *SD* = 5.43) being significantly higher than mean $\Delta_{cortisol}$ for the control group *(M* = -2.43, *SD* = 2.25) at $t(89) = 6.68$ ($p < .001$). Cohen's *d* was calculated at 1.38. This confirmed findings from previous studies with the TSST, and it supported our assumption that stress and control conditions would differ in their stress and cortisol levels due to the acute stressor (Kirschbaum, et al., 1993).

Reaction Time

A 2 (Cue Validity: Valid vs. Invalid) x 2 (Stress vs. Control) analysis of variance (ANOVA) was conducted on the Go-Trial RTs to determine if exposure to an acute stressor would impair inhibition. Go-Trial RTs on the Valid Go-Trial and Invalid Go-Trial measures of the GNGT did not differ between the stress group ($n = 44$) and the control group ($n = 47$) for Go-Cue/Go-Trial, $F(1, 89) = 1.84$, $p = .178$, Cohen's d = .269. The invalid cue however, was found to significantly increase reaction time, $F(1, 89) = 13.09$, $p < .001$.

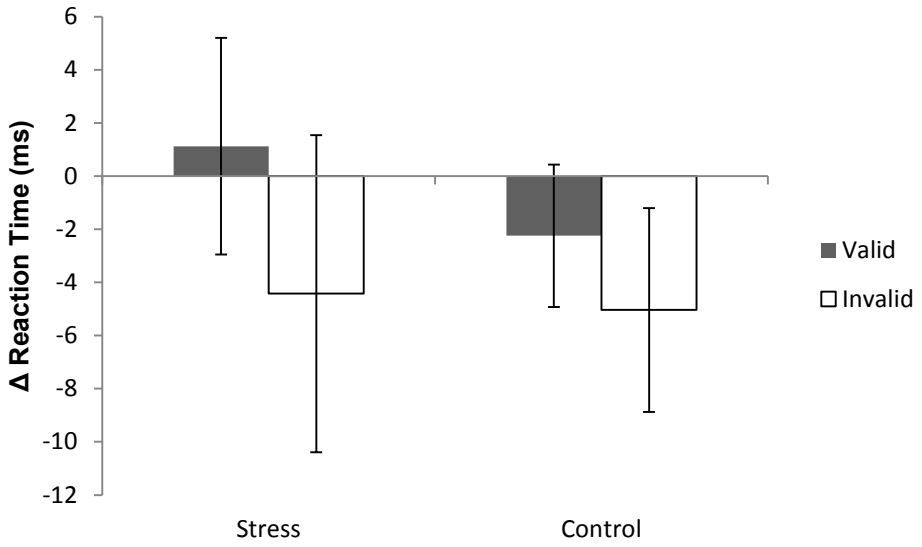

Figure 1: Differences in pre-TSST and post-TSST Go-Trial reaction time across conditions. There were no significant main effects or interactions. Error bars represent standard error of the mean.

Within the stress group, a number of participants were expected not to respond to the TSST with increased cortisol. The reason for non-responders, participants who do not produce a 10% increase in cortisol, to have a reduced response may be because their HPA and cortisol response have been damaged by chronic stress exposure or from differences in their early development (Kirschbaum et al., 1993; Elzinga & Roelofs, 2005). Because the responses to stress might differ from stress responders, the non-responders were separated and analyzed separately. To identify these non-responders, participants were separated in to two groups depending on their cortisol increase from baseline. Those participants who increased their cortisol by 10% or higher from baseline were categorized as responders while those with a less than 10% increase were categorized as non-responders. Participants were then separated by condition and cortisol

reactivity in to 3 groups: control (16 male, 31 female), responders (11 male, 17 female), and non-responders (1 male, 15 female).

