Effects of Post-Exposure Naps on Change in Autonomic Arousal to a Social Challenge Across Exposure Therapy for Social Anxiety

Effects of Post-exposure Naps on Change in Autonomic Arousal to a Social Challenge Across Exposure Therapy for Social Anxiety

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Abstract

Sleep promotes memory consolidation and regulates emotion. Sleep may strengthen therapeutic extinction learned during exposure therapy. We investigated effects of post-exposure naps on pre- to post-treatment changes in autonomic arousal during an experimental social stressor in people with Social Anxiety Disorder. Twenty-six participants aged 18-39 (16 females) with mean Liebowitz Social Anxiety Scale scores of 85 (96% > 60) completed a five-session group exposure therapy for social anxiety. A modified Tier Social Stress Test (mpTSST) was conducted before and after treatment. Heart rate (HR) and skin conductance level (SCL) were measured during the baseline, performance (speech and mental math), and recovery periods of the mpTSST. Heart rate variability (HRV) measures using time domain and frequency domain analysis with ratio of low- to high-frequency HR oscillations (sympathovagal balance) were computed. The third and fourth therapy sessions concluded with a speech exposure followed by either a 120-minute nap opportunity (nap, 14Ss) or a non-arousing video (wake, 11Ss). The nap group showed greater pre- to post-treatment decrease in SCL during and while recovering from the social stressor, $F(1, 23) = 4.54$, $p < .05$. The post- compared to pre-treatment SCL during mpTSST decreased in the nap group ($p < .05$) but not the wake group. There was a trend toward decrease in standard deviation of all successive R-to-R intervals (SDNN) across treatment, $F(1, 21) = 4.67$, $p < .05$, but no group differences. Although the pre-to-post treatment main effect for sympathovagal balance was not significant, a near trend was seen for the *Time* x *Group* interaction, $F(1,$

21) = 2.57, p = .12, with sympathovagal balance decreasing in the nap group—the same pattern that was seen for the other sympathetic activity index, SCL. Thus, therapy with post-exposure naps was associated with greater reduction in sympathetic activation to a social stressor from pre-to post treatment. Sleep augmentation of exposure therapy may benefit the treatment of Social Anxiety Disorder.

Dedication

I dedicate this thesis and give special thanks to my parents Dr. YJ Kim and HJ Jang for their unconditional love and support throughout the entire process. I appreciate their sacrifice as parents throughout these years and how they have helped me to achieve my highest potential. I also dedicate this thesis to my sister, MJ Kim, for encouraging me to believe in myself.

Acknowledgments

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Chapter I

Introduction

Social Anxiety Disorder (SAD) is an anxiety disorder that is characterized by excessive fear and anxiety in social situations, leading to a significant disability that interferes with education, employment, and relationships (American Psychiatric Association, 2013; Stein & Kean, 2000; Stein, Torgrud, & Walker, 2000). Treatments for anxiety disorders, including SAD, include medications and Cognitive Behavior Therapy (CBT) with exposure therapy, a form of CBT in which individuals get repeatedly exposed to feared objects, situations, or activities in a safe/clinical environment (Craske & Mystkowski, 2006) thereby encoding therapeutic fear-extinction memories (Craske, Kircanski, Zelikowsky, Mystkowski, Chowdhury, & Baker, 2008). Then, the extinction memories acquired during anxiety treatments compete with or inhibit the original fearful (or conditioned) memory (Milad & Quirk, 2012). Previous studies found that exposure therapy is an effective treatment for specific phobias such as those of spiders, heights, and snakes; however, not all SAD patients respond to exposure therapy, and relapse is common (Hofmann, 2007; Hofmann & Smits, 2008; Kleim, Wilhelm, Temp, Margraf, Widerhold, & Rasch, 2014). Researchers have stressed the importance of finding new ways to improve the outcome of exposure therapy. Sleep, which promotes memory consolidation (Rasch & Born, 2013) and regulates emotion (Goldstein & Walker, 2014), has been suggested as a possible means to augment the therapeutic effectiveness of exposure therapy as sleep may strengthen therapeutic extinction learned during the

treatment (Pace-Schott, Germain, & Milad, 2015).

Effects of Sleep on Memory

Extensive research has shown the effects of sleep on memory. Studies have found that sleep strengthens the consolidation of memories, regulation of emotional memories, stabilization of memories, and fear extinction (Rasch & Born, 2013; Pace-Schott, Verga, Bennett, & Spencer, 2012; Walker, 2008; Spoormaker et al. 2012). Specifically, sleep participates in improving synaptic and systems consolidation, the storing of newly encoded information into memory, which are major processes of memory consolidation (Maquet, 2001). Sleep deprivation and restriction can disrupt these consolidation and stabilization processes by interfering with NMDA receptors, which are involved with memory function, resulting in the dysfunction of memory consolidation (Davis, Harding, & Wright, 2003; Ishikawa et al., 2006; Kim, Mahmoud, & Grover, 2005; Kopp, Longordo, Nicholson, & Luthi, 2006). Another benefit is that sleep helps to lower levels of emotional arousal associated with a memory (van der Helm, Yao, Dutt, Rao, Saletin, & Walker, 2011). Van der Helm and her colleagues (2011) performed fMRI tests on participants and assessed the subjective emotional intensity of the participant after showing standardized affective pictures. People who slept after seeing emotional pictures had reduced reactivity in the amygdala, a region of the brain involved in emotional expression, compared with participants who remained awake. This was accompanied by reduced self-reported reactivity to emotional memories compared to people who remained awake. Furthermore, a study has demonstrated that one to two hours of sleep has a significant influence on memory (Diekelmann et al., 2008); however, at least 90

minutes of sleep is required to have the maximum memory enhancement (Diekelmann, Biggel, Rasch, & Born, 2012). Moreover, studies suggest that rapid eye movement sleep (REM) and non-rapid eye movement sleep (NREM) may be associated with different types of memories. It has been suggested that NREM strengthens hippocampus-dependent declarative memories (Rasch & Born, 2013) whereas REM affects emotional memories (Walker & van der Helm, 2009) and extinction of conditioned fear (Spoormaker et al., 2012). In addition, previous studies found significant positive effects of REM sleep on fear extinction memory in both animals and humans reviewed in Pace-Schott et al. (2015).

Effects of REM Sleep on Extinction in Rats

Early research relating sleep to extinction learning and memory was based on studies of rats. Fu et al. (2007) studied extinction training followed by REM sleep deprivation in rats. They found that after fear conditioning followed by extinction training 24 hours later, six hours of REM-sleep deprivation following the extinction training resulted in a reduced ability to recall extinction memories. In the study, male Sprague-Dawley rats were fear conditioned for two consecutive days using footshock. After the extinction training, rats were either placed in the six-hour sleep deprivation group or in the control group with no sleep deprivation. Researchers used the "flowerpot" technique to prevent rats from falling asleep. Sleep deprived rats were placed on an inverted flowerpot, and the pot was placed in water. Once they fall asleep, relaxed muscle tone during REM caused them to fall into the water. Then, they would have to climb back and remain awake to avoid drowning while rats in the control group remained

uninterrupted for six hours. On the third day, referred to a recall test phase, sleep-deprived rats showed freezing behavior whereas controls did not. This demonstrated that sleep-deprived rats were not able to recall extinction memories as effectively as the control, which resulted in continued physiological reactivity. Similarly, being REM-sleep-deprived after fear conditioning negatively impacted later extinction learning (Silvestri, 2005). Therefore, REM-sleep-deprived rodents oppose fear cues using extinction less successfully than controls. Both studies (Fu et al., 2007; Silvestri, 2005) have found that sleep allows rodents to better learn or consolidate extinction and thus respond less to fear cues than REM-sleep-deprived rodents. Interestingly, similar effects of sleep on extinction memory in humans have been supported by various studies.

Effects of REM Sleep on Extinction in Humans

The first human study on the effects of sleep on extinction suggested that sleep promotes generalization of fear extinction (Pace-Schott et al., 2009). Following experimental fear conditioning to two different stimuli, fear for one of these stimuli was extinguished. Participants then spent 12 hr. containing a normal night's sleep or an equal duration awake. The skin conductance responses (SCR) during the presentation of both extinguished and un-extinguished stimuli were then measured to investigate the influence of sleep on recall and generalization of extinction memories. Extinction recall and generalization are essential factors for regulating fear. After the fearful stimulus is encountered, the extinction memories must first be recalled in order to inhibit fear memories to the specific conditioned stimulus, and they must be generalized to ensure the reduction of reactivity to similar stimuli (Vansteeenwegen et al., 2007). The decrease in

physiological arousal, measured using SCR towards to the fear-conditioned and then extinguished stimulus was due to extinction recall (Pace-Schott et al., 2009). In addition, the decrease in physiological arousal to the fear conditioned but un-extinguished stimulus was due to extinction generalization. Participants who slept after fear conditioning followed by extinction learning had significantly lower physiological reactivity to the un-extinguished stimulus compared to persons who stayed awake, $F(1, 26) = 8.33$, $p < 0.01$. However, subjects who did not sleep after extinction learning maintained physiological reactivity to the unextinguished stimulus, suggesting that they were able to recall extinction memories but were unable to generalize extinction to similar cues. As a result, the study claimed that sleep supports generalization of fear extinction. This finding had important clinical implications, especially for anxiety disorder treatments. Anxiety patients must generalize therapeutic extinction memories that were acquired during the exposure therapy to situations outside of the clinic in order to cope with their stressor. This finding suggests that sleep can benefit therapeutic effectiveness of exposure therapy by helping anxiety patients to efficiently apply therapeutic extinction memories to their general experience.

