Title: Stress, Sex, and Fear
NCT number: 03786952
Document date: 10/7/2019

Study protocol

Participants reported to the PI's laboratory for 1-hr sessions on two consecutive days. On Day 1, informed consent was obtained from participants, after which they completed a short demographics (i.e., age, sex, ethnicity) questionnaire. Participants were then randomly assigned to experience acute stress (socially-evaluated cold pressor test; SECPT) or a control manipulation. Participants assigned to the acute stress condition placed their dominant hand in ice cold (0-2 degrees Celsius) water for up to 3 min. They were also misleadingly informed that they were being videotaped for the analysis of facial expressions and were asked to stare at a camera throughout the manipulation (social evaluative component). Participants assigned to the control condition placed their dominant hand in lukewarm (35-37 degrees Celsius) water for up to 3 min. The water was maintained at the appropriate temperature by a circulating water bath. Participants rated, at 1-min intervals, the painfulness and stressfulness of the stress or control manipulation on scales of 0-10 (0 = lack of pain/stress; 10 = unbearable pain/stress).

Immediately or 30 min later (determined via random assignment), participants underwent a fear-potentiated startle paradigm, in which differential fear conditioning was employed. The conditioned stimuli (CSs) and generalization stimuli (GSs) consisted of nine black circles of increasing diameter, which were presented on a white background of a computer monitor (via SuperLab software; Cedrus Corporation, San Pedro, CA, USA). The monitor was located approximately 124 cm in front of participants. The smallest circle was 8.5 mm in diameter, and each larger circle increased in size by 2.5 mm, ending with the largest circle, which was 29 mm in diameter. For approximately half of the participants, the smallest circle served as the CS+, and the largest circle served as the CS-. For the remaining participants, the largest circle served as the CS+, and the smallest circle served as the CS-. The circles sized between the CS+ and the CS- were the GSs and were only presented to participants on Day 2. The unconditioned stimulus (US) was a 250-ms, 140-p.s.i. airblast aimed at the larynx. The startle probe was a 40-ms, 108-dB burst of white noise that was delivered to participants through a pair of headphones. During CS+ trials on Day 1, the startle probe was presented 6 s after onset of the CS, which was followed 500 ms later by presentation of the US; the CS+ terminated 500 ms after the onset of the US. During all GS and CS- trials (and CS+ trials on Day 2), the startle probe was presented 6 s following the onset of the stimulus, without any presentation of the US; stimulus presentation ended 250 ms after the startle probe. On noise alone (NA) trials, only the startle probe was presented while participants looked at the white background on the computer monitor; the length of NA trials matched the length of the startle probe (i.e., 40 ms).

Fear acquisition on Day 1 began with three NA trials, followed by a habituation phase that included four NA, CS+, and CS- trials. The CS presentations during habituation were not followed by the US. After habituation, participants underwent the conditioning phase, which included three blocks of four trials of each stimulus type (NA, CS+, CS-); this resulted in 12 trials per block and 36 total trials. During conditioning, the CS+ was always paired with the US (i.e., 100% reinforcement rate). On Day 2, participants completed the generalization phase of the paradigm. This phase began with three NA trials, followed by three blocks of one trial of each stimulus type (NA, CS+, seven GSs, CS-) for a total of 10 trials per block and 30 total trials. No CS or GS presentation during the generalization phase was reinforced with the US. Fixed trial orders were used for all participants. The only restrictions were that, during acquisition, there were 4 trials of each trial type (i.e., NA, CS+, CS-) for each block of 12 trials and, during generalization, there was 1 trial of each trial type (i.e., NA, CS+, seven GSs, CS-) for each block of 10 trials. The fixed trial order involved randomizing the trial types within each block. The intertrial interval was random, between 9 and 22 s in duration.

