Does Propranolol Attenuate Inflammatory Responses to a Psychological Stressor?

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Beta-Blockers & Stress Study Protocol

Complete Title: The effects of propranolol on psychological and physiological responses to acute stress
Short Title: Beta-Blockers and Stress Study
Drug or Device Name(s): Propranolol
Sponsor: Dr. Muscatell UNC laboratory start-up funds
Protocol Date: 9/26/2016
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PROTOCOL TITLE: Beta-Blockers and Stress

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Protocol Version 2
Version Date: October 12, 2016

I confirm that I have read this protocol and understand it.
Principal Investigator Name: Keely Muscatell

Date: October 12, 2016

TABLE OF CONTENTS

ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
SNS	Sympathetic nervous system; part of the autonomic nervous system that is engaged during physiological arousal
CTRC	Clinical and Translational Research Center; center at UNC-CH that provides investigators with research support services, including on-location nursing services utilized in the present study
TSST	Trier Social Stress Task; a commonly-used, acute, laboratory stress paradigm for inducing physiological and psychological stress in participants
IDS	Investigational Drug Services
MLM	Multilevel Modeling
PEP	Pre-Ejection Period; a measure of sympathetic nervous system activation derived from electrocardiogram and impedance cardiography.
RSA	Respiratory Sinus Arrhythmia; a measure of parasympathetic nervous system activation derived from electrocardiogram.

PROTOCOL SYNOPSIS

LIMIT SYNOPSIS to no more than 2 - 3 pages. The synopsis should provide an overview of the study. Keep brief and use bullet points.

Study Title	Beta Blockers and Stress
Funder	Dr. Muscatell's laboratory start-up funds provided by UNC-CH
Clinical Phase	Phase IV
Study Rationale	This randomized, double-blind, placebo-controlled study of propranolol will shed important light on how sympathetic nervous system (SNS) activation influences psychological and inflammatory responses to acute stress. Results from this study will inform both the basic science literature that is attempting to map the physiological mechanisms by which psychological stress may lead to poor mental and physical health, and may also ultimately have therapeutic relevance for individuals who are experiencing high levels of stress that is putting their health at risk. Utilizing a psychopharmacological approach allows for the circumvention of many of the challenges of conducting this research in human populations, and will allow for conclusions regarding causality, given that SNS activation will be experimentally manipulated, rather than relying on correlational measures of SNS activity that are difficult to assess and are not appropriate for asking if SNS activity *causes* changes in psychology and biology.
Study Objective(s)	Primary Objective 1. To determine if blocking beta-adrenergic receptors with the drug propranolol will attenuate inflammatory responses (i.e., interleukin-6; IL-6) to an acute, laboratory stressor. Secondary Objective 1. To examine if blocking beta-adrenergic receptors with the drug propranolol will attenuate cortisol responses to an acute, laboratory stressor. Secondary Objective 2. To examine if blocking beta-adrenergic receptors with the drug propranolol will attenuate salivary alpha amylase responses to an acute, laboratory stressor. Secondary Objective 3. To evaluate if blocking beta-adrenergic receptors with the drug propranolol will attenuate sympathetic nervous system responses (i.e., pre-ejection period; PEP) to an acute, laboratory stressor. Secondary Objective 4. To examine if blocking beta-adrenergic receptors with the drug propranolol will change parasympathetic nervous system responses (i.e., respiratory sinus arrhythmia; RSA) to an acute, laboratory stressor. Secondary Objective 5. To examine if blocking beta-adrenergic receptors with the drug propranolol changes self-reported affective responses (i.e., negative affect) to an acute laboratory stressor.
Test Article(s)	Propranolol (FDA approved)