Table 2

Mean accuracy and reaction time across blocks and conditions

Condition	**Variable**	**Block 1**	**Block 2**	**Block 3**	**Block 4**
Control	Valid-Cue/No-Trial Accuracy	97.23 ± 4.76	98.30 ± 3.18	98.19 ± 3.52	97.98 ± 4.13
	Invalid-Cue/No-Trial Accuracy	95.70 ± 9.37	98.30 ± 5.64	97.90 ± 6.23	95.70 ± 9.27
	Valid-Cue/Go-Trial Reaction Time	327.0 ± 39.1	309.8 ± 33.2	312.7 ± 30.4	311.3 ± 27.6
	Invalid-Cue/Go-Trial Reaction Time	333.4 ± 44.8	314.8 ± 36.2	323.6 ± 36.2	315.3 ± 30.2
Stress Responder	Valid-Cue/No-Trial Accuracy	96.61 ± 4.52	97.68 ± 4.61	97.50 ± 8.55	96.61 ± 8.28
	Invalid-Cue/No-Trial Accuracy	93.60 ± 10.96	95.00 ± 10.36	95.00 ± 11.71	93.60 ± 12.24
	Valid-Cue/Go-Trial Reaction Time	323.6 ± 38.2	314.8 ± 29.4	310.5 ± 34.4	312.1 ± 35.4
	Invalid-Cue/Go-Trial Reaction Time	326.3 ± 42.4	312.9 ± 39,6	322.2 ± 43.9	323.7 ± 44.9
Stress Non-Responder	Valid-Cue/No-Trial Accuracy	.981 ± .03	.988 ± .02	.988 ± .02	.994 ± .02
	Invalid-Cue/No-Trial Accuracy	.963 ± .08	.988 ± .05	1.00 ± 0	.975 ± .07
	Valid-Cue/Go-Trial Reaction Time	336.96 ± 69.62	328.83 ± 21.39	331.11 ± 21.66	332.66 ± 31.49
	Invalid-Cue/Go-Trial Reaction Time	343.93 ± 41,71	343.99 ± 41.26	333.94 ± 24.49	343.19 ± 32.55

Means and standard deviations for No-Cue/No-Trial accuracy (adherence to task), Go-Cue/No-Trial accuracy (inhibition), Go-Cue/Go-Trial reaction time (base reaction time), and No-Cue/Go-Trial reaction time (inhibition).

A 2 (Cue Validity) x 2 (Condition) mixed ANOVA was conducted on difference scores between pre-TSST and post-TSST Go-Trial RTs. The results reported a trend for validly-cued go-trial tasks to have a faster RT compared to invalidly-cued go-trial tasks, F (1,73) = 3.73, p = .057. Exposure to stress was found to have negligible effect on

reaction time, F (1, 73) = .006, p = .94, and there was no interaction, F (1,73) = 1.62, p = .21.

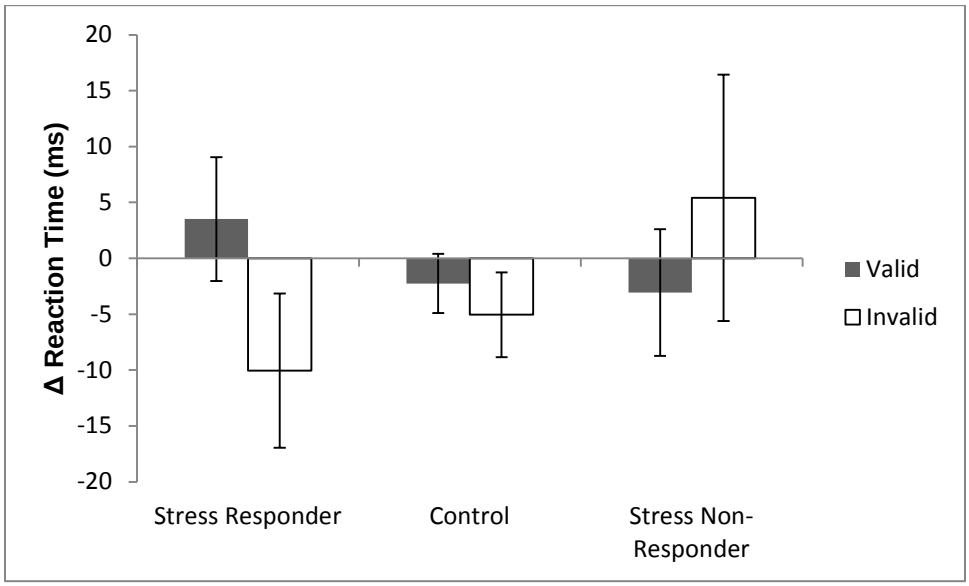

Figure 2: Differences in pre-TSST and post-TSST Go-Trial reaction time across conditions. There were no significant main effects or interactions. Error bars represent standard error of the mean.

