Cover Page: Study Protocol

Official Title of Study: Stress and the Sympathetic Nervous System in Adults with Depression

NCT04838262

Document Date: Study Protocol Document (PRS Review 05/22/2022); IRB Approval Letter + Protocol (05/23/2023)

ClinicalTrials.gov PRS
Protocol Registration and Results System

ID: MH123928 Stress and the Sympathetic Nervous System in Adults With Depression NCT04838262

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Protocol Section

Study Identification

Unique Protocol ID: MH123928

Brief Title: Stress and the Sympathetic Nervous System in Adults With Depression

Official Title: Daily Stress Processes and Sympathetic Reactivity in Depression

Secondary IDs:

Study Status

Record Verification: April 2022

Overall Status: Recruiting

Study Start: May 1, 2021 [Actual]

Primary Completion: March 2023 [Anticipated]

Study Completion: March 2023 [Anticipated]

Sponsor/Collaborators

Sponsor: The University of Texas at Arlington

Responsible Party: Sponsor

Collaborators: Penn State University

Oversight

U.S. FDA-regulated Drug: No

U.S. FDA-regulated Device: No

U.S. FDA IND/IDE: No

Human Subjects Review: Board Status: Approved Approval Number: 2019-0266
Board Name: Institutional Review Board

Board Affiliation: The University of Texas at Arlington
Phone: 817-272-1234 Email: kmorning@uta.edu
Address:
Center for Innovation, Suite 300, Box 19188
Arlington, TX 76010

Data Monitoring: No

FDA Regulated Intervention: No

Study Description

Brief Summary:

To test our hypotheses, we will enroll healthy adults having no history of mood disorders and adults with major depressive disorder (MDD) having a broad range of depressive symptom severity. After screening, subjects will meet with the research coordinator or an investigator for a discussion, with opportunity for questions, before applicable consent forms are obtained. Daily stress processes will be assessed using an ecological momentary assessment approach for 8 consecutive days. On the last day of the daily stress assessment, we will directly measure muscle sympathetic nerve activity, blood pressure, and heart rate during acute laboratory-based cognitive, emotional, and physiological interventions to induce a stress response. A venous blood sample will be taken for measurements of metabolic and renal health and systemic inflammation.

Aim 1: To examine the effect of daily psychosocial stressor exposure on acute sympathetic stress reactivity in MDD. Two stressor exposure indicators will be calculated: stressor frequency (i.e., percentage of interview days during which at least one stressor occurred) and total stress (i.e., total number of stressors reported across all interview days) and will be related to the magnitude of responsiveness to the acute stress interventions. We hypothesize that the slope of this relation will be steeper in adults with MDD compared to healthy non-depressed adults.

Aim 2: To determine the relation between negative affective reactivity to daily psychosocial stressor exposure and acute sympathetic stress reactivity in MDD. Negative affective reactivity will be calculated as the change in affect on days when stressors occurred compared to one's typical affect on non-stressor days and will be related to the magnitude of responsiveness to the acute stress interventions. We hypothesize that the slope of this relation will be steeper in adults with MDD compared to healthy non-depressed adults.

Detailed Description:

Conditions

Conditions: Major Depressive Disorder

Keywords:

Study Design

Study Type: Interventional
Primary Purpose: Basic Science
Study Phase: N/A
Interventional Study Model: Single Group Assignment
Number of Arms: 1
Masking: None (Open Label)
Allocation: N/A
Enrollment: 100 [Anticipated]

Arms and Interventions

Arms	Assigned Interventions
Experimental: Assessment of Daily Stress Processes Subjects will report cumulative exposure, perceived severity, and emotional responsiveness to commonly occurring everyday psychosocial stressors utilizing an ecological momentary assessment approach for 8 consecutive days.	Acute Stressors Sympathetic nervous system activity and blood pressure will be measured before, during, and after several acute laboratory-based cognitive (Stroop Color Word Test), emotional (International Affective Picture System), and physiological (Cold Pressor Test) stressors.

Outcome Measures

Primary Outcome Measure:

1. total number of daily stressors
   [Time Frame: 8 days before intervention]
2. change in negative affect in response to daily stressors
   [Time Frame: 8 days before intervention]
3. change in muscle sympathetic nerve activity in response to acute stress (compared to resting baseline activity)
   [Time Frame: during laboratory-based intervention (3 minutes)]

Eligibility

Minimum Age: 18 Years
Maximum Age: 30 Years
Sex: All
Gender Based:
Accepts Healthy Volunteers: Yes

Criteria:

Inclusion Criteria:

- All participants will be 18-30 yrs.
- Healthy non-depressed men and women will have no history or evidence of psychiatric illness and will not have a family history of MDD or major psychiatric illness.
- Men and women with MDD will have symptomatic depression that meets diagnostic criteria and will be non-medicated.
- The capacity and willingness to provide written informed consent, to attend all study related visits, and to comply with the study protocol.

Exclusion Criteria:

Subjects will be excluded at the discretion of the PI/collaborating clinician or for any of the following reasons:

- psychiatric illness aside from MDD (including current or past psychotic disorders, bipolar disorder, schizophrenia or schizoaffective disorder, panic disorder, post-traumatic stress disorder, obsessive compulsive disorder)
- subthreshold depression
- current use of psychotropic medications (including major classes of antidepressants, anxiolytics, antipsychotics, mood stabilizers)
- active suicidal or homicidal ideation
- active substance dependence or eating disorders
- current use of any medications that could alter sympathetic reactivity
- diagnosed or suspected cardiovascular, renal, or metabolic disease (hypertension, heart disease, diabetes, hyperlipidemia)
- autonomic disorders
- tobacco use (including electronic cigarettes)
- obesity (body mass index > 30 kg/m2)
- breastfeeding or pregnancy
- <18 or >30 yrs

Contacts/Locations

Central Contact Person: Jody Greaney, PhD
Telephone: 817-272-7891
Email: jody.greaney@uta.edu

Central Contact Backup:

Study Officials:

Locations:

United States, Texas

The University of Texas at Arlington Recruiting
Arlington, Texas, United States, 76010
Contact: Jody Greaney, PhD 817-272-7891
jody.greaney@uta.edu
Principal Investigator: Jody Greaney, PhD

IPD Sharing Statement

Plan to Share IPD: Yes

Per the data sharing policy of the National Institute of Mental Health (NIMH), we will deposit all phenotypic, psychosocial stress, and sympathetic reactivity data from this study to the NIMH Data Archive. We will use standard data dictionaries (NIH Toolbox Cognition Battery, NIH Toolbox Emotion Battery, PHQ-9) and will collect the data necessary to generate global unique identifiers for each study subject.

Supporting Information:

Time Frame:

Access Criteria:

URL:

References

Citations:

Links:

Available IPD/Information:

Document Section

[No PDF/A documents uploaded.]

5/12/2023

IRB Approval of Full Board Continuing Review + Modification

PI: Jody Greaney
Department: Kinesiology
IRB Protocol #: 2019-0266.13
Study Title: *Stress Reactivity in Human Depression*

Effective Approval: 5/12/2023

Protocol Details

- Original Protocol Approval Date: 6/28/2019
- Protocol Expiration Date: 5/14/2024
- Federally Funded: NIH, Mentis BlueSheet #2020-825
    - Subject to 45 CFR 46, Revised 2018
- FDA Regulated: IND
    - Subject to FDA Regulations 21 CFR parts 5, 56, 312, and 812
- Continuing Review required: Yes, standard

This continuing review and modification was reviewed and approved with conditions at the convened IRB full board meeting on 05/09/2023; the status is continuing for one year. Conditions for approval were met on 05/11/2023. The approved modifications are limited to:

- Add protocol personnel: Tracy Greer, Cassie Argenbright
- Remove protocol personnel: Brandi Stephens, Ben Young, Damsara Nandadeva, Zachary Martin, Rachel Skow, Madeleine Harris, Jose Coello, and Aleema Haq.

Principal Investigator and Faculty Advisor Responsibilities

All personnel conducting human subject research must comply with UTA's IRB Standard Operating Procedures and RA-PO4, Statement of Principles and Policies Regarding Human Subjects in Research. Important items for PIs and Faculty Advisors are as follows:

- **Notify Regulatory Services of proposed, new, or changing funding source**
- Fulfill research oversight responsibilities, IV.F and IV.G.
- Obtain approval prior to initiating changes in research or personnel, IX.B.
- Report Serious Adverse Events (SAEs) and Unanticipated Problems (UPs), IX.C.
- Fulfill Continuing Review requirements, if applicable, IX.A.
- Protect human subject data (XV.) and maintain records (XXI.C.).
- Maintain HSP (3 years), GCP (3 years), and RCR (4 years) training as applicable.

UNIVERSITY OF TEXAS ARLINGTON

INSTITUTIONAL REVIEW BOARD (IRB) FOR THE PROTECTION OF HUMAN SUBJECTS
APPLICATION FOR RESEARCH INVOLVING HUMAN SUBJECTS

Faculty, staff, or students who propose to engage in any research, research development, testing or evaluation with human subjects must have review and approval from the IRB prior to initiation. Some activities involving humans are not considered human subject research requiring IRB review (i.e., class projects, program evaluation, oral histories, quality improvement). Refer to the Research Project Chart for more information.