(If Applicable)	
Study Design	This is a randomized, double blind, placebo-controlled study of the effects of propranolol on psychological and physiological responses to stress. It will consist of a telephone screening, an initial in-person session when consent will be obtained, and a subsequent longer, intervention session.
Subject Population key criteria for Inclusion and Exclusion:	Inclusion Criteria 1. Healthy individuals from the UNC-Chapel Hill community 2. Ages 18-25. 3. Fluent in English (spoken and written) 4. In good overall health. Exclusion Criteria 1) Presence or history of chronic physical illness (especially disorders with an inflammatory component, such as rheumatoid arthritis, asthma, allergies, or issues that can affect the heart, including low-blood pressure or other heart conditions) 2) Presence or history of psychiatric illness (depression, anxiety) 3) Any current prescription medication use 4) Currently pregnant or planning to become pregnant (self-reported) 5) Engagement in a number of health-compromising behaviors that may affect levels of pro-inflammatory cytokines, including cigarette smoking, excessive caffeine intake and sleep disturbance (e.g., working night shifts) 6) Body Mass Index (BMI) greater than 30, given that adiposity is known to relate to baseline levels of inflammation; 7) Anxiety about or previous history of problems with blood draws (e.g., fainting) 8) Any reported heart conditions
Number Of Subjects	95 (45 per condition; we will run 5 "extra" subjects in the case of data loss)
Study Duration	Each subject's participation will last 5-6 hours. The entire study is expected to last 1-2 years.
Study Phases Screening Study Treatment Follow-Up	(1) Screening: screening for eligibility will occur via telephone interview, and consent will be obtained in-person during a first study session. (2) Intervention: study intervention/experimental treatment will occur during a second study session. Participants will be randomly assigned to take either a single, 40 mg dose of propranolol, or a placebo, and will go through a standardized psychological stress task. Blood draws and saliva samples will be taken throughout, and will be assayed for inflammation and hormones, respectively. (3) Follow-Up: n/a
Efficacy Evaluations	This does not apply, as this is not a true interventional trial. These are healthy participants.

Safety Evaluations	The safety of the participants will be ensured by trained research staff and nurses from the UNC Clinical and Translational Research Center (CTRC), who will be on-hand for the intervention sessions, with the supervision of the PI. The co-investigating physician (Samantha Meltzer-Brody) will be on-call during all sessions, and the investigational drug service at UNC is available for consultation. A single does of propranolol is expected to be very well-tolerated in this young, healthy population.
Statistical And Analytic Plan	To address our primary aim, we will conduct multilevel model (MLM) analyses examining levels of inflammation, with time (baseline, pre-stress, 30-, 60-, 90-min post-stress) as a within-subjects factor and condition (propranolol vs. placebo) as a between-subjects factor. We hypothesize that individuals in the placebo condition will show an increase in levels of the inflammatory marker IL-6 from pre- to post-stress, while those in the propranolol condition will show significantly lower levels of inflammation over time compared to the placebo condition (i.e., no stress-related increase in inflammatory markers). We will also conduct similar multilevel modelling analyses on our other secondary endpoints (i.e., cortisol, salivary alpha amylase, PEP, RSA, negative affect).
DATA AND SAFETY MONITORING PLAN	The PI and the project coordinator will be responsible for data quality management and ongoing assessment of safety. All data collected for the study will be stored on secure, password-protected servers maintained by the Department of Psychology and Neuroscience (or on secure, password-protected computers in the laboratory. Safety monitoring will be provided by the PI and the study physician, together with nurses from the UNC CTRC and the study coordinator.

1 BACKGROUND AND RATIONALE

1.1 Introduction

Psychological stress is implicated in the onset and progression of many common and costly chronic diseases, including cardiovascular disease, chronic pain conditions, and major depressive disorder (Cohen et al., 2007; Kendler et al., 1999; Steptoe and Kivimäki, 2012). An emerging body of evidence suggests that inflammation, indexed via levels of pro-inflammatory cytokines and reactive proteins, may be a key biological mechanism by which stress affects health (Baker et al., 2012; Miller et al., 2009; Slavich et al., 2010). Indeed, psychological stressors can induce increases in inflammation (Slavich and Irwin, 2014; Kiecolt-Glaser et al., 2003; Rohleder, 2014; Steptoe et al., 2007), and greater levels of inflammation may contribute to the development of disease (Capuron and Miller, 2004; Choy and Panayi, 2001; DellaGiola and Hannestad, 2010; Raison and Miller, 2013; The Emerging Risk Factors Collaboration, 2010). Despite this growing literature linking stress, inflammation, and poor health, little is known about the precise physiological mechanisms linking psychological stress and increases in inflammation.