Electromyographic (EMG) recordings of the right orbicularis oculi muscle were used to measure participants' startle responses. Ag/AgCl electrodes (5-mm) that were filled with electrolyte gel were placed 1 cm below the pupil of the right eye, 1 cm below the lateral canthus, and over the mastoid behind the right ear (ground). The impedance levels for each participant were less than 6 kΩ. Electrodermal activity (EDA) was measured by placing Ag/AgCl electrodes on the hypothenar surface of the hand. EMG and EDA activity were sampled at 1000 Hz and amplified and digitized with the appropriate Biopac modules of the Biopac MP150 system (Biopac Systems, Inc., Aero Camino, CA, USA) and its accompanying Acqknowledge data acquisition and analysis software (Biopac Systems, Inc., Aero Camino, CA, USA). The Acqknowledge data files were imported into the MindWare EMG or EDA analysis program (MindWare Technologies, Ltd., Gahanna, OH, USA). This program was used to filter and smooth the EMG and EDA signals. The EMG signal was amplified with a gain of 2000; it was filtered with low and high-frequency cutoffs at 28 and 500 Hz, respectively, and a 60-Hz notch filter was applied. The peak EMG signal 20–200 ms after each startle probe was used as a measure of the startle response. Skin conductance responses to the CSs and GSs were quantified by calculating the average increase (from a 1 s pre-stimulus baseline) 3-6 s after stimulus onset.

Participants used a three-button response keypad (SuperLab software, Cedrus Corporation, San Pedro, CA, USA) to report their expectation of the US during each CS+, GS, and CS- trial on Days 1 and 2. When each stimulus was presented on the computer monitor, participants pressed an “AIR” key if they expected the US to occur, a “NO AIR” key if they did not expect the US to occur, and a “?” key if they were unsure if the US would occur. For data analysis, “AIR” responses were scored as +1, “?” responses were scored as 0, and “NO AIR” responses were scored as -1.

Participants’ heart rate was measured for 1 min prior to and continuously throughout the stress or control manipulation via the PPG module of the Biopac MP150 system.

Saliva samples were collected at several time points on Day 1 (4 samples) and Day 2 (2 samples) to measure salivary hormones [cortisol, alpha-amylase, estradiol (in females), progesterone (in females)]. Saliva samples were collected via the SalivaBio Oral Swab (Salimetrics LLC, State College, PA). The samples stored at -20°C until assayed in duplicate by enzyme immunoassays (EIAs) (Salimetrics LLC). Saliva samples 1 (baseline) and 4 (25 min post-stress) from Day 1 were assayed for cortisol to assess stress-induced changes in hypothalamus-pituitary-adrenal axis activity. Saliva samples 1 (baseline) and 2 (post-generalization test) from Day 2 were assayed for cortisol to control for the influence of salivary cortisol levels on generalization testing. Saliva samples 1 (baseline), 2 (middle, or 1.5 min mark, of stress or control manipulation), and 3 (immediately following stress or control manipulation) from Day 1 were assayed for alpha-amylase to assess rapid stress-induced changes in sympathetic nervous system activity. Saliva samples 1 and 4 (from females) from Day 1 and saliva sample 1 (from females) from Day 2 were assayed for progesterone. The first saliva samples (from females) from Days 1 and 2 were assayed for estradiol.

Following the completion of generalization testing on Day 2, participants completed the Spielberger State-Trait Anxiety Inventory (STAI-Trait) to quantify participants’ state and trait anxiety levels, the Anxiety Sensitivity Index (ASI) to assess participants’ anxiety sensitivities, the PTSD Checklist – Civilian Version to examine symptoms of post-traumatic stress disorder, the Center for Epidemiological Studies Depression Scale (CES-D) to measure symptoms of depression, and the Childhood Trauma Questionnaire (CTQ) to quantify childhood abuse and neglect.