Utilize the **IRB Submission Checklist** to guide you through the full IRB application process. **NOTE**: **All study personnel must have completed Human Subjects Protection (HSP) Training prior to study approval. HSP Training expires and must be retaken every 3 years.**

*If you require assistance to complete this form or need additional information, please contact Regulatory Services at 817-272-3723 or regulatoryservices@uta.edu. Regulatory Services also has open office hours every Thursday from 9:00 – 11:00am. The **UTA IRB Website** also has lots of helpful guidance– check it out!*

SECTION A: GENERAL INFORMATION

1. **Non-UTA Personnel:** *Enter all individuals that are **NOT affiliated with UTA** who will interact or intervene with human subjects for the research study OR who will access identifiable subject data. **UTA-affiliated** personnel should be listed on the electronic portion of the protocol (#3) in the electronic submission system.*

   ***Note:** In the electronic submission system, upload a completed Non-UTA Collaborator Form and Human Subject Protection training for each listed Non-UTA individual.*

Name:	Organization:
Bau Tran, PA-C, PharmD	UT Southwestern Medical Center
Jacqueline Mogle, PhD	The Pennsylvania State University

2. **Expected Start Date and Completion Date:** May 2019/Dec 2021 *(You are not authorized to start any research on human subjects including subject recruitment until the IRB has approved the research protocol.)*

3. **Funding:** *Indicate existing, potential, or pending sources of funding below (you may select more than one).*
   ***Note: If you do (or may) receive funding from NSF, NIH, CMMS, DOD, DOJ, DOE, DOEd, DOT, or any other federal agency, you MUST disclose this funding source below to ensure that your study is reviewed in accordance with the appropriate federal regulations for that specific federal funding source.**

   External:

   ☒ **Federal (Sponsor:**NIH**)** ☐ **State (Sponsor:)** ☐ **Industry (Specify Sponsor:)**

   Grants & Contracts Bluesheet Number from Mentis: 126602571 and 126602810

   Other:

   ☐ **UTA Department Account** ☐ **Personal Funds** ☒ **Other:** start-up ☐ **None (*No funding*)**

SECTION B: RESEARCH CLASSIFICATION, RATIONALE, PROCEDURES, SITES, QUALIFICATIONS, OVERSIGHT

4. **Research Classification:** *Indicate if this study is categorized as **Minimal Risk (MR)** or **Greater than Minimal Risk (GMR).** "Minimal Risk (MR)" means that the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in the subjects' daily life or during the performance of routine physical or psychological examinations or tests. "Greater than Minimal Risk (GMR)" refers to research activities that do not meet the definition of "Minimal*

Risk." *Throughout this application form, there are additional questions or information requested for studies categorized as GMR; these instructions will be presented in purple.*

☐ **Minimal Risk (MR)** ☒ **Greater than Minimal Risk (GMR)**

***Note:** Studies that are federally funded and/or FDA regulated will be further classified into exempt, expedited, or full board in accordance with the Common Rule 45 CFR 46 and/or 21 CFR parts 50 and 56. See **Flowchart**.*

5. Rationale: *List the primary research questions, hypotheses, and / or objectives guiding this study.*

***Note*: This is a companion project to "Central mechanisms of neurovascular dysfunction in human depression" (IRB #2019-0127). The PI currently holds a FDA IND (125,994) for the procedures and drugs used in these studies; this IND is being modified for use in the current study.**

The proposed studies are complementary to those outlined in the PI's funded NIH K99/R00 grant and, although not formally listed in the specific aims in the grant, these studies are described in the research strategy as alternative approaches to generate pilot data for future grant submissions.

Rationale:
Major Depressive Disorder (MDD) manifests in >15% of the population and is directly linked to the development of cardiovascular disease (CVD), independent of traditional cardiovascular risk factors. Importantly, CVD risk remains elevated even with the remission of depressive symptoms. Although multiple factors likely contribute, accumulating evidence indicates that vascular dysfunction plays a pathogenic role in depression-CVD comorbidity.

Exposure to psychological stress plays a critical role in the etiology of MDD and is itself associated with endothelial dysfunction, accelerated atherosclerosis progression, and increased CVD risk. Despite this clear epidemiological association, no studies have examined the relation between everyday psychosocial stressors and direct measures of neurovascular reactivity to acute stress in adults with MDD. Exposure to acute stressors includes activation of the autonomic nervous system, which can induce transient vascular dysfunction and exaggerated increases in blood pressure. Collectively, chronic and repetitive exposure to psychosocial stress, or a maladaptive response to repeated stressors (i.e., exaggerated stress reactivity), may accelerate the atherosclerotic process and precipitate the development of CVD.

In the laboratory, we propose to conduct a comprehensive examination of cardiovascular stress reactivity. We will then relate cardiovascular reactivity to daily psychosocial stressors in adults with MDD. Our **central hypothesis** is that adults with MDD will demonstrate altered sympathetic neural control of cardiovascular function and this impairment will be modulated by exposure to everyday psychosocial stressors; the resultant imbalance in vasodilator and vasoconstrictor regulation serves as a potential mechanistic link between daily stress and depression in increased CVD risk. Although the effects of daily stressors on activation of the hypothalamic-pituitary-adrenal axis are well-established, particularly in MDD, our proposed series of studies will test the novel concept that naturally-occurring daily stressors also directly influence sympathetic neural reactivity of the cardiovascular system.

Specific Aim 1: To examine microvascular stress reactivity in adults with MDD.
Hyp 1: Microvascular adrenergic sensitivity will be greater in adults with MDD compared to healthy non-depressed adults, due to a reduction in the buffering capacity of β-adrenergic receptor-mediated vasodilation to offset α-adrenergic receptor-mediated vasoconstriction.
Hyp 2: Adults with MDD exposed to everyday psychosocial stress will exhibit more severe alterations in the mechanistic control of microvascular adrenergic reactivity.

Specific Aim 2: To examine cardiovascular stress reactivity in adults with MDD.
Hyp 1: Blood pressure reactivity will be greater in adults with MDD compared to healthy non-depressed adults, due to exaggerated increases in sympathetic outflow and augmented vascular resistance.
Hyp 2: Adults with MDD exposed to everyday psychosocial stress will exhibit more severe alterations in sympathetic control of blood pressure during acute cardiovascular stressors.

Preliminary Results:
The PI has extensive experience assessing the mechanisms and modifiers of cardiovascular function in a variety of pre-clinical populations (e.g., primary aging, hypertension, psoriasis, MDD). Specific to MDD, the PI recently demonstrated that acute exposure (within 1 day) to any naturally occurring everyday psychosocial stressor was associated with greater impairments in microvascular endothelial function in treatment-naïve young otherwise healthy adults with MDD (Greaney JL *et al J Am Heart Assoc*, 2019). Further, our exciting preliminary data suggests that psychosocial stress is related to greater norepinephrine-induced vasoconstriction, the mechanistic control of which appears to be altered in adults with MDD.

Rationale for Addition of Accelerometry:
Indirect evidence suggests that **habitual physical activity (PA)** increases positive affect, decreases negative affect, and decreases overall perceived stress, all of which improve overall mood. Because PA is a powerful lifestyle intervention to reduce the psychobiological effects of stress, whether habitual PA also blunts NA-R to daily stress is a clinically relevant research question. Importantly, reducing NA-R has potential to reduce not only the psychological but also the cardiovascular consequences of daily stress. Also, increased sedentary time is now increasingly recognized for its negative implications for CVD risk, independent of habitual PA. Specifically, increased sedentary time is associated with greater 24-hour BP, vascular endothelial dysfunction, and CVD risk. Therefore, it is important to understand, separately, whether sedentary time sensitizes the relation between NA-R and BP reactivity. We do not have any specific aims/hypotheses related to sleep duration/heath, but these metrics are provided by the accelerometers.

6. Procedures: *Describe the procedures step-by-step, including details on all methods that will be used to collect human subject data from the beginning to the end of the study. Describe what data will be collected (and if it will be individually identifiable); when and where the data will be collected; and how it will be collected (instruments or other measures). Use clear, concise layman's language that can be easily understood by persons outside your field and provide definitions for any technical terms. Add pictures if needed.* ****Note: Refer to the Types of Research guidance page for a list of specific information required for different types of research.*** *For GMR research, it is also helpful to provide references or pilot data to support the proposed procedures.*

Note: This study does not meet the definition of a Clinical Trial, because no human subjects are "prospectively assigned to an intervention." Rather, subjects are classified according to defined parameters based on their psychiatric profile and all subjects undergo the same experimental tests. Please see the confirmation email from the NIH Program Officer regarding its Clinical Trial designation. **The PI currently holds a FDA IND (125,994) for the procedures and drugs used in these studies; this IND is being modified for use in the current study.**

This is a cross-sectional study. Two subject groups will be recruited: healthy control (HC) subjects will not have a family history of MDD or major psychiatric illness, and MDD patients will have clinically significant depression (non-medicated).