One hypothesized mechanism that may translate psychological stress into increases in levels of inflammation is activation of the sympathetic nervous system (SNS). The SNS is part of the autonomic nervous system and is primarily indexed by release of the catecholamines epinephrine (adrenaline) and norepinephrine (noradrenaline). Prior research in non-human animal models has shown that stress-induced SNS activation leads to increases in levels of pro-inflammatory cytokines inflammation (Bierhaus et al., 2003; DeRijk et al., 1994; Kop et al., 2008; van Gool et al., 1990), while pharmacologically blocking sympathetic activation attenuates the inflammatory response to stress (Bierhaus et al., 2003). However, no known human studies to date have examined the relationship between psychological stress, SNS activation, and inflammation. The present study is designed to address this major gap in our knowledge of the physiological mechanisms that may link stress and disease.

A potential reason for the lack of human research linking stress, SNS activation, and inflammation is that SNS activity is difficult to measure. Indeed, adrenaline and noradrenaline are released into the bloodstream very rapidly during a stressor, making their kinetics difficult to capture during typical laboratory-based stress paradigms. Indirect measures of SNS activity may be acquired using psychophysiological approaches that involve peripheral measures of electrical activity and efficiency of the heart; however, these methods provide only indirect indicators of SNS activity, making them subject to criticism in the psychoneuroimmunology community.

To circumvent these issues with assessment of SNS activity, the present study will employ a psychopharmacological approach to experimentally block SNS activity using the drug propranolol. Propranolol is a beta-blocker medication that is very commonly prescribed by physicians in the United States for the treatment of hypertension, given that it blocked adrenergic receptors that lead to relaxation of the cardiac muscle and smooth muscle tissue. Interestingly, propranolol is also sometimes prescribed to individuals who have performance anxiety (i.e., public speaking anxiety), as reducing SNS activity (i.e., eliminating the increased heart rate, blood pressure, sweaty palms, etc., that typically accompany anxiety-provoking situations) has been anecdotally observed to decrease perceptions of stress during these situations. Psychological scientists have recently become more interested in the role SNS activity may play in the formation and reconsolidation of fear memories, and a number of studies have now used propranolol to investigate if blocking SNS activity may help treat individuals with Post-Traumatic Stress Disorder (PTSD; Pitman et al., 2002; Vaiva et al., 2003). However, only one known study to date has investigated if propranolol reduces stress-induced immune system activation (Benschop et al., 1994), and this (now dated) study did not specifically explore if propranolol reduces *inflammatory* responses to stress. Furthermore, no known studies have examined if blocking SNS activity with propranolol changes individuals' appraisals of the stressful situation, or their affective responses to stress. Results from this study will complement and extend the existing work on how SNS activity affects fear memories and stress by focusing on how propranolol affects inflammatory and psychological responses to a stressor.

In addition to these primary goals of the present study, we will also explore the role of SNS activation in a number of additional tasks that are hypothesized to be affected by sympathetic arousal. More specifically, we will examine if exposure to propranolol eliminates implicit biases toward out-group members (in this case, African Americans), given that a very large literature suggests that many White Americans hold implicit biases against African Americans (Witenberk et al., 1997; Nosek et al., 2002). While it has been hypothesized that sympathetic arousal based on cultural stereotypes associating African Americans with negativity may be leading to these implicit biases, no known studies have investigated this issue. We will also explore of SNS activation is critical for empathy, or our ability to understand the emotional states of others, for avoiding risky decisions, and for moral judgments. Thus, this study will also answer a number of important, unanswered questions in social psychology regarding the role that sympathetic arousal plays in some of our most fundamental psychological processes.