As the preliminary research investigating impacts of sleep on therapeutic extinction memories found positive effects, the current study extends these findings to include additional physiological indices of fear. It is possible that the extinction memories from the exposure therapy suppress fear, which may cause less activation of the sympathetic nervous system. The reduced sympathetic nervous system activity will cause changes in psychophysiological reactivity such as skin conductance level (SCL), the sympathetic components of heart rate variability (HRV), and heart rate (HR).

Effects of Sleep After Different Types of Exposure Therapy

Because exposure therapy is effective for anxiety disorders but does not offer complete relief, the use of sleep to augment the effectiveness of exposure therapy seems promising. Exposure therapy provides learning situations where new therapeutic extinction memory traces are formed that then need to be consolidated and integrated with pre-existing memories and experiences (Kleim et al., 2014). Studies that investigated the effects of sleep on various types of exposure therapy for spider phobias have demonstrated that sleep can enhance therapeutic extinction memories. The first study of sleep following exposure therapy tested the effectiveness of sleep after simulated exposure therapy for spider fear (Pace-Schott et al., 2012). Young adult, spider-fearful women viewed repeated videos of one spider in Session 1 followed by 12 hr. of continuous wakefulness or 12 hr. containing a normal night's sleep. In Session 2, participants watched videos of the same spider that they watched in Session 1 as well as videos of a different spider. At the second session, the wake group demonstrated a significant increase in both subjective ratings and physiological responses to the original spider compared with these measures at the end of Session 1. In contrast, in the sleep group, physiological reactivity when a new spider video was shown in Session 2 did not differ from the responses to the original spider video in Session 1 when it was first shown. However, in the wake group, responses to the new spider were actually stronger than to the original spider video in Session 1 when it was first shown (sensitization). The result suggested that, in the wake group, some of the extinction learned in Session 1 was lost over the delay whereas this learning was maintained across the delay in the sleep group. In addition, for people from the wake group, extinction from Session 1 was not

sufficiently generalized to prevent their being sensitized to the new spider. This suggests that the simulated exposure therapy for reducing the fear of spiders was not as effective with people who remained awake. Physiological reactivity to the new spider video in Session 2 increased only in the wake group. Thus, people who slept were able to apply therapeutic extinction memories from the exposure during Session 1 to the new spider video in Session 2. Therefore, the generalization of extinction learning was enhanced by post-exposure sleep. Thus, sleep following exposure therapy may promote retention and generalization of extinction learning, which may reduce clinical symptoms in some anxiety disorders.

Another study of sleep following exposure therapy for spider phobia suggested that sleep improved therapeutic effectiveness (Kleim et al., 2014). This study tested the effectiveness of sleep following virtual reality exposure therapy on extinction memory consolidation. Individuals with spider phobia received one session of exposure and either slept for 90 minutes or remained awake. One week later, participants who napped immediately after exposure had a significant decrease in self-reported fear ($p=0.045$, $d=0.47$) compared to participants who remained awake. The study demonstrated that extinction memory traces formed during the exposure therapy might have been strengthened by even a brief nap, resulting in subjects being less fearful towards spiders an entire week later. From the clinical view, it is important that fear extinction generalizes so that the individuals can be less reactive to the fearful stimuli encountered outside of therapy (Rowe & Craske, 1998; Vansteeenwegen et al., 2007). Although preliminary studies (Pace-Schott et al., 2012; Kleim et al., 2014) that investigated the influence of sleep on exposure therapy have used different types of exposure therapy,

both have shown that sleep enhances the effectiveness of clinical outcomes by strengthening extinction memory.

Study Aim & Hypothesis

The purpose of this study is to expand the limited research on sleep and extinction memory into the clinical setting, and the study is the first to investigate experimental evidence of therapeutic sleep-dependent extinction memory consolidation using changes in heart rate variability (HRV)--the variation of beat to beat intervals in the electrocardiogram, from which measures of autonomic nervous system activity can be derived. Whereas Pace-Schott et al. (2012) and Kleim et al. (2014) examined post-treatment sleep in spider phobia, this study examines reducing the fear of social demands in people with SAD. Moreover, no previous studies have specifically investigated the clinical benefit of post-treatment sleep for people with SAD. This study seeks to test the hypothesis that SAD participants who sleep rather than remain awake after exposure therapy will show greater decreases from pre- to post-treatment in both self-reported distress levels (Hypothesis 1) and indices of sympathetic activation (Hypothesis 2) during an experimental social stressor.

Significance of Study

With the exception of one sleep deprivation study (Labbate et al., 1998), no previous study has examined the effect of sleep on symptoms of social anxiety disorder. The current study examines changes over 5 weeks of exposure therapy treatment in autonomic nervous system activity in response to an experimental social challenge and

compares exposure treatment with and without post-exposure naps. In anxiety provoking situations, the sympathetic nervous system stimulates the fight-or-flight response and causes secretion of hormones such as cortisol and adrenaline, which results in physiological effects such as an increase in heart rate and sweating and a decrease in clear hearing and vision (Gleitman, Fridlund, & Reisberg, 2004). In contrast, the parasympathetic nervous system stimulates the rest-and-digest response when stresses are no longer present and it causes a decrease in heartbeats and constricts bronchi as it tries to calm down physiological changes that might have resulted from the sympathetic nervous system (Gleitman et al., 2004). The study recorded subjective measurements of anxiety during the experimental stressor from participants using the Subjective Units of Distress Scale (SUDS) (Heimberg, 1991) as well as objective measurement of physiological arousal such as heart rate variability (HRV), heart rate (HR), and skin conductance level (SCL). All of these measures can help to identify the effectiveness of sleep augmentation during the exposure therapy. All of the objective measurements of this study, HRV, HR, and SCL, are good indexes of sympathetic nervous system activation as these measures are highly influenced by the fight-or-flight response. Because the fight-or-flight response causes an increase in sweating, SCL, which is a measurement of skin conductance, also increases and reflects the vigorous activity of the sympathetic nervous system. The HRV, which represents regulation of circulatory function (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), indexes the balance in the autonomic nervous system between sympathetic nervous system and parasympathetic nervous system activity. The variability of the heartbeat (R-to-R) intervals displays changes during the fight-or-flight response. These physiological

measures are essential measurements of the study since they represent a natural reactivity to the stressor of which participants may not be consciously aware. One possible mechanism of changed reactivity is that exposure therapy creates new therapeutic extinction memories, which diminish fear. When fear is suppressed, it may result in a reduction in the fight-or-flight response, which in turn, impacts HRV, HR, and SCL. The strength of this study is that it provides a clearer picture of the therapeutic effects of sleep during the exposure therapy in both objective and subjective measurements.

Chapter II

Method

The study was conducted at the MGH Center for Anxiety and Traumatic Stress Disorders (CATSD). The study consisted of baseline and final assessments before and after a five-week group exposure therapy treatment. The baseline and final assessments included the Tier Social Stress Test modified for psychophysiology (mpTSST), a modification of a standardized laboratory protocol to induce psychological stress that adds psychophysiological measures to the traditional TSST measures of SUDS and salivary cortisol (Kirschbaum, Pirke, & Hellhammer, 1993). After two exposure therapy sessions, a nap versus wake intervention was conducted at the MGH sleep laboratory in the Wyndham Hotel in Boston.

Participants

Participants were recruited from the general public. Potential subjects completed a preliminary telephone screening to exclude people who had a lifetime history of any neurological illness, psychosis, bipolar or neurodevelopmental disorders, substance abuse, sleep disorders, shift work, use of psychiatric medication within four weeks of the study, or who were receiving CBT for social anxiety. The inclusion criteria included being 18-40 years of age, proficient in English, and willing to comply with the study protocols (see Appendix A). Those subjects who passed exclusion and inclusion criteria were scheduled for a psychiatric interview using the Structured Clinical Interview for

DSM-IV-TR Axis I Disorders–Non-Patient Edition (SCID 1/NP) (First, Spitzer, Gibbon, & Williams, 2007), sleep disorders interview using the Pittsburgh Structured Clinical Interview for Sleep Disorders (PSCID; unpublished investigator-administered instrument), completed the Liebowitz Social Anxiety Scale (LSAS), and had a urine toxicology test for substances abuse. Subjects, who met inclusion and not exclusion criteria, scored at least 60 on the LSAS and whose urine toxicology was negative were eligible to participate in the study.

Table 1

Demographic Analysis

Variables	Level	N	%
Gender	Female	16	61.54
	Male	10	38.46
	Total	26	

Variables	Level	N	%	Mean	SD
Age	18-19	3	11.54	18.33	0.58
	20-29	15	57.69	23.80	3.53
	30-39	8	30.77	34.88	3.00
	Total	26		26.58	6.66

A total of 32 participants aged 18-39 with mean LSAS scores of 85 (96% > 60) completed a five-session group exposure therapy for social anxiety; however, only 26 produced physiological data of sufficient quality for all of the analyses reported here (see Table. 1). Among these 26 participants, mean age was 26.58 (*SD* = 6.66). The sample consisted of 60% of females and 40% of males. Subjects were randomly distributed to

nap (N = 14, 53.8%) and wake (N = 12, 46.2%) groups using a predetermined randomized schedule, but they did not know their assignment before the beginning of each of their 2 nap or wake periods. All participants provided written informed consent. Participants received free treatment and were paid up to $400 with a pro-rated schedule of payment for partial completion.