Please see below for a detailed description of all of the techniques and measurements to be utilized and/or obtained, as well as a complete description of the experimental protocol. Participants complete a screening/familiarization visit (~2 hours), an assessment of daily stress processes (~2 hours in total over the course of 8 consecutive days), and one experimental visit (~4 hours) to assess stress reactivity.

Pre-Screening:
Interested people may contact us. The script of the study information (see attached document labeled "stress recruitment script") will be provided verbally (if initial contact is by phone) or in writing (if initial contact is by email). We conduct a basic telephone or electronic interview and health history [age, height/weight, medications taken regularly, health history (e.g., any known diseases), history of tobacco use, etc.] with the potential subjects and discuss the study with them (see attached document labeled "recruitment interview form"). The pre-screening information is provided by the subject either verbally over the phone or electronically via Qualtrics.

In addition, during initial contact, subjects perform the PROMIS Emotional Distress-Depression Short Form (see attached document labeled "PROMIS"). This scale can be administered over the phone or electronically via Qualtrics. A raw PROMIS score > 18 is indicative of mild depressive symptoms. The PROMIS is used to "pre-screen" adults for depressive symptoms and thus limits the burden of scheduling and conducting screening visits in patients who are ultimately not likely to meet all eligibility criteria. Given the relative ease of recruiting young non-depressed adults into our research study, conducting the PROMIS prior to the on-site screening visit allows us to further target the patient population of interest. *These pre-screening interviews and questions present no more than minimal risk of harm to subjects and involve no procedures for which written consent is normally required outside of the research context.*

If, after completion of this initial pre-screening interview, the subject meets study eligibility and is interested in enrolling, we will schedule a screening visit (see below for details). If enrolled, the PROMIS form will be coded with subject number. Pre-screening 'failures' will be tracked for date of contact and reason for ineligibility; however, if not enrolled, all pre-screening information is de-identified and destroyed.

Screening/Familiarization Visit (see attached document labeled "screening protocol"):

In the event that a subject does not meet the inclusion criteria following the screening visit, we suggest that the follow-up with a physician or mental healthcare provider (see attached document labeled "script study exclusion").

1. The participant signs the informed consent before screening procedures begin. After the participant signs the consent, we provide the participant a photocopy.
2. Participants complete a standard health history form (see attached document labeled "medical history form").
3. The investigator measures height (stadiometer), weight (digital scale), waist circumference (tape measure), blood pressure (mercury sphygmomanometer), heart rate (Welch Allyn Connex), and temperature (Welch Allyn SureTemp Plus).
4. Premenopausal women will provide a urine sample for a pregnancy test.
5. Dr. Greaney or a trained investigator administers the Mini International Neuropsychiatric Interview (MINI) to determine depression status (see attached). The MINI has 16 sections and takes ~20 minutes to complete.
    - If a participant indicates an illness other depression, we give the participants a copy of the "Local Mental Health Providers" document (see attached document labeled "mental health providers"), strongly encourage them to follow-up with a clinician, and exclude the participant from the study.
    - If a participant indicates a "high" level of suicidality (coded as scoring ≥17 on the Suicidality Module) and immediate risk with intent to harm, MHMR Tarrant County Crisis Relief will be called for an external evaluation and study personnel will ensure the participant's safe transfer to MHMR personnel or facility.
6. Participants are familiarized with, and practice, the experimental measurements/procedures described in detail below.

If, after completion of the Screening Visit, the subject meets study eligibility and is interested in continuing with enrollment, we will schedule the experimental visits (see below for details). Screening 'failures' will be tracked for date of screening and reason for ineligibility; however, if not enrolled in the experimental visits, all screening information, data, and blood samples are de-identified and destroyed.

In order to provide the most flexibility to participants in terms of time constraints regarding scheduling, the following procedures may occur at either the screening visit or the experimental visits:

1. An approved investigator or research nurse performs standard venipuncture to obtain a blood sample (100 ml/~6.7 tbsp) for complete blood count, chemistry analysis, lipid profile, and other substances of interest (NOx metabolites, inflammatory cytokines, indices of oxidative stress, peripheral blood mononuclear cells). The researchers do not perform genetic analyses on the blood nor do they look for the presence of disease (e.g., HIV). The blood draw may occur at either the screening visit or the experimental visit.
    - The lipid and basal metabolic panel will be analyzed by Labcorp Inc., the same facility that processes blood samples from Student Health Center at UTA. Each blood sample container is labeled with two identifiers, the subject initials+study number, and the subject's date of birth, as required by Labcorp (e.g., Subject: ABC 01234, DOB 10/14/65) and placed in a locked drop-box. Labcorp is contacted and a courier retrieves the sample from the drop-box on the 1st floor of the Science and Engineering Innovation and Research building (SEIR) the same day. In case the subjects present any abnormal lab results from any of the bloodwork, we will inform them about their results and suggest to schedule an appointment with their primary-care physician. No medical diagnosis will be provided.

- Blood will also be stored in a -80 degree freezer (in the corridor space designated for freezers and refrigerators) in the 1st floor clinical space of the Science and Engineering Innovation and Research building (SEIR) in order to allow samples for certain assays to be run all at one time and avoid variability. The code-key will be kept in the PI's lab in a locked cabinet. Only PI and study personnel on this IRB will have access to the files (via key). No other students or faculty will have access. All stored data will be de-identified using a coding system and frozen until analysis. After successful analysis, blood will be discarded into a container labelled with a biohazard sign and stored in secondary waste containers.

2. An investigator administers a cognitive function assessment on an iPad using select measures of the Cambridge Neuropsychological Test Automated Battery (CANTAB). This assessment takes ~45 minutes. The CANTAB tests include: Rapid Visual Information Processing (RVP) to assess sustained attention; Delayed Matching to Sample (DMS) and Match to Sample Visual Search (MTS) to assess visual memory; Spatial Working Memory (SWM) to assess working memory; One Touch Stockings of Cambridge (OTS) to assess executive function; Emotion Recognition Task (ERT) and Emotion Bias Test (EBT) to assess emotion processing; and the Cambridge Gambling Task (CGT) to assess reward-based decision making. CANTAB is language-independent, culturally neutral, non-invasive and require no technical knowledge or prior familiarity with computers making them suitable for large, multi-site studies and diverse participant groups. This assessment may be administered at either the screening visit or the experimental visit.
3. An investigator administers an emotional state assessment on an iPad using the NIH Toolbox Emotion Measures Battery. This assessment takes ~10 minutes. It entails survey-style questions for the assessment of positive affect, general life satisfaction, emotional support, friendship, loneliness, perceived rejection, perceived hostility, self-efficacy, sadness, perceived stress, fear, and anger. Participants are asked to evaluate each question along a continuum from "strongly disagree" to "strongly agree" or from "not at all" to "very much" or from "extremely untrue of me" to "extremely true of me" etc. This assessment may be administered at either the screening visit or the experimental visit.
4. Participants undergo 24-hour ambulatory blood pressure monitoring (Oscar 2; SunTech Medical). We place a blood pressure cuff around the non-dominant upper arm. We plug the cuff into the control unit, which attaches to a strap placed around your waist or hung on your shoulder. The monitor makes a blood pressure measurement 3 times per hour while the participant is awake and 1 time per hour while he is asleep. To take a measurement, the cuff inflates, records blood pressure, and deflates, in the same manner as other automated brachial blood pressure devices used in this study. The subject will receive specific verbal and written instructions for the use of the ambulatory blood pressure monitor (see document labeled "ABPM instructions"). The participant will be instructed to return the unit to the investigators at the experimental visit.
5. Flow-mediated dilation (FMD) to assess peripheral vascular function. During this procedure:
    - We place a small cuff around the forearm, just below the elbow.
    - Above the elbow, we use a Doppler ultrasound probe covered in ultrasound gel to image the brachial artery. The ultrasound measures blood vessel size and blood velocity.
    - After a 3-minute resting measurement, we tightly inflate the cuff for 5 minutes to occlude blood flow to the forearm.
    - After the cuff deflates, we continue to image the artery for ~5 minutes. We may repeat this measure several times.
6. Pulse wave analysis (PWA) and pulse wave velocity (PWV) to assess arterial stiffness. To measure PWA, a standard blood pressure cuff is placed on the subject's upper arm. The cuff will inflate/deflate to determine brachial artery blood pressure; after 5 seconds, the cuff will inflate again and automatically capture the PWA waveform. To measure PWV, a blood pressure cuff is placed on the subject's upper leg. A pen-like probe (applanation tonometer) will be placed against the skin over the carotid artery to continuously measure the pulse wave while the blood pressure cuff on the upper leg is inflated and then deflated.