In sum, this randomized, double-blind, placebo-controlled study of propranolol will shed important light on how SNS activation influences our psychological and inflammatory responses to stress. Results from this study will inform both the basic science literature that is attempting to map the physiological mechanisms by which psychological stress may lead to poor mental and physical health, and may also ultimately have therapeutic relevance for individuals who are experiencing high levels of stress that is putting their health at risk. By utilizing psychopharmacological approaches, we will circumvent many of the challenges of conducting this research in human populations, and we will also be in a place to draw strong conclusions regarding causality, given that we will have experimentally manipulated SNS activation, rather than relying on correlational measures of SNS activity that are difficult to assess and are not appropriate for asking if SNS activity *causes* changes in psychology and biology.

1.2 Name and Description of Investigational Product or Intervention

This study entails a one-time, 40 mg dose of short-acting propranolol to assess its immediate/short-term effects on inflammation, stress hormones, autonomic physiology, and psychological responses to an acute stressor. Propranolol is typically prescribed to treat hypertension and other heart-related conditions, but it sometimes used to treat performance anxiety. The participants enrolled in this study are medication-naïve, thus the administration of propranolol departs from the participant's routine clinical care.

1.3 Non-Clinical and Clinical Study Findings

Propranolol blocks the effects of epinephrine and norepinephrine by competitively binding to both β_1- and β_2-adrenergic receptors in both the peripheral and central nervous system. It was originally used to treat hypertension, approved by the FDA in 1965. Since then it has been FDA-approved for a variety of other indications, including migraine prevention, and essential tremor. It is even approved for certain pediatric conditions. Propranolol is one of the historically most commonly-used prescription medications, and it is generally viewed as a safe, nontoxic medication in the daily dosage range of up to 640 mg, divided into 2-4 doses per day. The 40mg dose to be given here is expected to be well-tolerated, with the most adverse side effect anticipated being the possibility of allergic reaction. Propranolol rarely causes side effects, including: hypotension, insomnia, fatigue, light-headedness, nausea, brochospasm, dizziness, headache, hyperglycemia, and muscle weakness. Each of these side effects is uncommon at the low, single dosage being utilized in these studies. Moreover, these side effects are not only rare, but also quite mild and rarely prolonged (i.e. < 1-2 hours) when they do occur. If any of these side effects do occur, the subjects will be given a comfortable place to lie down until the symptoms pass, and will be monitored by the CTRC nurse and the project coordinator.

Studies of the pharmacokinetics of propranolol suggest that peak plasma concentrations following a 40 mg dose occur 1-2 hours after administration; as such, we will have participants rest quietly for 1 hour following administration of the medication before the start the stress task. Plasma half-life of propranolol is 3-6 hours, and as such, we will examine our more exploratory aims at the end of the session, which is still within the half-life of the drug.

The study MD will be on-call during all intervention sessions to assess any side effects and prescribe appropriate management as needed, and a CTRC nurse will be present throughout the majority of study procedures. Given that we do not anticipate any serious side effects to occur and that there is currently no data

to suggest that an acute dose of propranolol leads to serious side effects in healthy populations, we believe the facilities at Howell Hall are sufficient to manage the mild, transient side effects that may occur in response to taking propranolol.

1.4 Relevant Literature and Data

See Section 1.1 (Introduction) above and references at the conclusion of this proposal for a literature review and references.

2 STUDY OBJECTIVE

The purpose of this study is to determine if SNS activation is a key physiological mechanism linking psychological stress and inflammation. Thus, we will experimentally-block SNS activation using propranolol, a beta-blocker, and examine if exposure to propranolol attenuates inflammatory responses to a psychological stressor.

2.1. Primary Objective

Primary Objective 1. To examine if blocking sympathetic nervous system activity via the beta-blocker propranolol will attenuate inflammatory responses to stress.