Procedure

This study was approved by the Partners Human Research Committee (Institutional Review Board) and required seven weeks to complete.

Table 2

Study Timeline and Procedure Outline

Baseline Assessment	Five-week exposure therapy program					Final Assessment
	Week 1	Week 2	Week 3	Week 4	Week 5	
Pre-treatment mpTSST	Psycho-education	Psycho-education & Speech Exposure	Speech Exposure Then 2hr Nap or Video	Speech Exposure Then 2hr Nap or Video	Relapse prevention	Post-treatment mpTSST

Note. This table shows the timeline of the study. The pre-treatment mpTSST was conducted a week before the starting date of the exposure therapy program. The post-treatment mpTSST was conducted a week after the completion of exposure therapy.

Schedule of Visits

A week before the five-week exposure therapy started, individuals came to the

CATSD for the pre-treatment mpTSST (see Table 2). Once participants successfully completed the mpTSST, the five-week exposure therapy started the following week. The nap versus wake treatment interventions were performed after the third and fourth exposure therapy sessions. One week after the completion of the fifth therapy session, the post-treatment mpTSST was conducted.

mp-TSST Procedures

This study modified the standard TSST (Kirschbaum et al., 1993) procedures by the addition of psychophysiological measures and a fear-potentiated startle phase. The rest of procedure followed Kirschbaum et al. (1993). Psychophysiological measures added included the electrocardiography (ECG), skin conductance level (SCL) and two measurements of facial electromyography (EMG, not reported here). The mpTSST was conducted in a participant room and all data was recorded in an adjacent control room with viewing through a one-way mirror (see Figure 1). The pre- and post- mpTSST followed identical procedures except that audiences were different. The mpTSST contained seven different phases and the following is an overview of each of the phases.

Orientation. The participant was first taught how to complete the SUDS (Heimberg, 1991) which was completed at six specified times with the training serving as the first. For SCL, researchers placed two BIOPAC EL504 disposable adhesive sensors filled with isotonic paste on the hypothenar surface of the non-dominant handpalm. For the electrocardiogram (ECG), two BIOPAC EL503 disposable electrodes were placed on the right torso over the first intercostal space and below the lowest rib on the left. In order to ensure responsivity of SCL, participant was asked to hold breath or hyperventilate.

Baseline phase. The participant sat in the room alone while SCL and ECG were recorded for five minutes. Then, a researcher placed headphones over participant's ears and an initial startle habituation phase lasted an additional 10 minutes. Randomly spaced sudden-onset tones were played. Then, the participant was asked to complete the SUDS rating #2 (see Appendix B).

Instruction phase. A two-person "audience" (one male and one female) entered the room and placed a video camera, tape recorder, and microphone in front of the participant (see Figure 1). One audience member gave instructions to the participant to prepare a five-minute speech for an interview for their ideal job.

Preparation phase. The participant sat in the room alone for 10 minutes and prepared for their job interview. During this time, additional 10-startle tones were played through headphones at different randomly chosen but fixed intervals. Then, the participant was asked to complete the SUDS rating #3.

Performance phase. The audience reentered the room and the participant started the eight-minute speech. If the participant finished the speech early, the audience asked the participants questions to prolong the speech. Next, the participant was asked to perform as quickly and accurately as possible a series of subtractions by 7 starting at 996. If participant made a mistake, an audience member asked the participant to start again from the beginning. After five minutes the participant was stopped, then asked to complete the SUDS rating #4. The audience then left the room.

Recovery phase. The participant remained alone for 10 minutes while completing the SUDS rating #5. Then, participant remained alone for another 10 minutes and completed the last SUDS rating #6.

Debriefing phase. Researchers entered the room and removed all adhered sensors. For the pre-treatment mpTSST debriefing, researchers ensured that the participant dissipated the stress engendered by the mpTSST and explained that the pre-mpTSST was not a part of therapy treatment and was more to measure biological response to stress. For the post-treatment mpTSST debriefing, researchers told participants that there were no evaluations performed by the audience and neither video nor audio recordings took place. Then, the purpose of the mpTSST, which is designed to be stressful, and the goals of the study were explained to subjects.

Exposure Therapy Procedures

A week after the baseline mpTSST test, both nap and wake groups received the same five-week exposure therapy conducted by two experienced clinical psychologists at the CATSD (see Table 2). The group exposure therapy was conducted with either 3 or 4 participants who were randomly assigned to either the sleep or nap groups but were not made aware of which until right before the nap/wake period. Each therapy session lasted 90 minutes and was conducted once a week. The first session involved psychoeducation, providing participants with an orientation to the model of SAD, a rationale for exposure treatment, and the use of didactic materials. The second session involved additional psychoeducation as well as an exposure exercise in which each participant described the SAD treatment model. The 3rd and 4th sessions continued with exposure exercises, this time delivering a videotaped speech to the therapy group members on a topic decided by participant and therapist. The fifth session addressed relapse prevention and reflected on

participant progress. At this session, an individual post-treatment evaluation was scheduled.

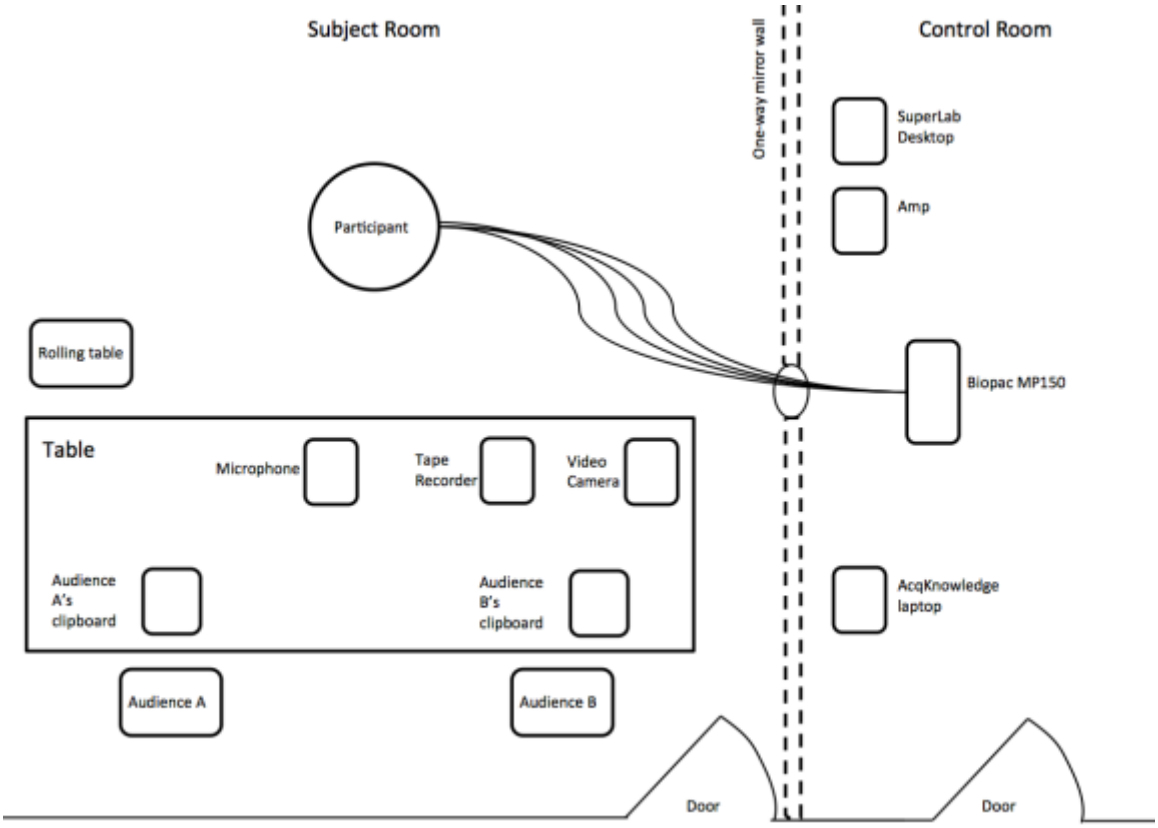


Figure 1. Overview of the mpTSST Setup. This shows an overview of a subject room, where the mpTSSTs were conducted, and control room, where all psycholophysiological measurements were recorded. All equipment that was used during the mpTSST is also shown.

Sleep Lab Procedures

On the third week, right after the group exposure therapy, researchers escorted all

subjects to the MGH sleep laboratory in the Wyndham Hotel in Boston. Participants had a fully equipped hotel room with night vision cameras. Both groups were instrumented for polysomnography to track participants' sleep activities but instrumentation of wake participants was sham. Individuals in the sleep group were asked to take a nap and they achieved approximately 69 minutes of sleep across both nap interventions while individuals in the wake group were asked to watch an episode of "Planet Earth" for two hours. Research assistants monitored subjects in the wake group to ensure wakefulness.