Assessment of Daily Stressor Processes

1. Participants will complete the Daily Inventory of Stressful Events (DISE; see attached document) survey once per day, for 8 consecutive days. The DISE survey will be completed using online software (Qualtrics; accessed via institutional contract), which significantly minimizes participant burden. The DISE survey can be completed on any device with internet connectivity (smartphone, tablet, laptop, desktop computer, etc.).
2. This survey takes ~10-15 minutes to complete per day and consists of ~100 questions. The main types of information that will be obtained using the DISE include questions about the types of daily stressors (e.g., argument with a friend, work/school deadlines, malfunctioning computer), how the stressor made you feel

(e.g., angry, nervous), feelings of psychological distress (e.g., anxiety, irritability), and daily physical symptoms. Participants will also be asked questions about stress related to the COVID-19 pandemic. Participants are free to skip any questions that they prefer not to answer.

3. Participants are familiarized with the organization, flow, and types of questions in the DISE prior to participation.
4. A laboratory team member will send the participant both a text message and an email every day for the 8 consecutive days of the assessment, and these reminder texts/emails will contain information on how to access the DISE survey.
5. The DISE survey will be scheduled such that on Day 8 (i.e., the last day of the daily stressor assessment), the participant will return to the lab for one of the experimental visits (described below). The second experimental visit will be scheduled on Day 9 (i.e., one day after the last day of the daily stressor assessment).

Assessment of Daily Physical Activity and Sleep

1. 24-hour movement behaviors will be assessed using an accelerometer. This assessment will occur during the same sampling timeframe as the Assessment of Daily Stressor Processes described above. Participants will be asked to wear an accelerometer on the wrist of their non-dominant hand or hip for 24 hours a day except during aquatic activities, and to keep wearing it for 8 consecutive days. The accelerometer will be numbered to each participant with his/her age, gender, height, and weight information uploaded in advance. The participant will receive specific verbal and written instructions for the use of the accelerometer (See "Actigraph instructions").
2. Participants will return the accelerometer to the lab when they report for the Experimental Visit to Assess Cardiovascular Stress Reactivity (described below; this occurs on Day 8 of the DISE). The data captured by the accelerometer will be downloaded immediately on a password-secured computer in the Greaney lab. No GPS data can be collected in this assessment.
3. The monitor will be cleaned between uses according to manufacturer recommendations.

Experimental Visit to Assess Cardiovascular Stress Reactivity:

1. Vital signs (oral temperature, heart rate, and blood pressure) are measure upon arrival to the lab. Women who are premenopausal submit urine for a pregnancy test if they have not been tested within 2 weeks prior to the experiment.
2. Participants complete the Patient Health Questionnaire (PHQ9) to assess depressive symptom severity. The PHQ9 has 9 questions (see attached document labeled "PHQ-9").
3. Participants complete the International Physical Activity Questionnaire (IPAQ; see attached document labeled "IPAQ long-form") to assess habitual physical activity.
4. Participants complete the Daily Inventory of Stressful Events, an interview-based approach to the measurement of multiple aspects of daily stressors (see attached document labeled "daily stress survey").
5. Heart Rate (electrocardiogram, ECG): We tape 3-5 ECG leads to the chest to measure heart rate throughout the experiment (BioAmp, AD Instruments).
6. Respiration: We place an elastic band (Pneumotrace II, UFI) around the abdomen to measure the rate and depth of breathing throughout the experiment.
7. Blood Pressure: A blood pressure cuff will be wrapped around the upper arm to obtain blood pressure via a standard automated oscillometric device (BP Monitor, Welch-Allyn). In addition, beat-by-beat blood pressure will be obtained via finger photoplethysmography (Finger BP Monitor, Finapres). Blood pressure is measured continuously throughout the experiment.
8. Sympathetic Nerve Activity (SNA): We measure SNA throughout the experiment using a technique called microneurography (Nerve Traffic Analyzer, University of Iowa/AD Instruments).
    - First, we apply a mild external electrical stimulus to the lower leg or upper arm using a pencil-like device to find the approximate location of the nerve beneath the skin. The nerve from which activity is recorded is located close to the skin's surface on the lower leg just below the knee or on the upper arm near the elbow. When the nerve is stimulated, involuntary twitching and/or tingling sensations of the foot or hand will occur. The twitching or tingling will disappear when the stimulation is stopped.
    - After finding the general location of the nerve, two tiny, sterile, microelectrodes will be inserted through the skin. One is a reference electrode placed just above the nerve site (2 cm) and the other is the recording electrode. The recording electrode is advanced into the nerve.

- When the tip of the electrode enters the nerve, the subject may briefly notice either pressure or tingling sensations. At this point, minor adjustments in the position of the electrode will be made until an optimal nerve signal is obtained.
- If necessary, SNA measurements conducted within 3 weeks of each other will be done on alternate legs or arms. This will in no way impact the subject, and there are no additional risks associated with alternating legs or arms. If the same leg or arm is used, experimental visits will be separated by at least 4 weeks.

9. Peripheral Blood Flow: Blood flow will be determined by using pulsed Doppler ultrasound to non-invasively measure mean arterial blood velocity and diameter. Peripheral blood flow (e.g., femoral artery, brachial artery) and central blood flow (e.g., carotid artery) can be obtained by placing a Doppler flow probe on the surface of skin over the respective artery. Ultrasound imaging of artery diameter will be performed at a site matching that at which velocity is measured. The following formula is used to calculate blood flow: blood flow = π * (radius^2) * velocity. For these measurements, when requested by the participants for "comfort" purposes, a lab member of their gender preference will be present to conduct these measurements during the protocol.
10. Cardiovascular Reactivity is measured during a variety of perturbations that elicit transient increases in blood pressure and SNA. We continuously record blood pressure, heart rate, and muscle sympathetic nerve activity for each of the perturbations. Subjects may be asked to repeat a perturbation if necessary.
    - Isometric handgrip and post-exercise ischemia
        - Maximal voluntary contraction of the dominant hand will be measured. The subject will squeeze the device at maximal effort 3-5 times, and the highest value will be used as the maximum. This will be used to calculate relative work rates ranging from 20-40%.
        - An inflatable cuff will be place around the upper arm on the dominant side and connected to a rapid cuff inflator, which will be attached to an external air source.
        - After 5 minutes of baseline, subjects perform isometric handgrip when they squeeze the handgrip device at the target intensity for 2 minutes. At the completion of exercise, we ask the subject to rate the perceived difficulty of the exercise on a numerical scale ranging from 6-20.
        - During the last 5 seconds of handgrip, we rapidly inflate the outer cuff on the upper arm to supra-systolic blood pressure for 3 minutes (post-exercise ischemia). At the completion of post-exercise ischemia, we ask the subject to rate the perceived discomfort of the ischemic period on a numerical scale ranging from 1-11.
        - Then we deflate the outer cuff and continue to collect data during a 2 minute recovery period.
    - Cold pressor test
        - After 5 minutes of baseline, the subject immerses one hand (up to the wrist) into an ice-water slurry (~4°C) for 2 minutes.
        - Data are recorded for an additional 2 minutes of recovery.
        - We ask the subject to rate the perceived discomfort of the cold pressor test on a numerical scale ranging from 1-11.
    - Mental arithmetic
        - After 5 minutes of baseline, subjects complete a series of simple arithmetic problems. Subjects are encouraged to respond as quickly and correctly as possible. Subjects perform the test for 2 minutes.
        - Data are recorded for an additional 2 minutes of recovery.
        - We ask the subject to rate the perceived difficulty of the mental arithmetic on a numerical scale ranging from 0-4.
    - Stroop color word test
        - After 5 minutes of baseline, subjects will be asked to name the ink color of a color word, a psychological task that is more difficult if there is a mismatch between the ink color and the word (e.g., the word ‘green’ printed in red ink). Subjects are encouraged to respond as quickly and correctly as possible. Subjects perform the test for 3 minutes.
        - Data are recorded for an additional 2 minutes of recovery.
        - We ask the subject to rate the perceived difficulty of the test on a numerical scale ranging from 0-4.
    - N-Back test
        - After 5 minutes of baseline, subjects will be shown a sequence of letters/geometric shapes one at a time. Subjects will be asked to state if the letter/geometric shape that they are currently viewing matches the letter/geometric shape shown 1 (“1-back”) or 2 screens previously (“2-back”). Subjects are encouraged to respond as quickly and correctly as possible. Subjects perform the test for 3 minutes.

- Data are recorded for an additional 2 minutes of recovery.
- We ask the subject to rate the perceived difficulty of the test on a numerical scale ranging from 0-4.

- Operation span task
  - After 5 minutes of baseline, subjects will be shown a sequence of simple arithmetic problems followed by a word/letter one at a time. Subjects are encouraged to complete the arithmetic problems as quickly and correctly as possible. Subjects will be asked to state the order of words/letters shown to them at multiple time points throughout the task. This task is self-paced for a total of 75 math problems and 75 letters/words.
  - Data are recorded for an additional 2 minutes of recovery.
  - We ask the subject to rate the perceived difficulty of the test on a numerical scale ranging from 0-4.
- Emotional stress
  - After 5 minutes of baseline, subjects will be shown a picture slide show, using pictures from the International Affective Picture System. The neutral (e.g., a table lamp) and negative (e.g., a wounded child) picture slide show each contains one blank slide and 59 pictures. Subjects will view each picture for 3 seconds (total viewing time of 3 minutes).
  - Data are recorded for an additional 2 minutes of recovery.
  - We ask the subject to rate the perceived stress of the picture viewing on a numerical scale ranging from 0-4.