2.2 Secondary Objectives

Secondary Objective 1. To examine if blocking beta-adrenergic receptors with the drug propranolol will attenuate cortisol responses to an acute, laboratory stressor.

Secondary Objective 2. To examine if blocking beta-adrenergic receptors with the drug propranolol will attenuate salivary alpha amylase responses to an acute, laboratory stressor.

Secondary Objective 3. To evaluate if blocking beta-adrenergic receptors with the drug propranolol will attenuate sympathetic nervous system responses (i.e., pre-ejection period; PEP) to an acute, laboratory stressor.

Secondary Objective 4. To examine if blocking beta-adrenergic receptors with the drug propranolol will change parasympathetic nervous system responses (i.e., respiratory sinus arrhythmia; RSA) to an acute, laboratory stressor.

Secondary Objective 5. To examine if blocking beta-adrenergic receptors with the drug propranolol changes self-reported psychological responses (i.e., negative affect) to an acute laboratory stressor.

3 INVESTIGATIONAL PLAN (brief overview)

3.1 Study Design

This is a double blind, randomized, placebo-controlled trial of the effects of propranolol on inflammatory responses to psychological stress in healthy participants. We will use a between-subjects manipulation, given that repeated exposures to the stress task may lead to habituation in both psychological and physiological responses, introducing a potential confound in order-effects. It consists of three stages: 1) telephone screening 2) in-person Session I and 3) in person Session II.

Telephone screening. The telephone screening will be conducted by trained research assistants in the Carolina Social Neuroscience and Health Laboratory, under the supervision of the PI. Inclusion and exclusion criteria will be assessed, and study procedures will be described. If participants meet inclusion criteria and are interested in participants, they will scheduled for Session I.

Study Session I. Session I will last approximately 30-60 minutes. Participants will enter the lab in Howell Hall at UNC-Chapel Hill, meet the research assistant who is running the session, and read through the consent form. Once participants have been given a chance to ask any questions about the consent form and it is clear that they understand the protocol and are comfortable with proceeding, they will sign a copy of the General Consent Form. Next, subjects are informed in greater detail about the procedure for a heartbeat detection task as a measure of their interoceptive or inner bodily awareness. Task descriptions, instructions, and an opportunity to ask questions will be provided to each participant. The heartbeat detection task will take about 20 minutes to complete, and will be administered by trained research assistants. Following completion of the

heartbeat detection task, the experimenter will reiterate to the participant the general purpose of Session II and remind participants of their scheduled date for Session II.

Study Session II. Session II will last approximately 5 hours and will take place in the emotion induction laboratory in Howell Hall at UNC-CH. When the participant arrives for the session, they will meet the lab manager and the nurse supplied by CTRC. The lab manager will give the participant a chance to review the consent form the signed during Session I, and will then remind the participant of the protocol and tasks in Session II. Once the participant understands the protocol and is comfortable with proceeding, the nurse will insert a catheter into the participant's non-dominant forearm, which will be used to draw all the blood samples. Over the course of the five hours, there will be five blood draws, four saliva samples, and a series of questionnaires. The participants will also complete the Trier Social Stress Task during. For this, the participant will give a short speech (10 minutes) while they are evaluated by trained research assistants. Participants will also complete a number of computerized tasks designed to assess our secondary aims. Participants will be fully debriefed at the end of the study.

3.2 Allocation to Treatment Groups and Blinding

The UNC Investigational Drug Service (IDS) will randomly assign patients to the experimental group (propranolol) or the placebo group. The randomizer will be instructed that groups should be matched on age, gender, and racial composition, and will thus be informed of the participant's demographic information in advance of randomization. Research staff who have direct contact with the participant will be blind to the condition of the participant. The psychiatrist who is monitoring safety and possible adverse events while the participants are on propranolol or placebo will be unblinded during the study and therefore able to identify which pill was taken if there are any significant adverse effects felt by the participant after taking the pill. The psychiatrist will not have direct contact with participants during the experimental visits unless the participant is experiencing adverse side-effects. Thus, no study team member who interacts with participants on the propranolol/placebo Session II visit will know whether a participant is on placebo or propranolol. After data collection for the study is completed, the pharmacist who made order assignments will reveal which group participants were in (propranolol vs. placebo) so appropriate analyses can be conducted.