A 2 (Cue Validity) x 2 (Condition) mixed ANOVA was conducted on difference scores between pre-TSST and post-TSST No-Trial accuracy percentages. These results reported no significant main effects for condition, F (1,73) = .02, p = .91, cue, F (1,73) = .36, p = .55, nor any interaction F (1,73) = .27, p = .60.

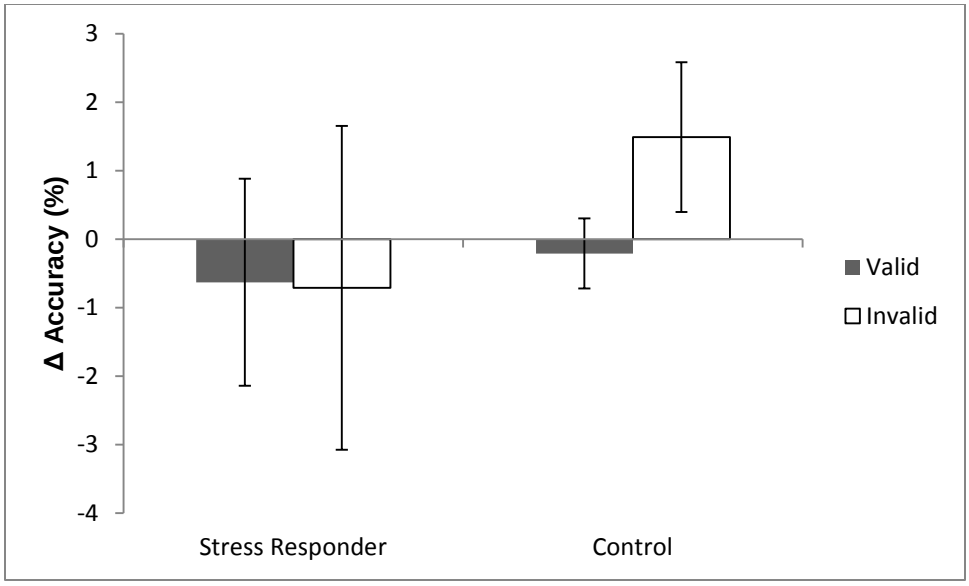

Figure 3: Differences in pre-TSST and post-TSST No-Trial accuracy percentage for control and stress responders. There were no significant main effects or interactions. Error bars represent standard error of the mean.

Additional Analyses

There are a number of factors that affect cortisol and inhibition. These factors must be accounted for when directly analyzing cortisol. Females exhibit a similar cortisol response as males, but female cortisol change is generally not as pronounced as male cortisol change, resulting in a lower $\Delta_{cortisol}$ (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Second, negative emotions such as anger and depression have been known to increase the likelihood of impulsive behaviors (Bunce, Handley, & Glance, 2008; Eckhardt & Cohen, 2007). Momentary negative emotions such as anger and depression were collected using the Brunel Scale following exposure to the TSST and analyzed in an additional analysis. Finally, the prefrontal cortex (PFC), a region of the brain responsible for EF and inhibition, is known to mature between the ages of 18 through 28 (Webster, Weickert, Herman, & Kleinman, 2002; Davies,

Segalowtiz, & Gavin, 2004). Considering the mean age of the sample is 20 years, the maturation and development of individual EF will vary greatly with age. To account for any possible effects of cortisol, a post-test using a 2 (cued validity) x2 (condition) mixed Analysis of covariance was conducted on Go-Trial RTs and No-Trial accuracy to determine the unique contributions of cortisol in inhibition after accounting for differences in gender, age, and mood (Table 3). After accounting for those potential confounds, the effects from the acute stressor from the TSST and cortisol reactivity were found to have an insignificant effect on GNGT reaction time.