11. At the conclusion of the experiment, we de-instrument the subject.
12. We measure blood pressure and heart rate before the subject departs the laboratory.

Subject Participation: During the course of the study, a subject may decide not to participate in a particular experimental measurement or procedure; therefore, this portion of the protocol will not be completed. However, all other measurements and procedures will be performed. This will not affect the scientific value of the subject's participation as each experimental measurement and procedure provides important and, in most cases, independent information.

Subject Re-enrollment: The principal investigator may decide to re-enroll subjects in the study if previous testing was unsuccessful or certain experimental measurements and procedures were not initially performed. The re-enrollment of subjects has no additional safety risks other than those inherent to the protocol and this will assist in the scientific merit of the project by providing additional information.

Undergraduate Student Roles: All undergraduates involved in this project have completed all required EHS trainings for handling biological specimens for humans, and have been trained by the PI and graduate students for each specific procedure (eg, centrifugation, pipetting, storage). Invasive aspects of the study refers to those techniques that are invasive (eg, microneurography) or require a high degree of skill (eg, Doppler ultrasonography). They will have limited direct interaction with participants. They mostly function in a supporting role and assist all aspects of the study (eg, running the data acquisition computer -- this is a task that requires being present for the duration of the experiment (~4 hrs) but does not require undergraduate students (usually) to ever physically touch the participant or speak to them).

7. Duration: *Indicate how many participation sessions, interactions, or follow ups are expected for each subject participant, including the amount of time required for each visit and how long their total participation is expected to take (weeks, months, years, etc.) over the entire duration of the study.*

Screening Visit: no more than 2.5 hours
Assessment of Daily Stressor Processes: no more than 3 hours per 8-day assessment (no more than 6 hours total)
Experimental Visit to Assess Cardiovascular Reactivity: no more than 5 hours
Total: no more than 13.5 hours

8. Alternatives to Participation: *Describe subjects' available options if they choose not to participate in the research study and clarify whether individuals that decline participation will still be subjected to the intervention (even if their data will not be utilized for research purposes). If research involves students,*

describe their alternatives to obtain course / extra credit if applicable. If research involves a health intervention, clarify whether individuals that decline will continue to receive standard care.

The alternative to participation is choosing to decline participation. Participants may withdraw at any time. We may end the participant's role in the study without her consent if we determine that her health or behavior adversely affects the study or increases the risks beyond those approved by the Institutional Review Board and agreed upon by her/him in the informed consent. Participants will be withdrawn if they cannot follow or understand the rules of the protocol. Participants will also be withdrawn if they cannot hold their appointments.

9. **Location(s) and Site(s):** *Specify all locations where research procedures are expected to take place and which study procedures will take place at each site. Studies that take place online should specify the websites where data will be collected. Describe if any of the research will take place internationally. For multi-site research studies, review the web page for Collaborative Research. If any part of this study will be conducted in an institution or location administratively separate from UTA, indicate the institution(s) and upload a site permission letter.*

All studies will be performed in the clinical laboratories located on the 1st floor of the SEIR. These laboratories are BSL2 certified.

10. **Personnel Qualifications:** *Describe the relevant qualifications, special training, and experience of the research team/personnel as it pertains to the specific procedures or population of the study. If you (and your faculty advisor, if applicable) do not have any relevant qualifications or experience, please state that; the IRB will consider the risk level of the study and evaluate if additional oversight or input is necessary.*

Dr. Greaney, has extensive knowledge of and experience with human subjects research pertaining to neuro-cardiovascular physiology in adults with pre-clinical cardiovascular disease (e.g., primary aging, hypertension, psoriasis, depression). Dr. Greaney has shadowed her collaborating psychiatrist, Erika Saunders, MD (co-mentor for her NIH-funded K99/R00), in outpatient psychiatry clinics to observe patients with psychiatric illness in the clinical setting, to learn the background of psychiatric diagnosis, and to receive psychiatric interview training. The training she received is fully adequate to ensure the accurate diagnosis of MDD.

Dr. Greer will provide support and oversight for issues related to major depression and psychiatric illness. Dr. Greer is a Professor at UTA in the Dept of Psychology and has an appointment in the Dept of Psychiatry at UT Southwestern Medical Center. She has extensive experience conducting research and safety evaluations in vulnerable populations.

Dr. Fadel is a Co-Investigator on this project. He too has extensive knowledge of and experience with human subjects research pertaining to neuro-cardiovascular physiology in adults with pre-clinical cardiovascular disease. He is an expert in human neural control of the cardiovascular system.

Dr. Siddiqui is the Medical Director for Clinical Translational Science at UTA. He is a practicing surgeon with extensive expertise in neural cardiovascular physiology in health and disease and will provide medical input and oversight of our translational science team. He will assist in reviewing health history information of the participants. However, he will not be heavily involved in the administration of these studies. Carrie Arena-Marshall and Yaewon Seo will provide nursing support.

These trainees have completed the required trainings, are familiar with the experimental measurements and procedures, and will assist with all phases of the research study: Ashley Darling, Jeremiah Joseph, Cynthia Dominguez, Cecilia Nguyen.

These trainees have completed the required trainings, are familiar with the experimental measurements and procedures, and will primarily assist with the administration of the CANTAB cognitive function battery: Cassie Argenbright.

11. **Study Oversight:** *The Principal Investigator has ultimate responsibility for the conduct of this research, protection of subjects, and supervision of all protocol personnel. Describe your plan for oversight and communication to ensure that the entire research team: conducts the research ethically and in accordance with the approved protocol, creates/maintains appropriate study documentation and research records, and protects confidentiality of data.*

Dr. Greaney is responsible for conducting the study in compliance with this protocol, with institutional and IRB policies, with Good Clinical Practice guidelines and any other applicable regulatory requirements. She is assisted by members of her lab group. The monitoring is ongoing. Dr. Greaney and other investigators will hold regular lab meetings where relevant data safety and management procedures, interim data evaluation, untoward events (rare in this research), new developments in related research, and quality control issues are discussed. Regular bimonthly meetings and teleconference calls as deemed necessary will be scheduled with our collaborators. Collaborating investigator Dr. Mogle will assist with all phases of the implementation of the DISE survey (survey programming, data reduction/analysis), data interpretation, dissemination (abstracts/manuscripts), and the development of future grant proposals.

SECTION C: POPULATION & ENROLLMENT

12. **Population(s):** *Describe the target population(s) of the study, for example: UTA students, competent or healthy adults, children, prisoners, non-English speaking, pregnant women, individuals with impaired decision making capacity, other vulnerable populations.*

Competent adults; UTA students

Note: Additional forms may be required for your population. Obtain these from the Forms & Templates Page.
For Individuals with Impaired Decision Making Capacity: Upload Form 2A.
For Pregnant Women, Fetuses, Women Undergoing In-Vitro Fertilization, or newborns: Upload Form 2B.
For Prisoners (Individuals involuntarily detained): Upload Form 2C.
For Children (Under 18 or the local legal adult age): Upload Form 2D.

13. **Inclusion Criteria:** *List all criteria for including subjects, and explain the methods you will use to determine whether a subject is eligible based on your criteria (i.e. pre-screen, medical chart review). If your study is/will be funded, ensure that the inclusion criteria listed here match the details in your proposal.*

- Men and women 18 – 55 years,
- Two subject groups will be recruited:
  1. Healthy control (HC) subjects will not have a history of MDD or other major psychiatric illness; PROMIS score < 18; and as confirmed by MINI
  2. MDD patients will have clinically significant depression (non-medicated); PROMIS score > 18; and as confirmed by the MINI

14. **Exclusion Criteria:** *Explain any specific factors or contraindications that would make a subject ineligible to participate in this study, even if they would otherwise meet the inclusion criteria listed above. If your study is/will be funded, ensure that the exclusion criteria listed here match the details in your proposal.*

The exclusion criteria is applied to all groups. We will not enroll Non-English speaking individuals. This protocol involves the use of numerous questionnaires, including a detailed neuropsychiatric interview with the investigators. In addition, participants need to understand English in order to follow instructions and comply with procedures conducted during the screening and experimental visits.