3.3 Study Duration, Enrollment and Number of Subjects

The study is expected to be active for 1-2 years, while we enroll 95 subjects (45 in the placebo condition, 45 in the propranolol condition; with some potential for data loss).

Each subject's participation will last approximately seven hours over the course of approximately two weeks. They will complete a telephone screening for eligibility (~15-20 minutes), an in-person session finalizing eligibility, consent and completing a measure of interoceptive sensitivity (~30-60 minutes), and the intervention session, which will involve taking the study medication, blood draws, saliva samples, the psychological stress task, and computerized tasks and questionnaires (~5 hours). We expect approximately 1 week to pass between Session I and Session II.

3.4 Study Population

Participants in this study will be healthy, undergraduate students from the UNC- Chapel Hill community, between the ages of 18-25 who are fluent in English (spoken and written) and in good overall health.

Exclusion Criteria

1) presence or history of chronic physical illness (especially disorders with an inflammatory component, such as rheumatoid arthritis, asthma, allergies, or issues that can affect the heart, including low-blood pressure or other heart conditions)
2) presence or history of psychiatric illness (depression, anxiety)
3) any current prescription medication use
4) currently pregnant or planning to become pregnant;
5) engagement in a number of health-compromising behaviors that may affect levels of pro-inflammatory cytokines, including cigarette smoking, excessive caffeine intake and sleep disturbance (e.g., working night shifts);

6) body mass index (BMI) greater than 30, given that adiposity is known to relate to baseline levels of inflammation;
7) anxiety about or previous history of problems with blood draws (e.g., fainting)
8) any reported heart conditions

4 STUDY PROCEDURES (what will be done)

4.1 Screening Procedures and Baseline Visit

Prospective participants who email the Social Neuroscience and Health Lab regarding participation will first be screened via telephone by the lab manager or other trained research assistant in the lab to ensure they meet eligibility requirements. Eligible participants will then be scheduled for Session I. Participants who endorse one or more exclusionary criteria will be notified that they are not eligible for the present study, but will be encouraged to keep an eye out for flyers and announcements regarding other research projects in our lab if they are interested in participating in other studies. See attached telephone screening script for more information.

Session I will last approximately 30-60 minutes. Participants will enter the lab, meet the research assistant who is running the session, and read through the consent form. Once participants have been given a chance to ask any questions about the consent form and it is clear that they understand the protocol and are comfortable with proceeding, they will sign a copy of the General Consent Form. They will then follow the experimenter to a private testing room in our lab in Howell Hall, where our psychophysiological equipment for the heartbeat detection task is located (Howell 119). There, subjects are informed in greater detail about the procedure for the heartbeat detection task as a measure of their interoceptive or inner bodily awareness and also the conceptual knowledge questionnaires. Task descriptions, instructions, and an opportunity to ask questions will be provided to each participant. The heartbeat detection task will take about 20 minutes to complete, and will be administered by trained research assistants. Following completion of the heartbeat detection task, the experimenter will reiterate to the participant the general purpose of Session II and remind participants of their scheduled date for Session II. Participants are told that they do not have to complete Session II if they do not wish, and that they will still receive $20 for Session I. Those participants who do *not* wish to continue the study will be paid and dismissed. Participants who ARE continuing with Session II will be reminded to get a good night's sleep before the next session, to not exercise or use over-the-counter medication the morning of the session, and to not intake any caffeine in the two hours before the beginning of the session. These instructions are given because sleep, exercise, anti-inflammatory use and caffeine intake are all known to impact levels of inflammation. Participants will also be asked to eat a satisfying meal and to be well-hydrated before coming to the lab for Session II, to lessen the likelihood of fainting during blood draws or fatigue during the session.