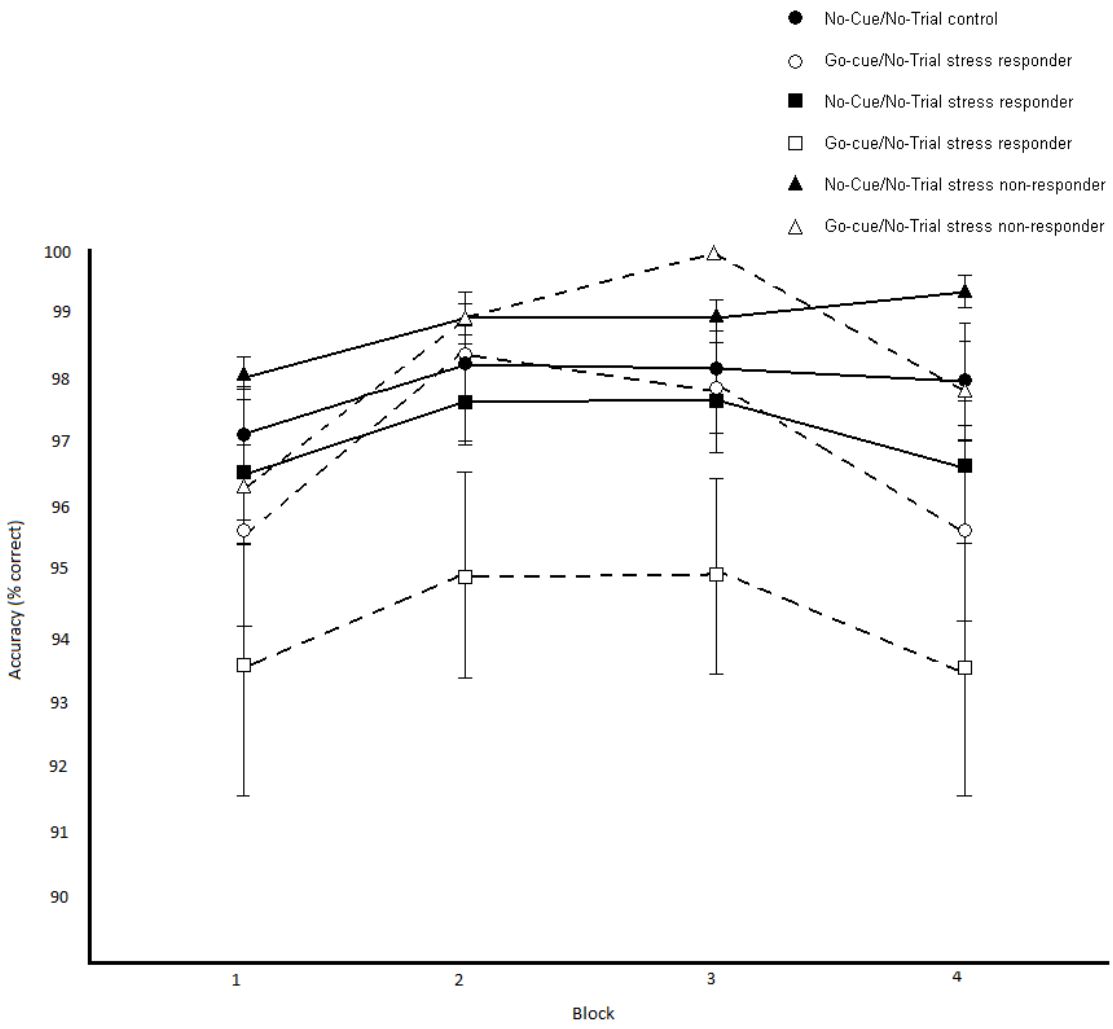

Figure 4. Mean accuracy (successful inhibitions) across blocks by cue validity and stress condition for the No-Go trials. Blocks 1 and 2 are from the pre-test; Blocks 3 and 4 are from the post-test. The black filled shapes correspond to trials with No-Go cues (i.e., valid cueing for No-Trials); the unfilled shapes correspond to trials with Go-cues (i.e., invalid cueing for No-Trials). Circles correspond to the non-stress exposure group; squares indicate the stress responders from the stress-exposure group; triangles indicate stress non-responders from the stress-exposure group. Go-cue/No-Trial for stress non-responders was significantly higher compared to control and stress responder. There was no interaction between conditions and blocks. Error bars represent one standard deviation. The data for Block 1 is invalid because participants were still in the process of acquiring the task. It is presented above for reference only.