Measurements

The study included both objective and subjective measurements to examine therapeutic effects of post-exposure sleep.

Psychophysiological Assessments

Skin conductance level (SCL), also known as the electrodermal activity, refers to how well skin conducts electricity which reflects how close sweat is to the skin surface which, in turn, depends on the level of sympathetic nervous system activity. The BIOPAC GSR100C Electrodermal Activity Amplifier Module was used to measure the SCL. Data were transmitted to Biopac AcqKnowledge 4.1.5 data acquisition software. Recordings were measured in microsiemens, which is a unit of electrical conductance.

Electrocardiogram (ECG) was recorded using the Biopac MP150 system (Biopac Systems, Inc., Goleta, CA) with a BIOPAC ECG100C Biopotential amplifier module. The ECG was in millivolts, was sampled at 2000 Hz, and was transmitted to the Biopac AcqKnowledge 4.1.5 data acquisition software. Using the ECG, intervals between

successive R peaks of the QRS complex were extracted to calculate the heart rate variability (HRV) and heart rate (HR). The 300 seconds of ECG segments during the beginning of baseline, performance-speech, performance-math, and recovery phases of the mpTSST were extracted and computed seven HRV parameters and HR using the Acqknowledge 4.1.5 software. ECG recordings obtained during the speech and math periods were excluded for the HRV analysis since respiration occurring while subjects talk produces artifact in HRV analysis, thus altering the ECG. All of the HRV time domain parameters were measured in milliseconds (ms) and the frequency domain parameters were measured in milliseconds squared (ms^2). HR readings were measured in beats per minute (BPM).

Questionnaires

The Subjective Units of Distress Scale (SUDS) (Heimberg, 1991) is among the oldest self-report scales used both by clinicians and researchers for cognitive-behavioral treatments of anxiety symptoms. Subjective distress that a participant is experiencing at the time is self-reported by marking a scale of 0 to 100 where 100 indicates being out of control as in a "nervous breakdown" and 0 being in a total relief and free from anxiety of any kind (see Appendix B). It takes up to five minutes to complete. The purpose of this questionnaire is to let participants monitor and think about the change in their anxiety levels and physiological reactivity (Kim, Bae, & Park, 2008). In this study, participants were asked to complete the SUDS six times during the orientation, baseline, preparation, performance, and recovery phases of the mpTSST.

The Liebowitz Social Anxiety Scale (LSAS) (Liebowitz, 1987) is a self-report

questionnaire, which measures social phobia that interferes with subject's daily life. It describes 24 social situations and asks how anxious or fearful one feels in the given situation and how often one avoids the situations presented using four-point Likert Scales (see Appendix C). Out of 144 points, scoring up to 30 is considered as unlikely SAD, 60 as probable SAD, 90 as very probable SAD, and over 90 as highly probable SAD (Rytwinski et al., 2009). With respect to another commonly used social anxiety self-report questionnaire, the Social Interaction Anxiety Scale (SIAS), the LSAS has validity over 0.73 (Heimberg et al., 1999). Correlations among LSAS subscales range from 0.68 to 0.98 and the LSAS total score is highly correlated with its total fear and total avoidance subscales (r=0.98). The LSAS presents high reliability of 0.81 and internal consistency between 0.81 and 0.96 (Heimberg et al., 1999). The entire questionnaire required up to 45 minutes to complete, and it was conducted through phone calls.

Dependent Variables and Analyses

The dependent variables were *Heart rate variability (HRV), Heart rate (HR), Skin conductance level (SCL), and Subjective Units of Distress Scale (SUDS).*

Heart Rate Variability (HRV)

HRV is based on the interval between heartbeats and is measured by the variation in the beat-to-beat intervals usually measured as the interval between successive R-waves (R-R interval) in sinus rhythm. HRV was calculated using eight different quantities divided between time domain and frequency domain. The time domain analysis focuses on a statistical variance between beat-to-beat intervals, which represents the activity of

autonomic nervous system. The time domain includes the following: standard deviation of all successive RR intervals (SDNN), root mean square of successive RR interval differences (RMSSD), standard deviation of successive RR interval differences between adjacent RR intervals (SDSD), and the percentage of the number of pairs of successive RR intervals that differ by more than 50ms (pNN50). Studies found that the SDNN reflects overall autonomic activity and HRV while the RMSS, SDSD, and pNN50 represents vagal tone (parasympathetic activity) and correlates with high frequency from the frequency domain, also considered indicative of parasympathetic activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

The frequency domain describes the "periodic oscillations of the heart rate signal" using the fast Fourier transformation (FFT) to distinguish power at different frequencies of fluctuations in the duration of R-R intervals (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The frequency domains represent the balance of sympathetic and parasympathetic activity in the autonomic nervous system (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) and it includes the power of High Frequency (HF) which ranges from .15 to .4 Hz and Low Frequency (LF) which ranges from .04 to .15 Hz. Previous studies found that the HF reflects parasympathetic nervous system whereas the LF is associated with both of the sympathetic and parasympathetic nervous system. However, the LF is generally a dominant indicator of the sympathetic nervous system **(**Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology,

1996). Therefore, it is important to compute the low-to-high frequency ratio, which is also known as sympathovagal balance, to see the change of the sympathetic and parasympathetic nervous system (Pagani et al., 1984).

In this study, all seven parameters from the time domain and frequency domain were measured to analyze the HRV change during the baseline and recovery periods of the mpTSST through Acqknowledge 4.1.5. Once the ECG was recorded using the "digital filter" tool, all signal artifacts were eliminated. Then, the first 300 seconds of the baseline and last 300 secs of the recovery periods were extracted. Those intervals were chosen because the first 300 seconds of the baseline reflects subjects' initial physiological level right after all the equipment was set and were uninfluenced by the assessment. The last 300 sec of the recovery is when subjects were sitting and resting without any interruptions. The SDNN were computed using the "find cycle" to extract the successive RR interval channels from the ECG and the "measurement box" to calculate the mean and standard deviation of the RR intervals. To calculate the RMSSD, RR interval was plotted using the "find rate" tool, and root mean square waveform was computed using the "transform" function. After duplicating the waveform and eliminating the first two and three samples, the differences of the root mean square were calculated using the "measurement" tool. The SDSD was computed after shifting the RR interval plot by one sample and subtracting it from the original RR interval plot using the "find rate" and "transform" tool. Then, a standard deviation of the difference waveform was computed by the "measurement" tool. To compute the pNN50, the RR difference waveform from the SDSD was used. The number of peaks that were more than 50 msecs (NN50) was extracted using the "threshold transform" function then it was divided by the total

number of samples using the "measurement" tool to acquire a percentage of the NN50. Both the power of the LF and HF were calculated using the "HRV analysis" tool. Then, LF/HF ratio was computed in Microsoft Excel by dividing LF over HF.

Heart Rate (HR)

The HR was calculated using the "find rate" tool in AcqKnowledge 4.1.5. The calculation was based on the mean of R-to-R intervals over 300 seconds. ECG segments from the baseline, two separate sections in performance (speech and math), and recovery periods of the mpTSST were analyzed.

Skin Conductance Level (SCL)

Averages of the SCL recordings from the baseline, two separate sections in performance (speech and math), and recovery periods of the mpTSST were calculated in AcqKnowledge 4.1.5. using the "measurement" tool.

Subjective Units of Distress Scale (SUDS)

Although six ratings were collected, only four ratings from the orientation, performance, and recovery phases of the mpTSST were analyzed in the study. These intervals were chosen because the orientation phase reflects subjects' initial distress level without any influence from the assessment. Performance phase reflects subjects' distress level from the speech and mental math tasks. Early recovery phase reflects subjects' distress level ten minutes after the performance and late recovery phase reflects subjects' distress level while resting and sitting without any interruptions. Averages of four SUDS

from the orientation (1st SUDS), performance (4th SUDS), end of early recovery (5th SUDS), and end of late recovery (6th SUDS) of the mpTSST were calculated in Microsoft Excel.

Statistical Analyses

The independent variables were *Group* (between-subjects) which had two levels: *nap* and *wake*, *Time* (within-subject) which had two levels, *pre-treatment* and *post-treatment*, and *Phase* (within-subject) which had 4 levels for SCR and HR (*baseline, performance-speech, performance-math, recovery*), 2 levels for HRV measures *(baseline, recovery*), and 4 levels for the SUDS (*orientation, performance, early recovery, late recovery*).

Heart Rate Variability (HRV)

Each of the seven HRV parameters (RMSSD, SDNN, SDSD, pNN50, LF, HF, LF/HF) had two levels of *Phase* (baseline, recovery) within-subject factor. A mixed analysis of variance (ANOVA) consisting of one between-subjects factor *Group* (nap, wake) and two within-subject factors *Time* (pre-treatment, post-treatment) and *Phase* (baseline, recovery) was conducted to analyze changes in HRV variables before and after treatment.

Heart Rate (HR)

The HR had four level of the *Phase* (baseline, performance-speech, performance-math, recovery) within-subject factor. A mixed ANOVA consisting of one between-

subjects factor *Group* (nap, wake) and two within-subject factors *Time* (pre-treatment, post-treatment) and *Phase* (baseline, performance-speech, performance-math, recovery) was conducted to investigate the HR change to the stressor before and after the treatment.