Subjects will be excluded at the discretion of PI/collaborating psychiatrist/examining clinician or for any of the following reasons:

- Psychiatric illness aside from MDD (including bipolar disorder, panic disorder, schizophrenia)
- Psychiatric treatment with medication
- Active suicidal/homicidal ideation
- Active substance dependence
- Eating disorders
- Medications that could conceivably alter neurovascular function
- Changes or alterations in medication status (starting a new, additional, or different medication)
- Cardiovascular, renal, or metabolic disease (hypertension, heart disease, diabetes, hyperlipidemia)
- Tobacco use
- Pregnancy
- Latex allergy
- G6PDSickle cell anemia
- Lack of access to a device with internet connectivity

15. Number of Subjects: *Provide the number of subjects (or subject records/data sets) you intend to enroll over the course of the study. This information will be utilized by the IRB to understand the scope and logistics of the study; you may provide a projected range.*

Based on power calculations and previous experience using these techniques, up to 100 subjects per group (total n=200) will be included. This estimate will permit appropriate statistical comparisons and accounts for the technical difficulties of obtaining high-quality experiment measurements in all subjects and accounts for technical failure and subject dropout.

***Note:** For MR research, there is no cap on enrollment (enrollment can exceed the number provided here when needed for the study).*

For GMR research, the proposed number of subjects must be supported by statistical justification and/or references; please provide that information here. Enrollment for GMR research is capped (IRB will approve a specific range or maximum number of participants and enrollment must not exceed that approved number unless the IRB approves a modification request).

16. Recruitment Strategies: *Describe how you will identify and contact potential participants, and how you will obtain their contact information. Upload permission letters/emails as needed from individuals or organizations providing access to private contact information. Upload a copy of all planned recruitment materials (i.e. letters/emails; website/social media posts; printed flyers; telephone scripts; subject pool posts (SONA, Mechanical Turk, Research Match); scripts for recruitment in-person).*

Subjects will be recruited primarily from the University of Texas at Arlington and the greater Dallas-Fort Worth area. We use recruiting methods that will reach a large percent of the general population. We advertise for subjects and interested persons contact us. We post recruitment flyers in various campus (e.g., student union, classroom building bulletin boards, dormitory bulletin boards, campus eateries, etc) and community (e.g., coffee shops, bookstores, local businesses, etc) locations. We will obtain permission to provide recruitment flyers to local physicians' offices (family physicians, mental healthcare providers/specialists) and other locations specifically targeted to recruit patients with depression (Counseling and Psychological Services). Additionally, the text from the fliers may be utilized in "word of mouth" recruiting if the opportunity presents itself (see attached document labeled "stress recruitment ad"). Recruitment may also occur via ResearchMatch.com and other online resources such as Craig's List and Facebook. If we recruit by Research Match, we will not do so until we receive approval from all parties involved.

We also avail ourselves of lists of potential subjects maintained by other labs conducting human subjects research at University of Texas Arlington. The people on these lists have consented for other IRB-approved studies and indicated that they wished to be maintained on a list of potential subjects to be contacted in the event that they may qualify

for additional studies. Also, other researchers may agree to send an email (provided by us; see attached document labeled "neural recruitment email") to their research subjects who have indicated a willingness to be contacted for future studies for which they may qualify.

SECTION D: COMPENSATION AND COSTS

***Note:** You are responsible for maintaining accurate and confidential records regarding payment of your subjects. Per Accounting Services procedures, compensation must be documented for tax purposes using a W-9 form unless an exception is granted by the Accounting department. Obtaining an exception should be considered for cases of sensitive research or when disclosure of a subject's identity would expose them to high risk. Exception requests are submitted through the Business Affairs Exceptions Tracker (BAET) in SharePoint. Refer to knowledge base article KB0010632 for guidance. Contact Business Technology Services at 817-272-2155 or submit a ServiceNow ticket at https://uta.service-now.com/selfservice/ for assistance.*

17. **Compensation:** *Describe any compensation to subjects for participation, including monetary payments, gift cards, course/extra credit, raffle prizes, goods or services, donations to charity, etc. Describe how and when you will provide the payment to the subjects, and how confidentiality will be maintained (for example, use of coding in payment log books/receipts). If you intend to hold a raffle, explain when you expect that the raffle will be drawn, and how participants will be contacted if they win the drawing. For course / extra credit, alternative non-research assignments must be offered for an equal amount of credit.*

    Participants will be compensated at the completion of the study or after the final visit to the lab.

    For the screening visit, subjects will be compensated $30. This compensation will only be provided to participants who qualify. That is, if the MINI or medical health history indicate an exclusion criterion and the subject is not enrolled in the study, he will not be compensated.

    For the experimental visit, subjects are compensated $75. Additional compensation is not provided if the participant is asked to repeat a trial within the same experimental visit. However, if a participant is asked to repeat the experimental visit, they will be compensated for the completion of the second visit as described above.

    For the assessment of daily stressors, there is a base compensation of $15, with a $5 bonus for the completion of the DISE survey on all 8 days. Thus, participants who complete the entire 8-consecutive day DISE assessment will be compensated $20. Participants who do not complete the DISE survey on all 8 days will receive the base compensation of $15. This compensation scheme will also be followed for participants who may complete each of two 8-day assessments. Participants will be compensated in the form of an electronic gift card for completion of the DISE surveys. This compensation scheme will significantly reduce participant burden and, given the ongoing COVID-19 pandemic, will help to limit the number of times they are asked to come to campus and to the lab.

    If a participant is asked to repeat certain experimental measurements or procedures that were not initially performed, they will be compensated $15/hour.

18. **Costs:** *Describe any costs or expenses (monetary or non-monetary) subjects will incur as a result of participation.*

    N/A

SECTION E: INFORMED CONSENT

***Note:** The ethical foundation of human subject research is informed consent. It is important to ensure that subjects are provided with sufficient information to understand the requirements of their participation and the use/purpose of their data. You also cannot obtain information about a person through another individual (such as a family member) unless that person has undergone the informed consent process themselves. Use the*

Office of Human Research Protection (OHRP) informed consent checklist (http://www.hhs.gov/ohrp/policy/consentckls.html) and the IRB's Templates as guidance.

19. Informed Consent, Broad Consent, & Assent: *Describe the informed consent process, including when, where, and how subjects will be consented. If children or mentally disabled or incapacitated persons will be subjects, explain the assent process. If broad consent (consent to use data for future studies) will be requested, describe the scope and the process for tracking subjects' accept/decline responses. Upload finalized copies of all consent, assent, and / or verbal consent script documents in the electronic system.* ***There are several consent form templates available for your use on the Forms & Templates Page.***

Informed consent will be obtained at the screening/familiarization visit in the laboratory where the experimental visits will occur. These rooms are private. Access to these rooms is restricted. An investigator will review each section of the consent form with the participant. The participant will then be asked if they have questions. They will be explicitly given the option of taking the consent home for additional consideration and providing consent at a later date. Prior to any screening/testing, all subjects will provide written, informed consent.

19a. Requesting a Waiver of Consent or Waiver of Written Documentation: *If you wish to waive some or all of the requirements of informed consent, or the requirement for written/signed informed consent, please describe (if your study is federally funded or FDA-regulated, also upload Form 3 from the Forms Page).*

Please see Form 3.

20. Incomplete Disclosure / Deception: *Describe if your study will withhold information from subjects regarding the purpose of the research or the nature of the intervention, interaction, or procedures. Provide scientific justification for utilizing deception (if your study is federally funded, also upload Form 3).*

N/A

SECTION F: RISKS & BENEFITS

21. Risks to Subjects: *Explain any potential risks to subjects that could result from the research intervention/procedures, including* ***physical risks*** *(i.e. fainting, falls, infections, muscle soreness, pain, broken bones, physical fatigue, headache, burns, medication side effects);* ***psychological risks*** *(i.e. depression, anger, stress, guilt, embarrassment, damage to self-esteem);* ***social risks*** *(i.e. potential damage to financial standing, reputation, or employability);* ***risks to privacy or confidentiality*** *(i.e. exposing someone as a research subject, release or breach of sensitive data); and/or* ***risk of perceived coercion/undue influence*** *(i.e. if investigator could have influence by nature of their relationship or status, such as a teacher & student, manager & employee, doctor & patient).*

There is risk that the illness (MDD) will worsen during participation. All participants will be given materials on local resources for psychiatric care and suicide hotline information. During the interview process, subjects will be advised that they are able to stop the interview at any time or not answer questions if preferred. If during the course of the study, at any time, the participant experiences suicidal or homicidal ideation with intent to harm, MHMR Tarrant County Crisis Relief will be called for an external evaluation and transfer. Failing all of the above options, 911 will be called for immediate assistance. If a participant has significant worsening of MDD but no immediate safety concern, we will recommend that the patient seek psychiatric consultation.

Availability of Medical or Psychological Resources: Subjects will be free to contact the PI regarding any concerns or emergency during the study. The investigators also will provide the subjects with a detailed explanation of the neurovascular physiology of MDD. If participants have questions regarding clinical care, they will be referred to a psychiatric or medical provider. All participants are provided with a copy of the "Local Mental Health Providers" document. All subjects with MDD are highly encouraged to seek follow-up with a health care provider. If a subject

with MDD does not have a health care provider, the investigators will help them to identify local resources. Furthermore, the investigators will provide subjects with their screening data so that they may forward this information to a health care provider.