Discussion

The performance on the GNGT following exposure to the TSST was not found to be sufficiently different from the control. This failure to observe a demonstrable performance deficit was in contrast to the significant differences in cortisol reactivity between the stress responders and the control group. Consequently, we failed to reject the null hypothesis, implying that cortisol has no effect on motor inhibition. Moreover, the average performance in accuracy for the No-Trials of the GNGT across all conditions was 95% or higher, suggesting that any difference in inhibition as a cause of cortisol was negligible. There was also the possibility of a ceiling effect, which further complicated these findings. This conclusion should be taken with a note of caution however as the sample size after the stress group was diminished by separating the stress condition into stress responders and stress non-responders. These two conditions were considerably smaller than the control condition, resulting in a considerable loss of power as signified by the Cohen's *d*. A larger sample of stress responders and stress non-responders would yield more meaningful data. Furthermore, presenting only four blocks of the GNGT might not have been sufficient to establish a stable measure of participant performance.

Increasing the number of blocks in the GNGT would improve the quality of the data collected. Admittedly, this would come at the cost of participation fatigue, especially following the TSST condition. Further research will be required to determine the number of trials that a participant would reasonably complete and remain cooperative.

Future Studies

The GNGTremains a valid measure of inhibition following the TSST however. Previous research by Scholz, et al. (2009) has confirmed significant results in reaction time for the GNGTfollowing the TSST, albeit their presentation of the GNGT was administered without a cue. When comparing the results of the present study with the results of Scholz et al. (2009), the presence of a cue might provide a buffer against the effects of stress on inhibition. Research in to the effects of cue and other cognitive aids would further our understanding of the effects of cortisol on inhibition.

To counter the ceiling effect, future studies may consider another measure of motion inhibition, the Stop-Signal Task (SST) (Leotti & Wagner, 2010). The program presents the participant with a cue and a stimulus in much the same way as the cued GNGT. It differs in that it measures and sets the maximum time for the participant to respond to the 70-percentile level of their reaction time. The program also adjusts the delay time between presentation of the cue and stimulus so that accuracy is standardized at 50%. This has a net result of increasing the difficulty of the task and creating a standardized difference between stress and control groups. Compared to the GNGT, the SST is not as widely known or established, but the biggest limitation is its reliance on the participant to comply with instructions. Compared to the GNGT, the validity of the SST

is much more susceptible to non-compliance. This is especially relevant as participants asked to perform this task following the TSST may not always comply with instructions.

A topic of some controversy is the effect of mood and emotion on inhibition. The hierarchical MRC revealed that negative moods such as state depression, state anger, and state tension were also found to uniquely contribute to reaction time, but prior research indicated that depression did not significantly affect inhibition (Bunce, Handley, & Gaines, 2008). Manipulation of anger, however, was supported in the previous literature (Eckhardt & Cohen, 1997) and was seen to be associated with a number of inhibition tasks in the present study. These data are in contradiction to studies by Lok and Bishop (1999) that found no relationship between anger and inhibition. Admittedly, however, the test used in Eckhardt and Cohen's study was not a cognitive inhibition task instead of an emotional inhibition task, so the two measures may be distinct. All of these studies however have manipulated stress in different ways. The implication of differing reactions to these tests and manipulations is that emotion is prompted more readily depending on the type of stress presented whereas exposure to stress simply activates the HPA response and elevations in cortisol. Variations in the stress response could be considered a combination of emotion and cortisol. A subtle manipulation of emotion with a cortisol injection would provide support for this hypothesis when compared to tests known to correlate with higher state anger or depression measures like the TSST.

Finally, these results suggest that cortisol may not have an effect on inhibition. Previous research has also implicated DA as an effective neurotransmitter in the PFC and in certain tasks relating to EF (Broerson, Heinbroek, Bruin, & Olivier, 1996;

Vandenbossche et al., 2012). Arnsten (2009) reported that high concentrations of DA and norepenephrine (NE) are associated with reduced PFC activity and EF. Arnsten found that deficiencies in the participants' EF performance have been implicated to result from deficiencies in D_4 and α_2 receptors' ability to respond to those two neurotransmitters. The difficulty of this explanation is in its interpretation. Pruessner, Champagne, Meaney, and Dagher (2004) found in animal studies that early exposure to stress and cortisol impairs development of dopaminergic neurons and dopamine in the mesolimbic system from an early age. Exposure to acute stressors is also known to cause changes to these neurons (Brown, Henning, & Wellman, 2005). The changes to EF and inhibition may be a result of changes in these dopaminergic neurons rather than cortisol. Further study is required to determine how cortisol and dopamine affect EF and how stress non-responders might differ from control and stress responders.