Skin Conductance Level (SCL)

SCL had four level of the *Phase* (baseline, performance-speech, performance-math, recovery) within-subject factor. A mixed ANOVA consisting of one between-subjects factor *Group* (nap, wake) and two within-subject factors *Time* (pre-treatment, post-treatment) and *Phase* (baseline, performance-speech, performance-math, recovery) was conducted to investigate SCL changes to the stressors before and after treatment.

Subjective Units of Distress Scale (SUDS)

SUDS had four levels of the *Phase* (orientation, performance, early recovery, late recovery) within-subject factor. A mixed ANOVA consisting of one between-subjects factor *Group* (nap, wake) and two within-subject factors *Time* (pre-treatment, post-treatment) and *Phase* (orientation, performance, early recovery, late recovery) was conducted to investigate the SUDS change to the stressor before and after the treatment.

Chapter III

Results

Different significant main effects and interactions were found by subjective and objective measurements.

Self-report Measures

There was no main effect of the between-subjects factor *Group* on the SUDS rating ($p > .05$). However, main effects of the two within-subject factors *Time* and *Phase* were found. The *Time* main effect showed that the SUDS rating from the pre-treatment was significantly higher than the post-treatment SUDS, $F(1, 24) = 53.26$, $p < 0.0001$, indicating that all participants reported less distress during the final assessment after the five-week exposure therapy compared to the pre-treatment (see Figure 2). The significant main effect of *Phase* indicated that the SUDS rating changed over different phases of the mpTSST, $F(3, 72) = 47.03$, $p < 0.0001$. Post-hoc means comparisons revealed that the SUDS rating from the performance, which was reported right after the speech and mental math, was greater than orientation, early recovery, and late recovery. No significant difference was found between the orientation and the recovery ratings while the performance rating was significantly greater than the orientation and the early recovery ratings. There was a significant *Time* x *Phase* interaction across all participants ($p < .001$), showing that SUDS ratings for certain phases differed between pre-treatment and the post-treatment. Although performance ratings from both the pre- and the post-

treatment were significantly higher in relation to other phases ($p < .0001$ for both), it is apparent that during pre-treatment, the increase in SUDS during performance was higher relative to the other phases than at post-treatment, hence the significant interaction.

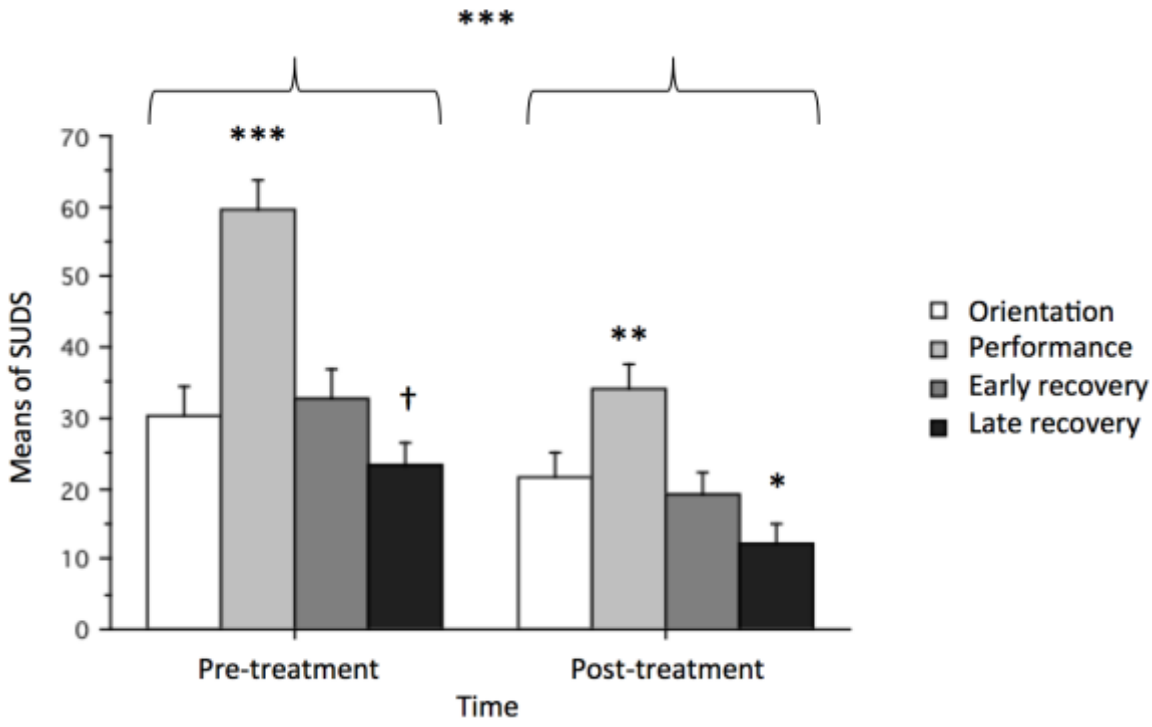


Figure 2. SUDS to Pre- and Post- mpTSST clasping across groups. Bars indicate standard error of the mean (SEM). *** $p < .0001$. ** $p < .001$. * $p < .05$. † $p < .1$.

In addition, late recovery ratings were also significantly lower than other ratings at both pre-and post-treatment ($p < .1$ and $< .05$, respectively), which implies that participants felt less distress at the end of mpTSST than even before the start of the mpTSST. However, there were no significant differences found between the groups.

Therefore, Hypothesis 1 was not supported.

Heart Rate (HR)

The *Group* x *Time* interaction for changes in HR was not significant ($p > .05$). Therefore, Hypothesis 2, that physiological arousal would decrease more over therapy in the sleep group, was not supported in terms of HR. Moreover, the fact that no significant *Time* main effect on HR was found ($p > .05$) indicated that mean HR in general was not changed by treatment. However, a significant *Phase* main effect was found, $F(3, 69) = 20.57$, $p < .0001$, showing that the performance phase had the highest HR at both pre- and post-treatment. Further analyses were conducted; however, no *Time* x *Phase* interaction or *Time* x *Phase* x *Group* interactions were found ($p > .05$ for both).

Skin Conductance Level (SCL)

There were no significant *Group* or *Time* main effects on SCL ($p > .05$ for both). However, a significant *Phase* main effect was found, $F(3, 69) = 35.98$, $p < .0001$, showing that the mpTSST had an effect on SCL across the different phases of the mpTSST (see Figure 3). Interestingly, there was a significant three-way *Time* x *Phase* x *Group* interaction, $F(3, 69) = 5.21$, $p < .05$. Because the *Time* x *Group* interaction was not significant ($p > .05$), the *Time* x *Phase* x *Group* interaction was examined for phase differences between the groups. As shown in Figure 3, baseline showed different pre-to-post changes compared to the other three phases (performance-speech, performance-math, recovery) in both groups. Thus, we first examined whether SCL during the baseline differed between the groups using mixed ANOVA with one between-subjects factor

Group and one within-subject factor *Time.* There were no main effects of *Group* or *Time* and no *Time* x *Group* interaction ($p > .05$ for all), showing that there was no difference between groups.

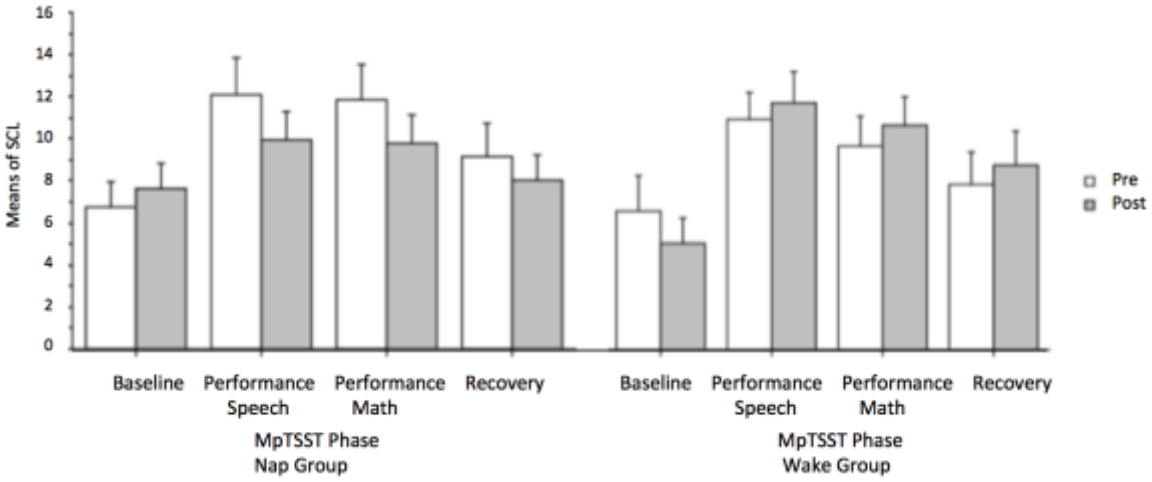


Figure 3. Bar graphs of SCL during the mpTSST by *Group*. Bars indicate SEM. The baseline of both groups had a different pattern as compared to other phases.