MINI/PROMIS/PHQ9: For the purposes of this study, we use these tests to identify potential subjects who have clinical depression and to identify for exclusion those who have psychiatric illness other than depression. Subjects answer 8 questions (PROMIS), 9 questions (PHQ9), and 16 sections (MINI) (see uploaded documents). A score of 18 or more on the PROMIS test is considered depressed. A score of 5-9 on the PHQ9 is indicative of mild depressive symptoms. The MINI has 16 sections with multiple questions per section and provides a more in depth examination. Dr. Greaney has learned to conduct the PROMIS, PHQ9, and MINI through extensive instruction and training by Dr. Saunders. Subjects may feel uncomfortable about answering the questions. They are reminded that they may decline to answer the questions and leave the study at any time. Some subjects may be disturbed if the test recommends their inclusion in the depression group. We remind them that the test is not intended to be a diagnosis or healthcare recommendation but is used as a tool to identify people who may have particular experiences or forms of psychological distress useful for the purposes of this study. If the subject has concerns about the results of the test, we suggest that she/he seeks follow-up with a healthcare or mental healthcare provider. If she/he does not have access to one or the other, we can provide her/him with contact information.

Flow Mediated Vasodilation (FMD) / Doppler Ultrasound: There is a small chance the probe could irritate the skin. Placing the probe on the arm's skin may cause temporary minor redness. The inflated cuffs may cause the participant's arms and feet to feel numb or tingly, and the skin's color to change slightly. The cuffs could cause mild bruising. The gel is the same as that used with medical ultrasound tests. The gel may feel cool or cold on the skin. A bad reaction to the gel is highly unlikely. The cuffs inflate for a minimal amount of time. The temporary redness from the probe is unlikely to have lasting ill effects. The participant may decline the test.

Microneurography: The technique of microneurography is an accepted and safe research technique. With regard to the assessment of nervous system activity, the use of the pencil-like device for external electrical stimulation may cause minor discomfort. There may also be mild discomfort when the fine wire needle is inserted through the skin; however, this needle is very small. Brief sensations of pins and needles and/or cramping are likely to be felt during the nerve search. This fine wire needle will be left in place for the duration of the experimental visit (approximately 3-4 hours). It is also possible that feelings of muscle weakness and/or pins and needles sensations can be felt after completion of the procedure. There is no specific treatment for these sensations, and in the small number of volunteers that have experienced them, they have disappeared spontaneously. As with venipuncture or any event that breaks the skin, there is also a small risk of infection at the site where the fine wire needle is inserted. The microneurographer (Jody Greaney, PhD) is proficient in this technique, using it in research for >7 years, and no subjects have previously reported any long-term adverse effects (>265 microneurography experiments performed).

Handgrip Exercise and Post-Exercise Ischemia: The muscles are likely to experience temporary fatigue and soreness during exercise. During and after cuff inflation, the arm may feel uncomfortable, numb, or tingly. These sensations cease rapidly, and there are no long-lasting adverse effects from these tests.

Cold Pressor Test: The subject's hand is likely to feel very cold. Subjects may stop the test at any time. They are unlikely to experience long-lasting adverse effects from this test.

ECG: The researchers attach 3-5 electrodes to the participant's chest and then attach the electrode wires to a ECG machine. The machine records the electrical activity of the heart. There are no adverse effects from this measure. A participant may be shy about electrodes applied to the chest. The staff carefully remove the tape afterward. They conduct the test professionally and privately.

PWA/PWV: A cuff inflates tightly on the upper arm and lower leg. Automated measurement technique minimizes the duration of cuff inflation.

Blood pressure: A cuff inflates on the upper arm. The inflated cuff may make the arm feel tingly and numb, and the cuff may temporarily bruise the arm. Efficient and competent measurement technique minimizes the duration of cuff inflation. The finger cuff (Finapres) may make the finger feel tingly and numb. The finger may be switched to a

different finger when necessary. These techniques to measure blood pressure are unlikely to produce lasting ill effects.

Blood draw: Blood draws can cause anxiety (with increased heart rate and blood pressure), mild pain, swelling, nausea, lightheadedness, fainting, or bleeding. There is a slight chance of infection or small blood clot. A competent nurse performs blood draws using standard venipuncture-procedure and techniques that minimize the chance of infection. Participants may recline for the procedure.

Cognitive Function Assessment: This test helps us to interpret the physiological data we record during the experiment. Subjects may feel shy or disturbed by the questions. They may choose not to answer the questions and decline to be in the study. We do not use the tools to decide a recommendation for healthcare. We keep the completed forms confidential and secure. Only approved staff may access the results.

Emotional State Assessment: This test helps us to interpret the physiological data we record during the experiment. Subjects may feel shy or disturbed by the questions. They may choose not to answer the questions and decline to be in the study. We do not use the tools to decide a recommendation for healthcare. We keep the completed forms confidential and secure. Only approved staff may access the results.

Surveys (Physical Activity Questionnaire, DISE): These surveys help us learn of a subject's experiences that are useful for the purposes of this study. The surveys help us to interpret the physiological data we record during the experiments. Subjects may feel shy or disturbed by the questions. They may choose not to answer the questions and decline to be in the study. We do not use the tools to decide a recommendation for healthcare. We keep the completed forms confidential and secure. Only approved staff may access the results.

Screening: The screening includes blood sample, height, waist circumference, weight, heart rate, blood pressure, pregnancy test, and history performed by the competent research staff. Participants may be uncomfortable giving medical information or being measured. The participants may decline to answer questions or participate in measurements. The researchers conduct screenings professionally and privately.

Latex: Some gloves and medical materials are made of latex rubber. Some people may be sensitive to latex. Screening identifies and excludes candidates having a known latex allergy.

Confidentiality: There is a risk of loss of confidentiality.

22. Strategies to Minimize Risks: *Explain the strategies that the research team will use to minimize the potential risks listed above.*

General Procedures

The research group's members are trained and competent in their duties. The group, led by Dr. Greaney, evaluates the effectiveness and safety of protocols and procedures in an ongoing fashion. They discuss the protocol with candidates, invite questions, and offer tours of the laboratory. Prior to medical screening, candidates read and sign informed consent forms detailing protocols, procedures, risks, sensations, compensation, etc. The researchers give candidates witnessed copies of the signed consent forms. After accepting participants into the study, the researchers discuss and review the procedures and protocols with them generally and at each step throughout the project. They frequently remind participants of the option to withdraw from the study at any time. Restricting access to experiments, data, and coding to authorized personnel maintains confidentiality. Lists of emergency numbers remain by lab telephones. At least one cell phone is present at each experiment. A hospital and emergency medical services are within 1-2 miles of the lab. An AED hangs nearby in the hallway.

The PI has obtained an IND number from the FDA for the pharmacological agents used with this protocol (IND 125,994). We are in complete compliance with FDA regulations. The agents are individually logged in a log book (quantity of agent, date of arrival and lot number). The date of use is then logged into the log book.

Availability of Medical or Psychological Resources: Subjects will be free to contact the PI regarding any concerns or emergency during the study. The investigators also will provide the subjects with a detailed explanation of the neurovascular physiology of MDD. If participants have questions regarding clinical care, they will be referred to a

psychiatric or medical provider. All participants are provided with a copy of the "Local Mental Health Providers" document. All subjects with MDD are highly encouraged to seek follow-up with a health care provider. If a subject with MDD does not have a health care provider, the investigators will help them to identify local resources. Furthermore, the investigators will provide subjects with their screening data so that they may forward this information to a health care provider. If a participant indicates a "high" level of suicidality (coded as scoring ≥17 on the Suicidality Module) and immediate risk with intent to harm, MHMR Tarrant County Crisis Relief will be called for an external evaluation.

Screening: Guided by the inclusion and exclusionary criteria approved by the IRB for the study, the investigators and clinical staff screen subjects before participation to insure that the subjects meet the study's requirements.

Stoppage Criteria: Although such events are extremely unlikely to occur, the investigators are prepared to immediately stop experiments and seek medical assistance if the subjects should experience the more serious reactions described in the informed consents and IRB applications such as signs and symptoms of an allergic reaction, anaphylactic shock, and fainting. In the case of a medical emergency, campus police will be contacted. The investigators end the experiments if the subject's blood pressure is greater than 220/110 mmHg or less than 90/60 mmHg, if heart rate is >120 bpm or <40 bpm, or the presence of symptoms. The investigators will stop the experiments at any time should a subject wish it. Also, the investigators will exercise the discretion to end a subject's participation if the subject should engage in behavior that could jeopardize his/her own health and well-being or that of others.

Previously Unknown Medical Conditions: It is possible that the investigators will discover a participant's previously unknown medical condition as a result of the screening. At the time of screening, the investigators inform subjects of any condition identified that might require further treatment, and will suggest follow-up with a healthcare provider. The investigators make the results from laboratory studies available to the subject as soon as possible.

Specific Procedures

Psychiatric Screening Instruments [MINI, PROMIS, PHQ9]: Subjects are reminded that they may decline to answer the questions and leave the study at any time. Some subjects may be disturbed if the test recommends their inclusion in the depression group. We remind them that the test is not intended to be a diagnosis or healthcare recommendation, but is used as a tool to identify people who may have particular experiences or forms of psychological distress useful for the purposes of this study.