References

Arnsten, A. F. T. (2009). Stress signaling pathways that impair prefrontal cortex structure and function. *Nature, 10*, 410-422.

Aron, A. R. (2007). The neural basis of inhibition in cognitive control. *The Neuroscientist, 13*(*3*), 1-15.

Aupperle, R. L., Melrose, A. J., Stein, M. B., & Paulus, M. P. (2011). Exectuve function and PTSD: disengaging from trauma. *Neuropharmacology, 62*, 686-694.

Broerson, L. M., Heinbroek, R. P. W., Bruin, J. P. C., & Olivier, B. (1996). Effects of local application of dopaminergic drugs into the medial prefrontal cortex of rats on latent inhibition. *Society of Biological Psychiatry, 40*, 1083-1090.

Brown, S. M., Henning, S., & Wellman, C. L. (2005). Mild, Short-term stress alters dendritic morphology in rat medial prefrontal cortex. *Cerebral Cortex, 15*, 1714-1722.

Bunce, D., Handley, R., & Gaines Jr., S. O. (2008). Depression, anxiety, and within-person variability in adults aged 18-85 years. *Psychology and Aging, 23*, 848-858.

Davies, P. L., Segalowtiz, S. J., & Gavin, W. J. (2004) Development of response-monitoring ERPs in 7- to 25-year-olds.*Developmental Neuropsychology, 25* (3), 355-376.

Eckhardt, C. I. & Cohen, D. J. (1997). Attention to anger-relevant and irrelevant stimuli following naturalistic insult. *Journal of Personality Individual Differences, 23* (4), 619-629.

Elzinga, B.M., & Roelofs, K. (2005). Cortisol-induced impairments of working memory require acute sympathetic activation. *Behavioral Neuroscience, 119*, 98-103.

Gunnar, M. R., Kryzer, E., Ryzin, M. J., & Phillips, D. A. (2010). The import of the cortisol rise in child care differs as a function of behavioral inhibition. *Developmental Psychology*. Advance online publication. Doi: 10.1037/a0021902.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The trier social stress test – a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*, 76-81.

Kofman, O. Meiran, N., Greenberg, E., Balas, M., & Cohen, H. (2006). Enhanced performance on executive functions associated with examination stress: evidence from task-switching and Stroop paradigms. *Cognition and Emotion, 5*, 577-595.

Lenbury, Y., & Pornsawad, P. (2005). A delay-differential equation model of the feedback-controlled hypothalamus-pituitary-adrenal axis in humans. *Mathematical Medicine and Biology, 22,* 15-33.

Leotti, L. A., & Wagner, T. D. (2010). Motivational influences on response inhibition measures. *Journal of Experimental Psychology: Human Perception and Performance*, *32* (2), 430-447

Levenstein, S., Prantera, C., Varvo, V., Scribano, M. L., Berto, E., et al. (1993). Development of the perceived stress questionnaire: A new tool for psychosomatic research. *Journal of Psychosomatic Research*, *37* (1), 19-32.

Li, G., Cherrier, M. M., Tsuang, D. W., Petrie, E. C., Colasurdo, E. A., et al. (2006). Salivary cortisol and memory function in human aging. *Neurobiology of Aging*, *27*, 1705-1714.

Lok, C. & Bishop, G. D. (1999). Emotion control, stress, and health. *Psychology and Health*, *14*, 813-827.

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., & Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: implications for the field of brain and cognition. *Brain and Cognition*, *65* (1), 209-237.

Marczinski, C. A. & Fillmore, M. T. (2003). Preresponse cues reduce the impairing effects of alcohol on the execution and suppression of responses. *Experimental and Clinical Psychopharmacology*, *11* (1), 110-117.