Thus, phase "baseline" was removed from analyses and an ANOVA with one between-subjects *Group* and two within-subject factors, *Time* and *Phase,* were conducted with three levels of within-subject factor *Phase* (performance-speech, performance-math, recovery). Once the baseline was removed, there still was a significant main effect of *Phase*, $F(2, 46) = 26.01$, $p < .0001$. Post-hoc analysis revealed that SCL during recovery was significantly different in relation to SCL during speech ($p < .0001$) and mental math ($p < .001$), indicating that reduction of SCL was significant after the stressor. Moreover,

there was now a significant *Time* x *Group* interaction, $F(1, 23) = 4.54$, $p < .05$. Therefore, to investigate changes by *Group* (nap, awake) over *Time*, we did repeated measures of ANOVA of the two groups individually with two within-subject factors *Time* and *Phase*. For the nap group, there was a significant main effect of *Time*, $F(1, 13) = 5.95$, $p < .05$, and *Phase,* $F(2, 26) = 10.64$, $p < .01$, showing that, in this group, the post-treatment SCL was significantly lower than the pre-treatment SCL and that SCL differed over the phases of mpTSST (see Figure 4). Both of the post-treatment SCL during performance-speech and performance-math were significantly lower than pre-treatment ($p < .05$ for both). Also, there was a trend-level interaction of *Time* x *Phase*, $F(2, 26) = 2.94$, $p = .096$, implying that post-treatment SCL was lower than pre-treatment SCL across all phase of mpTSST.

For the wake group, the main effect of *Phase* was also significant, $F(2, 20) = 20.29$, $p < .001$, but no main effect of *Time* was found ($p > .05$). Specifically, pre-treatment and post-treatment SCL did not significantly differ (see Figure 4). These findings show that in terms of SCL, Hypothesis 2 was supported, showing that the nap group compared to the wake group would show a greater decrease in psychophysiological arousals at the end of a five-week exposure therapy program. An additional analysis was conducted to examine gender effects on changes in SCL. An ANOVA with two between-subjects factors, *Group* and *Sex,* and two within-subject factors, *Time* and *Phase,* was conducted. There were no main effects of *Group* or *Time*; however, a significant main effect of *Sex,* $F(1, 21) = 8.39$, $p < .01$, showed that males and females had different SCL. Moreover, a significant main effect of *Phase* ($p < .0001$) indicated that SCL significantly changed over time and the *Phase* x *Sex* interaction ($p < .05$) showed that changes of SCL

in males within all mpTSST phases were different than in females.

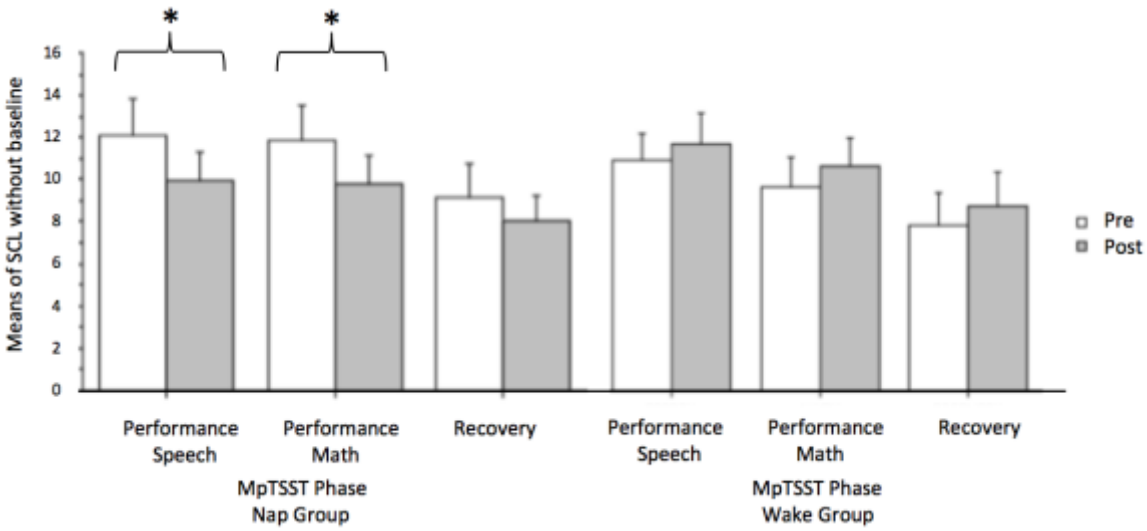


Figure 4. Bar graphs of SCL during the mpTSST by *Group* without the Baseline Phase. Bars indicate SEM. * $p < .05$.

Heart Rate Variability (HRV)

Mixed ANOVAs with one between-subjects factor *Group* and two within-subject factors, *Time* and *Phase,* were conducted for each of the seven HRV (SDNN, pNN50, RMSSD, SDSD, LF, HF, and LF/HF ratio) parameters.

SDNN

There were no significant main effects of *Group* and *Phase* ($p > .05$, for both); however, a significant main effect of *Time*, $F(1, 21) = 4.69$, $p < .05$, was found, showing that the post-treatment SDNN was significantly lower than pre-treatment. Lowered

standard deviation of RR interval represents a decrease in variation of RR interval, meaning that all participants' sinus rhythm was in a steadier state during the post- than during the pre-treatment mpTSST. Moreover, this suggests that overall autonomic activity during the pre-treatment mpTSST was greater compared to the post-treatment, meaning both the sympathetic nervous system and parasympathetic nervous system were more activated during the pre-treatment towards to the same stressor than the post-treatment mpTSST.

RMSSD

No significant main effects of *Group, Time, Phase* ($p > .05$ for all) or interactions were found.

SDSD

Similar to the RMSSD, there were no significant main effects of *Group*, *Time, Phase* ($p > .05$ for all) nor were any interactions found.

pNN50

Similar to the SDNN, a significant main effect of *Time,* $F(1, 23) = 6.50$, $p < .05$, was found, showing that the post-treatment pNN50 was significantly higher than the pre-treatment pNN50. It represents a percentage of RR intervals that were longer than 50ms increased, which implies the sinus rhythm was slower in the post- than the pre-treatment. This suggests that there was more parasympathetic nervous system activity during the post- than the pre-treatment mpTSST, meaning participants were more relaxed during the

post-treatment mpTSST. No other significant main effects of *Group* and *Phase* ($p > .05$ for both) were found.

Low Frequency (LF)

For LF, there were no main effects of *Group* or *Phase* ($p > .05$ for both). A main effect of *Time*, $F(1, 22) = 4.47$, $p < .05$, was found, implying that the LF was significantly higher during the pre-treatment than the post-treatment. This suggests more sympathetic nervous system activity, fight-or-flight response, was present during the pre-treatment mpTSST, meaning participants were more stressed during the pre-treatment mpTSST than the post-treatment mpTSST.

High Frequency (HF)

No significant main effects of *Group* or *Phase* ($p > .05$ for both) were found; however, a main effect trend for *Time* was found, $F(1, 22) = 3.24$, $p = .086$. Greater pre-treatment HF suggests more parasympathetic nervous system activation during the pre-treatment mpTSST. This contradicts the results of the pNN50, which correlates with HF and is also considered indicative of parasympathetic nervous system.

Low Frequency/High Frequency Ratio

There were no significant main effects of *Group* or *Time* on sympathovagal tone. However, a significant main effect of *Phase*, $F(1, 21) = 5.32$, $p < .05$, was found, showing that the sympathovagal tone changed across the two different phases of the mpTSST and the sympathovagal tone during recovery was significantly lower than

during baseline for all participants. Interestingly, a near trend was seen for the *Group* x *Time* interaction, $F(1, 21) = 2.57$, $p = .12$, with the sympathovagal tone decreasing in the nap group, which is the same pattern that was seen for the other sympathetic activity index, SCL (see Figure 5).

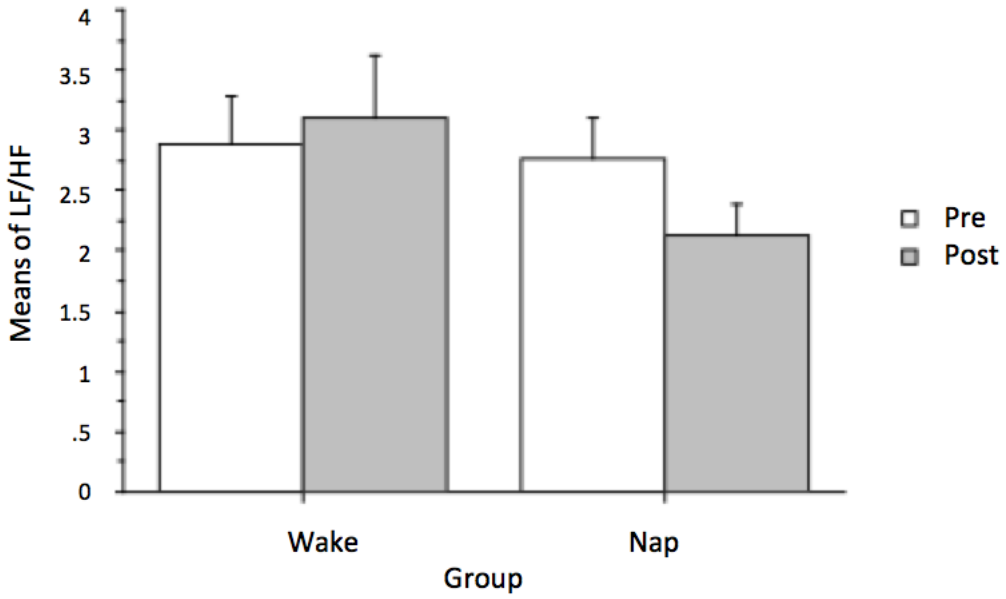


Figure 5. *Group* x *Time* interaction graph of sympathovagal tone. Bars indicate SEM. A near trend was seen in that the sympathovagal tone decreases during post- versus pre-treatment mpTSST in the nap group at $p = .12$.