Statistical analyses

To assess time-dependent changes in subjective and objective stress response measures, we performed separate mixed-model ANOVAs on each of the following measures: Day 1 cortisol levels, Day 2 cortisol levels, alpha-amylase levels, heart rate, subjective pain ratings, and subjective stress ratings. Stress, stress time point, and sex were entered as categorical, between-subjects factors. Time point of measurement for each measure was entered as the within-subjects factor in each analysis.

EMG difference scores, skin conductance responses, and US expectancy ratings from acquisition (Day 1) and generalization testing (Day 2) were subjected to separate mixed-model ANOVAs with stress, stress time point, and sex entered as categorical, between-subjects factors. Trial block (4 for acquisition; 3 for generalization) and trial type (acquisition: CS+, CS-; generalization testing: CS+, CS-, 4 generalization stimuli) were entered as within-subjects factors in these analyses.

For all statistical analyses, alpha was set at 0.05. In the case of post hoc testing, alpha was adjusted with Bonferroni corrections so that the family-wise error rate did not exceed 0.05.

A priori power analyses were performed with G*Power 3.1.9.2 (University of Kiel, Germany). The power analyses revealed that we would need approximately 565 participants to retain adequate statistical power ($1-\beta = 0.8$) to detect small-to-moderate effect sizes ($f = 0.145$) for main and interactive effects of all independent variables outlined above.

OHIO NORTHERN UNIVERSITY
CONSENT FOR INVESTIGATIONAL TREATMENT OR PROCEDURE

I, _____, hereby authorize or direct Dr. Phillip R. Zoladz or associates or assistants of his/her choosing, to perform the following treatments or procedures (described in general terms), upon _____.
(myself or name of participant)

The experimental (research) portion of the treatment or procedure is: You will be asked to submerge your dominant hand, up to and including the wrist, into a bath of water and then undergo a classical conditioning task.

This is done as part of an investigation entitled: Environmental influences on classically-conditioned responses

1. Procedures or treatments:

You will be asked to submerge your dominant hand, up to and including the wrist, into a bath of water and then undergo a classical conditioning task. You will be exposed to sudden tones and blasts of air throughout this task. We will measure your reaction by recording your eyeblink. We will do this by washing your cheek below your eye and behind your ear. A citrus-scented abrasive lotion will be used for this portion of the experiment, **so if you have any allergies or negative reactions to citrus-scented skincare products, please advise the experimenter**. A sticky tape will be used to attach two small electrodes (tiny metal discs with wires attached to them) to your face below your eye; a small amount of electrode gel will be applied to the electrodes to enhance conductivity. Throughout this procedure, your cardiovascular activity will be measured, the activity of your sweat glands will be monitored, and you will be asked to provide saliva samples for hormone analysis. You will be asked to return to the laboratory the day after the first experimental session to complete additional classical conditioning assessments. You will also complete questionnaires throughout each session to measure your mood. **You may be contacted via email following participation based on your responses to these questionnaires.**

Following completion of the two experimental sessions, you will be awarded 2 research experience credits and $20 cash. All data collected from this study will remain confidential, and your name will not be combined with your data.

2. Anticipated duration of participant's participation:

As a participant, you will complete two experimental sessions, separated by one day. Each session will last no longer than 1 hour.

3. Possible appropriate alternatives procedures or treatment (not to participate in the study is always an option):

The alternative is to not participate.

4. Discomforts and risks reasonably to be expected:

You are reminded that to participate in the present experiment, you should not meet any of the following conditions: diagnosis of Raynaud's disease or peripheral vascular disease; diagnosis of post-traumatic stress disorder (PTSD); presence of skin diseases such as severe psoriasis, eczema, or scleroderma; history of syncope (passing out) or vasovagal response to stress; history of any heart conditions or cardiovascular issues (e.g., high blood pressure); history of severe head trauma; current treatment with narcotics, beta-blockers, or steroids; pregnancy; substance use disorder; regular use of recreational drugs; regular nightshift work; and, hearing loss. In addition, you have been asked to refrain from drinking alcohol and to limit strenuous exercise for 24 hours prior to participation. You should not have eaten or drank anything but water for 2 hours prior to participation.