Microneurography: The microneurography procedure will be immediately discontinued if the subject experiences any excessive discomforts. According to the Eckberg et. al (Eckberg DL *et al*. Prospective study of symptoms after human microneurography. *Acta Physiol Scand*. 137(4):567-9, 1989), the risk of symptoms during or after microneurography are minimized when no more than 60 minutes are used for the nerve search; we generally avoid searing for more than 45 minutes—and consistent with Eckberg's recommendation, we never search for more than 60 minutes.

ECG: We carefully remove the tape afterward. We conduct the test professionally and privately. The subjects may request that a person of the same gender perform the test.

PWA/PWV: During the short time the cuff is inflated, the subject's arm may feel numb. The measure will be taken efficiently to minimize the amount of time the cuff is inflated. The investigator can manually deflate the cuff at any time during the procedure, if necessary.

Brachial blood pressure: During the short time the cuff is inflated, the subject's arm may feel numb. The measure will be taken efficiently to minimize the amount of time the cuff is inflated on the arm.

Finger blood pressure: The researcher will move the cuff to a different finger if the subject needs to give the first finger a rest.

Blood draw: Subjects may decline. Experienced personnel draw the blood. Sterile supplies are used. Subjects will only be subjected to a maximum of two attempts per arm by one person.

Surveys/Cognitive Function Assessment/Emotional State Assessment: The subjects may decline to answer questions or participate in measurements. We do not use the tools to decide a recommendation for healthcare. We keep the completed forms confidential and secure. Only approved staff may access the results.

Medical screening: The subjects may decline to answer questions or participate in measurements. Screenings are conducted privately. The subjects have the option of having a person of the same gender perform the measurements.

Tape and adhesive disks: The researchers remove the tape and adhesive disks carefully. Ointment is available, if needed.

Latex: Screening identifies and excludes candidates having a known latex allergy.

Confidentiality: Qualtrics is a secure website and survey application designed to support data capture for research studies. All data is encrypted. Data are deleted from Qualtrics' servers within 24 hours. The data files are downloaded and stored on an external password-protected hard-drive. Only authorized members of the lab have access to these files.

23. **Health & Safety Considerations:** *Specify whether the study involves any hazardous materials, locations, or equipment that is relevant to the health and safety of either the subjects or the protocol personnel (i.e. handling of human blood/body fluid/tissue, chemical or biological hazards, radiation/X-rays, lasers, or carcinogens). List any related authorizations/approvals from the Environmental Health & Safety Office.*

The laboratory space is designated as BSL-2 and approved for the collection and processing of blood samples.

24. **Benefits:** *List potential benefits that may accrue directly to the study subjects as a result of their participation, if any (other than compensation). Also describe the expected or potential benefits of this study to the field or society at large.*

There is no direct benefit to participation; however, there are several possible benefits to the subjects. Subjects receive a medical screening that could inform them about their health. They learn their blood pressure and blood cholesterol levels. We advise those with depression, high blood pressure, or blood cholesterol to follow-up with a health care provider.

Participants learn of the possible connection between depression and cardiovascular disease. Because impaired vascular function is an important contributor to the elevated cardiovascular risk, knowledge of the mechanisms underlying vascular dysfunction in adults with depression can be used to develop treatment plans to help decrease the incidence of disease in these individuals.

SECTION G: PRIVACY & CONFIDENTIALITY

25. **Privacy:** *How will the privacy of subjects be protected during the course of the study (privacy refers to controlling the environment and circumstances of interactions with subjects to prevent situations where they might be embarrassed, exposed, or stigmatized)?*

Subjects will be enrolled and tested individually in a private setting. Subjects may request an individual of the same sex to perform certain experimental measures and procedures. We tell participants that they may decline to answer questions and decline to participate in the study. Only authorized personnel are present during screening and experiments. On occasion (e.g. educational visit, visiting colleague, site visit) participants may give permission for visitors to observe a procedure or experiment.

26. **Confidentiality & Data Security:** *Explain if the data collected (including biospecimens) will be anonymous, identifiable/coded, or de-identified*. Explain the precautions that will be taken to protect confidentiality of subject data and information, and how these precautions will be communicated to subjects (during informed consent or another process). Security should be considered for each phase of data's life cycle,*

including: collection, transmission, accessing, collaboration, storage, analysis, reporting, and disposition. Consider the tools and resources that will be utilized for data collection, how access to identifiable data will be limited only to authorized research personnel, and who will be responsible for storage and disposition. ***Recordkeeping:*** *UTA and the IRB must be able to access research records and consent forms at any time; therefore,* ***all paper documents in their original form must be stored on the UTA campus*** *unless the IRB grants an exception.* ***All electronic data must be maintained on UTA servers utilizing sanctioned storage tools*** *unless the Office of Information Security grants an exception.* ***Record Retention Period:*** *All records (paper or electronic) must be maintained and kept secure for at least 3 years after the closure of the protocol or in accordance with funding agency requirements (whichever is longer). Student PIs should address long-term storage arrangements if planning to leave UTA prior to the end of the retention period.* **Visit the UTA IRB's Web Page on Human Subjects Data Security for allowable data storage options and more helpful information about DO's and DON'Ts with human subject data!**

All files will be coded with a unique code for each subject. Hard copies of all data entered for a given subject and informed consent form originals will be stored in a locked file cabinet in the laboratory. The computerized data files are password protected and automatically backed-up. All of the electronic data are coded and do not contain personal identifying information. These data are stored using UTA-sanctioned storage tools. Only the PI and study personnel on this IRB will have access to the files. No other students or faculty will have access.

Qualtrics is a secure website and survey application designed to support data capture for research studies. The participant may be concerned about data's security. All web traffic to and from the Qualtrics application website is done via a Secure Socket Layer (SSL) that encrypts the data in transmission. The questionnaire contains statements advising of the limitations of technology and that there is no confidentiality guarantee. Data is deleted from the Qualtric's servers within 24 hours after the user deletes it from the website.

Documents allowing identification of participants do not leave the investigator's labs and are only available to authorized persons. The investigators store any list linking the code to participants' identity in locked cabinets and on password-protected computers maintained in a locked room. Data forms containing identifiable information are shredded when no longer needed (3 years after publication of results). Screening data from subjects who are not accepted into the study are shredded immediately.

Subjects may give permission to have their contact information retained in the investigator's secured files if they wish to be considered for participation in future studies. After the investigators complete the study, they remove all identifiers from the data and store the data indefinitely. Individual data may be used without identifying the subject to illustrate representative response.

***Note:** "Anonymous" means that the data is unidentifiable (personally identifiable information will not be collected or accessed). "Identifiable" means that data obtained will be recorded in such a manner that subjects' identity can be readily ascertained, either directly or indirectly through identifiers linked to the subjects (research involving a coding mechanism that links to identifiable data is considered identifiable, but it is a helpful measure to protect confidentiality). "De-identified" means that all direct personal identifiers are permanently removed, no code or key exists to link the data to its original source, and the remaining information cannot reasonably be used by anyone to identify the source.*

26a. Legal Limits to Confidentiality: *If any part of this study could result in the potential identification of child abuse, elderly abuse, communicable diseases, or criminal activities that would / could not have been otherwise identified, explain this possibility and estimate the likelihood of disclosure. Describe the plan of action that you will take if this occurs. In rare circumstances when research reveals these issues, confidentiality should be maintained to the extent that the law allows.*

In accordance with UTA policy, if an incidence of abuse is reported or suspected, mandatory reporting will take place.

27. **Data Sharing:** *If you intend to share, release, or present any identifiable subject data from this study, explain where, when, and to whom the identifiable information will be shared, presented or released, and how this will be communicated to the subjects beforehand.*

N/A

SECTION H: CONFLICT OF INTEREST

28. **Conflicts of Interest (COI):** *Does the Investigator or any protocol personnel have an affiliation, arrangement, or financial interest that could be perceived as a conflict of interest*? *If yes, please describe.*

There are no Conflicts of Interest to report.

***Note:** All Covered Individuals in GMR research are required to have a current COI disclosure on file in Mentis (this must be complete prior to approval of the protocol). Covered Individuals are those with responsibilities for the conduct, design, or reporting of this research study.*

SECTION I: REQUIRED ADDITIONAL ATTACHMENTS

29. **Upload finalized versions of the following documents as applicable to your study in the electronic submission system***:*

- Survey instruments / questionnaires (and any versions translated into other languages)
- Demographics surveys
- Interview questions / prompts
- Focus group instructions / questions / prompts
- Observation data collection sheets
- Psychological & educational tests
- Educational materials
- All recruitment materials including flyers, ads, scripts, emails, social media posts, etc.
- Informed Consent Documents / cover letters and translated versions (See Forms Page for Templates)
- Permission letters from non-UTA study sites / collaborating organizations
- Signed Non-UTA Collaborator Forms & HSP Training (Collaborative Research Page)