An additional analysis was conducted to examine gender effects on changes in the sympathovagal tone. A mixed ANOVA with two between-subjects factors, *Group* and *Sex,* and two within-subject factors, *Time* and *Phase,* was conducted. There was a significant main effect of *Sex*, $F(1, 19) = 12.23$, $p < .01$, and *Phase,* $F(1, 19) = 7.24$, $p <$

.05, suggesting that males had a significant higher sympathovagal tone as compared to females (see Figure 6) and that the sympathovagal tone significantly changed between the baseline and recovery phases of the mpTSST.

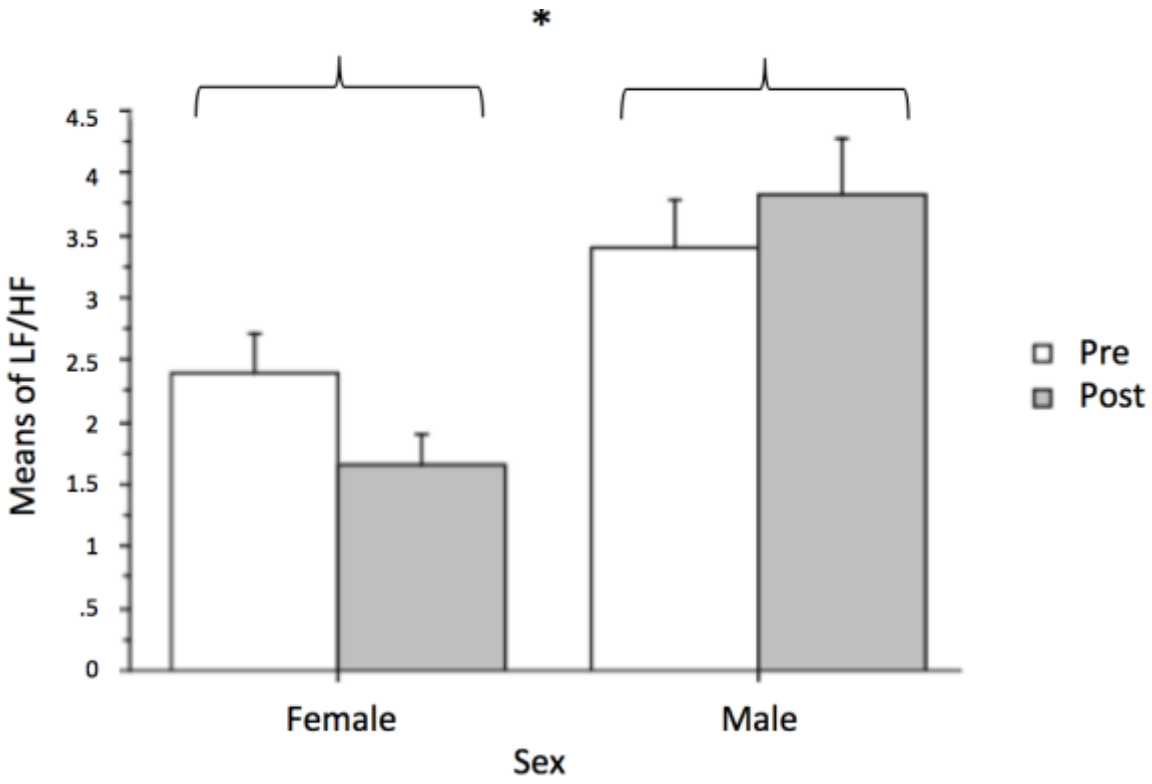


Figure 6. *Time* x *Sex* interaction graph of sympathovagal tone. Bars indicate SEM. * $p <$ .05.

A significant *Time* x *Sex* interaction, $F(1, 19) = 7.32$, $p < .05$, suggested that the change of the sympathovagal tone during the pre-to post-treatment in males was significantly different than in females (see Figure 6). In addition, a *Phase* x *Sex* interaction, $F(1, 19) = 5.46$, $p < .05$, showed that the change of the sympathovagal tone

during the baseline to recovery in females was significantly different than in males. When variability due to sex was added to the above ANOVA model, the *Group* x *Time* interaction, which was a near trend without the factor *Sex* (see Figure 5), became significant, $F(1, 19) = 4.49$, $p < .05$, showing that the change of the sympathovagal tone from pre- to post-treatment mpTSST in the nap group and the wake group was different.

Among HRV parameters, SDNN, pNN50, LF, and HF had significant main effects or a trend of *Time*; however, no main effects of between-subjects factor *Group* were found. Only the sympathovagal tone supported Hypothesis 2 that participants who slept rather than remained awake after exposure therapy would show greater decreases in sympathetic activation from pre- to post-treatment mpTSST.

Chapter IV

Discussion

This study examined pre- to post-treatment changes in response to a social stressor, a modified Trier Social Stress Test (mpTSST), among persons with social anxiety disorder (SAD) who underwent a five-week exposure-based therapy and compared those who napped versus those who remained awake following exposure sessions. The study hypothesized a reduced self-reported distress levels (Hypothesis 1) and lower indices of sympathetic activation (Hypothesis 2) from pre- to post-treatment social challenge for subjects who napped compared to those who remained awake. The main findings of this study were decreases in SCL and sympathovagal tone during the post- versus pre-treatment mpTSST in the nap group. Objective measurements of psychophysiological arousals in terms of SCL and sympathovagal tone supported Hypothesis 2 that the SAD participants who slept after exposure therapy had reduced psychophysiological arousals at the end of a five-week exposure therapy program compared to SAD participants who remained awake following treatment sessions. The subjective measure, SUDS ratings, showed no group differences at the end of a five-week exposure therapy program, thus failing to support Hypothesis 1. However, the SUDS ratings revealed that all participants reported a significantly lower distress level to the same stressor after the completion of the exposure therapy than before, which associates with other findings that greater autonomic nervous system activity during the pre-treatment and less of the sympathetic nervous system activation with an increase of the

parasympathetic nervous system activation during the post-treatment were found for all participants.

Based on the main findings, the therapeutic effect of exposure therapy may have been greater for the nap than the wake group when measured by objective measures of psychophysiological arousal-- SCL and sympathovagal tone-- but was not sufficiently strong to influence subjects to report a change in subjective experience. Alternatively, such subjective effects by sleep may be delayed.

Although the subjective measurement failed to find a significant difference between the groups, the reduced distress level during the post-treatment supports other indices that indicated less of autonomic nervous system activation during the post- than pre-treatment mpTSST, meaning all participants felt less anxiety towards the mpTSST after the completion of the exposure therapy program. SDNN, which represents overall HRV as well as autonomic nervous system activation, was higher during the pre-treatment mpTSST, indicating that both the sympathetic and parasympathetic nervous system were more activated compared to post-treatment mpTSST. In addition, LF, which predominantly indicates sympathetic nervous system activation, was also greater during the pre- compared to post-treatment mpTSST, indicating that all participants were more stressed during the pre-treatment. There was a trend to have greater parasympathetic nervous system activation during the pre- than post-treatment due to an increase of HF, which was unexpected. However, an increase of the parasympathetic nervous system during the pre-treatment could have occurred in order to maintain homeostasis within the body due to the elevation of the sympathetic nervous system (McCorry, 2007). Even though the activation of the sympathetic and the parasympathetic nervous system are in

opposition to one another, both can be activated at the same time (Berg & Jensen, 2011). It is possible that the sympathetic nervous system was more dominant during the pre- versus post-treatment mpTSST since the study found a statistical significance of the LF whereas a trend was found for the HF. Corroborating a significant increase in pNN50 during the post- than pre-treatment mpTSST, more of the parasympathetic nervous system was activated during the post- versus pre-treatment mpTSST, meaning the exposure therapy was an effective treatment for SAD participants in this study. However, it is possible that decreases in anxiety based on the findings of both subjective and objective measures during the post-treatment could have been caused by participants' habituation to the stressor. The effectiveness of exposure therapy could be assessed if a "wait-list" control group, a group with SAD who also have pre-and post-mpTSST but wait five weeks in between before starting therapy, was also included in a study. However, the main investigation of the current study focuses on the differences between nap versus wake group thus a "wait-list" control group was not necessary.

In addition, the fact that participants had the highest SUDS rating after the performance phase and the highest SCL and HR during the performance phase of the mpTSST shows that the mpTSST successfully influenced all participants and was a good experimental assessment to induce psychological stress.

It was interesting to see a significant gender differences in sympathovagal tone in this study. The overall sympathovagal tone was higher in males than females, which is consistent with previous findings (Barnett et al., 1999, Stolarz et al., 2003). The current study showed that the changes of the sympathovagal tone during the pre-to post-treatment and within the mpTSST were different in males and females. Since males show higher

responsiveness towards to the sympathetic nervous system and females react better to the parasympathetic nervous system (Dart, Du, & Kingwell, 2002), males may show a greater increase in sympathovagal tone (Valladares, Eljammal, Motivala, Ehlers, & Irwin, 2008), which can result in differences in changes of sympathovagal tone during the pre-to post-treatment and within the mpTSST.