Marczinski, C. A., Abroms, B. D., Van Selst, M., & Filmore, M. T. (2005). Alcohol-induced impairment of behavioral control: differential effects on engaging vs. disengaging Responses. *Psychopharmacology*, *182* (2), 452-459.

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual Review of Neuroscience*, *24*, 167-202.

Miyake, A., Friedman, N. P., Emerson, M. J., Witzki, A. H., & Howerter, A. (2000). The unity and diversity of executive functions and their contributions to complex "frontal lobe" tasks; a latent variable analysis. *Cognitive Psychology*, *41*, 49-100.

Pruessner, J. C., Champagne, F., Meaney, M. J., & Dagher, A. (2004). Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]raclopride. *Journal of Neuroscience*, *24*, 2835-2831.

Rochford, J., Beaulieu, S., Rousse, I., Glowa, J. R., & Barden, N. (1997). Behavioral Reactivity to Aversive Stimuli in a Transgenic Mouse Model of Impaired Glucocorticoid (Type II Receptor Function: effects of diazepam and FG-7142, *Psychopharmacology, 132* (*2*), 145-152.

Rubia, K., Russell, T., Overmeyer, S., Brammer, M. J., Bullmore, E. T., et al. (2001). Mapping motor inhibition: conjunctive brain activations across different versions of go/No-Go and stop tasks. *NeuroImage*, *13*, 250-261.

Scholz, U., La Marca, R., Nater, U. M., Aberle, I., Ehlert, U., et al. (2009). Go No-Go performance under psychosocial stress: beneficial effects of implementation intentions. *Neurobiology of Learning and Memory, 91* (1), 89-92.

Sprague, J., Verona, E., Kalkhoff, W., & Kilmer, A. (2011). Moderators and mediators of the stress-aggression relationship: executive function and state anger. *Emotion, 11*, 61-73.

Vandenbossche, J., Deroost, N., Soetens, E., Zeischka, P., Spildooren, J., et al. (2012). Conflict and freezing of gait in parkinson's disease: support for a response control deficit. *Neuroscience*, *206*, 144-154.

Vidal, F., Burle, B., Grapperon, J., & Hasbroucq, T. (2011). An ERP study of cognitive architecture and insertion of mental processes: Donders revisited. *Psychophsiology*, *48*, 1242-1251.

Webster, M. J., Weickert, C. S., Herman, M. M., & Kleinman, J. E. (2002). BDNF mRNA expression during postnatal development, maturation and aging of the human prefrontal cortex. *Developmental Brain Research, 139* (*2*), 139-150.

SAN JOSÉ STATE
UNIVERSITY

To: Daniel Miao

From: Pamela Stacks, Ph.D.
Associate Vice President
Graduate Studies and Research

Pamela C Stacks

Division of Academic Affairs
Associate Vice President
Graduate Studies & Research
www.sjsu.edu/gradstudies

One Washington Square
San José, California 95192-0025
Voice: 408-924-2427
Fax: 408-924-2612

www.sjsu.edu

Date: October 21, 2011

The Human Subjects-Institutional Review Board has approved your request to use human subjects in the study entitled:

"Relationship between Cognitive Functions and Hormones"

This approval is contingent upon the subjects participating in your research project being appropriately protected from risk. This includes the protection of the confidentiality of the subjects' identity when they participate in your research project, and with regard to all data that may be collected from the subjects. The approval includes continued monitoring of your research by the Board to assure that the subjects are being adequately and properly protected from such risks. If at any time a subject becomes injured or complains of injury, you must notify Dr. Pamela Stacks, Ph.D. immediately. Injury includes but is not limited to bodily harm, psychological trauma, and release of potentially damaging personal information. This approval for the human subject's portion of your project is in effect for one year, and data collection beyond October 21, 2012 requires an extension request.

Please also be advised that all subjects need to be fully informed and aware that their participation in your research project is voluntary, and that he or she may withdraw from the project at any time. Further, a subject's participation, refusal to participate, or withdrawal will not affect any services that the subject is receiving or will receive at the institution in which the research is being conducted.

If you have any questions, please contact me at (408) 924-2427.

Protocol #S1104056

cc. Cheryl-Chancellor-Freeland 0120