During some of the experimental manipulations, you might feel stressed or aroused. This is normal.

The citrus-scented abrasive lotion that is used to facilitate electrode conductance may irritate your skin. If you become uncomfortable with the cleansing process, you may notify the experimenter, and the electrodes

will be placed on your skin without further skin cleansing. If you have any allergies or negative reactions to citrus-scented skincare products, please advise the experimenter, and the electrodes will be placed on your skin without prior cleansing with the skincare product.

Importantly, if you feel uncomfortable and do not want to continue with any procedure, you are free to withdraw from the experiment without any penalty to you. Simply notify the experimenter.

5. Possible benefits to participants/society:

Participants will be paid $20 cash for participating and will be awarded 2 research experience credits that can be used for various courses at ONU. The research we are conducting may facilitate understanding factors that influence the development / maintenance of trauma-, stressor-, and anxiety-related psychological disorders.

A synopsis of this study and its aggregate, de-identified results will be posted at ClinicalTrials.gov within 12 months of study completion. De-identified information means that all personal information about research participants such as name, address, and phone number is removed and replaced with a code number. The results that are shared at this web site will inform other researchers who are conducting clinical trials about the results from the present study and facilitate future research directed at answering similar research questions.

Data from this study will also be submitted to the National Institute of Mental Health Data Archive (NDA). NDA is a data repository run by the National Institute of Mental Health (NIMH) that allows researchers studying mental illness to collect and share de-identified information with each other. A data repository is a large database where information from many studies is stored and managed. With an easier way to share, researchers hope to learn new and important things about mental illnesses more quickly than before. During and after the study, the researchers send de-identified information about your health and behavior and in some cases, your genetic information, to NDA. Other researchers nationwide can then file an application with the NIMH to obtain access to your de-identified study data for research purposes. Experts at NIMH who know how to protect health and science information will look at every request carefully to minimize risks to your privacy. You may not benefit directly from allowing your information to be shared with NDA. The information provided to NDA may help researchers around the world treat future children and adults with mental illnesses so that they have better outcomes. NIMH will also report to Congress and on its web site about the different studies that researchers are conducting using NDA data. However, you will not be contacted directly about the data you contributed to NDA. You may decide now or later that you do not want to share your information using NDA. If so, contact the researchers who conducted this study, and they will tell NDA, which can stop sharing the research information. However, NDA cannot take back information that was shared before you changed your mind. If you would like more information about NDA, this is available on-line at http://data-archive.nimh.gov.

I hereby acknowledge that I have been provided information about the procedures described above, about my rights as a subject and that all my questions have been answered to my satisfaction. If I have any further questions or concerns, I may contact XXX at XXX. If I wish to speak with someone not part of the research team, I may contact XXX (IRB member) at XXX. All of the risks described above have been fully explained to me, and I understand them. I understand that no compensation is available from Ohio Northern University and its employees for any injury resulting from my participation in this research.

I understand that records obtained during my participation in this study that may contain my name or other personal identifiers may be made available to the principal investigator(s) and his/her associates or assistants. Beyond this, I understand that my data will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time after notifying the principal investigator without penalty or loss of benefits to which I am otherwise entitled.

I understand in signing this form that, beyond giving consent, I am not waiving any legal rights that I might otherwise have, and I am not releasing the investigator, the institution or its agents from any legal liability for damages that they might otherwise have.

In the unlikely event of injury resulting in participation in this study, immediate medical treatment is available at the Ohio Northern University Health Center. The costs of such treatment will be at my expense; financial compensation beyond that required by law is not available.

I have read and fully understand the consent form. I sign freely and voluntarily. A copy of this consent form has been given to me.

Signed: _____ Date: _____

I certify that I have personally completed all blanks in this form and explained them to the subject or his/her representative before requesting the subject or his/her representative to sign the consent form.

Signed: _____ Date: _____