Similar to the findings of gender differences in the sympathovagal tone, significant gender differences were also found in SCL. The current study found that changes of SCL in females within all mpTSST phases were different than in males, which supports previous findings (Bianchin & Angrilli, 2011; Lithari et al., 2010) that females have a greater sensitivity of SCL to stressors than males, as they are more responsive to emotional stimuli. In addition, previous studies have indicated that females have higher electordermal activity (Shah, Pradeep, & Balasubramanium, 2012), which results in higher SCL. However, the current study found that males had moderately higher SCL than females. Based on studies that SCL differs by race and age (Juniper & Dykman, 1967; Zepelin & McDonald, 1987); therefore, it is possible that, in the current study, gender effects differ from previous findings because of the racial diversity of participants and/or the unbalanced gender ratio.

Previous studies (Garakani et al., 2009; Martinez, Garakani, Kaufmann, Aaronson, & Gorman, 2010) found a significantly lowered pNN50% in panic disorders relative to controls, suggesting that more anxiety caused a decrease in pNN50%. This study also found a significant increase of the pNN50 from the post- compared to the pre-treatment mpTSST, showing that participants had lower levels of anxiety during the post-treatment mpTSST. Therefore, the RMSSD, SDSD, and HF, which are also known to

represent vagal modulation (The North American Society of Pacing and Electrophysiology, 1996), were expected to have similar results to the pNN50. However, no significant effects or interactions of the RMSSD and SDSD were found. Moreover, the HF had a trend to be greater during the pre- versus post-treatment mpTSST, which contradicts not only the pNN50 but also our expectation. Since HRV parameters were only measured during the baseline and the recovery phases of the mpTSST (because respiratory artifacts that might have occurred during the performance phases), measuring only these two phases might not be sufficient measures for HRV because measurements when the stressor was present were not obtained.

Based on the findings from Brosschot, VanDijk, & Thayer (2007) and Martens et al. (2008) that lower HRV and higher HR associate with high levels of anxiety, we expected to find a significant change in HR after the treatment in this study. However, considering that the only significant changes in HR were found within the different phases of the mpTSST, it is possible that talking during the performance-speech and math phases produced HR artifacts. Based upon the finding that blood pressure and heart rate increase while talking (Freed, Thomas, Lynch, Stein, & Friedmann, 1989), participants' consistent talking during the social challenge could have caused excessive blood flow to the heart and resulted in artifacts in HR.

Limitations and Conclusions

The current study provides a new perspective on therapeutic effectiveness of exposure therapy for SAD. It expands the limited research on sleep and extinction memory in the clinical setting. This study is the first to investigate experimental evidence

of sleep-dependent consolidation of extinction memory in terms of HRV. Although both objective measurements of psychophysiological arousal and subjective measurement of distress levels were included to explore therapeutic effects promoted by the post-exposure sleep, only the objective measurements showed an advantage for post--exposure sleep.

The main finding of this study was that SCL and sympathovagal balance provided evidence that post-exposure naps versus waking promoted faster dissipation of anxiety from an experimental social stressor in persons with SAD. In sum, sleep augmentation of exposure therapy may show benefit in the treatment of Social Anxiety Disorder. It is therefore recommended that post-exposure sleep can be a new treatment that augments the therapeutic effectiveness of exposure therapy.

It is recommended that future studies could involve other anxiety disorders such as Post-Traumatic Stress Disorder, Generalized Anxiety Disorder, as well as mixed, co-morbid anxiety disorders. Based on the findings of previous studies (Pace-Schott et al. 2012; Kleim et al., 2014) and the current research, it seems possible that sleep augmentation of exposure therapy may benefit treatment of other anxiety disorders. Moreover, other physiological measurements of vagal tone such as respiratory sinus arrhythmia (RSA) as well as a larger sample sizes might reveal group effects not seen in the current study. Since the subjective measurement failed to support Hypothesis 1, it is also possible that a two-hour sleep intervention in a five-week exposure therapy program was not enough to have had a significant impact on subjective measures. Longer periods of post-exposure sleep might be tested in future studies. Altering the composition of post-exposure sleep by techniques such as sleep deprivation might also be tried. Additionally,

the future studies could modify post-treatment mpTSST tasks. Due to the participants' familiarity with the assessments, i.e. habituation to the social stressor, it is possible that the current study failed to find significant group effects in all variables. Since the study administered the same mpTSST assessment before and after the five-week exposure therapy, it is possible that post-treatment mpTSST psychophysiological reactivity will be lower or no different than pre-treatment mpTSST due to pre-exposure to the mpTSST. Future studies should control for this factor by modifying post-treatment mpTSST assessments and/or addition of a "wait-list" control group to assess the habituation and effectiveness of the exposure therapy. In sum, post-exposure sleep can reduce sympathetic activation to a social stressor from pre- to post- treatment and may augment the therapeutic effectiveness of SAD treatment.

Appendix A

Inclusion/exclusion criteria

a. Inclusion Criteria:

1) A score $\geq$ 60 on the Liebowitz Social Anxiety Scale (LSAS)[13]

2) 18-40 years of age

3) Proficient in English

4) Normal or corrected to normal vision

5) Able to give informed consent

6) Willingness and ability to comply with the requirements of the study protocol

7) Meets Psychophysiological Screening Test criteria (see below)

b. Exclusion Criteria

1) Any potentially confounding medical illness

2) Lifetime history of any neurological illness or injury including neurodegenerative disorders or dementia, stroke, seizure disorders, neurosurgical procedures, head injury resulting in loss of consciousness for greater than 5 min.

3) Lifetime history of bipolar disorder, schizophrenia or other psychotic disorder, pervasive developmental disorder, chronic mental disorder due to a medical condition or other potentially confounding chronic mental disorder.

4) Current major depressive disorder or current suicidal ideation or past hospitalization for suicidality.

5) Substance abuse or dependence within the last year, lifetime history of hospitalization

for substance abuse (determined at clinical interview) or positive urine toxicology screen at the time of the clinical interview

6) Any evidence of suicidal ideation, violent behavior or psychosis at the clinical interview

7) Use of psychiatric medication within 4 weeks of study (with the exception of 6 weeks for fluoxetine)

8) Current psychotherapy for Social Anxiety Disorder

9) Any indication of a sleep disorder, particularly sleep-disordered breathing, on the Pittsburgh Structured Clinical Interview for Sleep Disorders

10) Sleep onset latency > 1 hr, total sleep time < 5 hr or typical bed time later than 3 AM

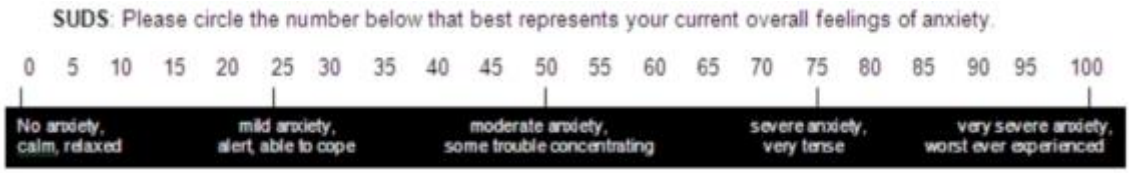
11) Overnight shift work or recent travel across multiple time zones

12) > 4 caffeinated beverages per day or > 11 alcoholic beverages per week

13) Nicotine use

Appendix B

SUDS rating



Appendix C

Liebowitz Social Anxiety Scale

Liebowitz Social Anxiety Scale Liebowitz MR. Social Phobia. Mod Probl Pharmacopsychiatry 1987;22:141-173

Pt Name:		Pt ID #:
Date:	Clinic #:	Assessment point:

Fear or Anxiety:
0 = None
1 = Mild
2 = Moderate
3 = Severe

Avoidance:
0 = Never (0%)
1 = Occasionally (1—33%)
2 = Often (33—67%)
3 = Usually (67—100%)

	Fear or Anxiety	Avoidance	
1. Telephoning in public. (P)			1.
2. Participating in small groups. (P)			2.
3. Eating in public places. (P)			3.
4. Drinking with others in public places. (P)			4.
5. Talking to people in authority. (S)			5.
6. Acting, performing or giving a talk in front of an audience. (P)			6.
7. Going to a party. (S)			7.
8. Working while being observed. (P)			8.
9. Writing while being observed. (P)			9.
10. Calling someone you don't know very well. (S)			10.
11. Talking with people you don't know very well. (S)			11.
12. Meeting strangers. (S)			12.
13. Urinating in a public bathroom. (P)			13.
14. Entering a room when others are already seated. (P)			14.
15. Being the center of attention. (S)			15.
16. Speaking up at a meeting. (P)			16.
17. Taking a test. (P)			17.
18. Expressing a disagreement or disapproval to people you don't know very well. (S)			18.
19. Looking at people you don't know very well in the eyes. (S)			19.
20. Giving a report to a group. (P)			20.
21. Trying to pick up someone. (P)			21.
22. Returning goods to a store. (S)			22.
23. Giving a party. (S)			23.
24. Resisting a high pressure salesperson. (S)			24.

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