4.1 Intervention/Treatment procedures (by visits)

Session II (the intervention/treatment visit) will last approximately 5 hours and will take place in the emotion induction laboratory in Howell Hall at UNC-CH. When the participant arrives for the session, they will meet the lab manager and the nurse supplied by CTRC. The lab manager will give the participant a chance to review the consent form the signed during Session I, and will then remind the participant of the protocol and tasks in Session II. Once the participant understands the protocol and is comfortable with proceeding, the nurse will insert a catheter into the participant's non-dominant forearm, which will be used to draw all the blood samples. We use the catheter method so that participants do not have to be stuck with needles multiple times throughout the experiment. Once the catheter is placed, the participant will be given at least 45 minutes to acclimate to the catheter, during which time they will be prepared for psychophysiological data collection. We are collecting Heart Rate, Respiratory Sinus Arrhythmia, Cardiac Impedance, and Blood Pressure measures from participants throughout the session and, which will allow us to get a good index of participants' autonomic nervous system responses to the stress task, and will provide a biological "manipulation check" to ensure that the propranolol has had the intended effect (it should lower heart rate and blood pressure). Again, as with

Session 1, female research assistants will be assigned to female participants, while both male and female research assistants will be assigned to male participants, in an effort to reduce participant discomfort with the procedure of attaching the sensors. Once sensors have been attached, we will collect 5 minutes of baseline data while the participant just rests and relaxes.

Upon completion of the baseline psychophysiological measures, participants will complete trait questionnaire measures while they continue to acclimate to the catheter and the laboratory environment. Once 45 minutes has passed (around 1:15 PM), the nurse will draw the first (baseline) blood sample, and participants will be asked to provide the first (baseline) saliva sample. Next, the oral tablet of propranolol or placebo will be administered to the participant by the nurse. Participants will continue completing trait questionnaires and once finished, they will be given neutral reading material (magazines) to read while drug activation takes place. One hour after administration of the medication (or placebo), the nurse will draw a second blood sample, and participants will be asked to provide a second saliva sample. We will also collect another 5 minutes of psychophysiological data.

Next, the Trier Social Stress Task (TSST; Kirschbaum, Pirkem, & Hellhammer) procedures will be explained to participants. The TSST is a commonly-used acute stress test to measure psychological and biological responses to stress in the laboratory. In this task, participants will be asked to prepare for and give a 10-minute speech and complete a difficult mental arithmetic task as they are evaluated by trained interviewers. The nature of the task is stressful and participants may experience varying levels of stress, anxiety, and possibly embarrassment. The consent form at the beginning of the study describes that they will take part in a "role play" during a cognitive performance task (see Consent Form). We describe the task in this vague way in the initial consent because we want to ensure that the baseline physiology data is clean and not impacted by participants' anticipatory stress about the upcoming task. Additionally, prior to the participant's participation in the TSST, participants will informed in greater detail about the procedure for TSST, and they will be asked if they would like to continue. If they do continue, but wish to stop at any time, they will be reminded that they can.

After being informed of the procedures and confirming participants would like to continue, the experimenter will introduce two more researchers who will act as the interviewers / judges. Participants will have 2 minutes to prepare for the speech, in which they will be asked to answer why they are the perfect candidate for their dream job. Participants will then give a 10 minute speech in front of the two interviewers and a video camera. After the 10 minute speech, participants will complete a verbal math task for 5 minutes. While participants are completing the tasks, interviewers will remain neutral (give neither positive nor negative feedback). The experimenter leading Session II will also be present throughout, to help with timing the tasks, etc.

Following completion of the TSST procedures, participants will complete a second round of questionnaires to measure their affective responses, somatic responses, and interpersonal appraisals during and after the TSST. Fifteen minutes after termination of the TSST, a third saliva sample will be taken, and 30 minutes after the termination of the TSST, a fourth (and final) saliva sample, and third blood sample, will be taken. Additional blood samples will be taken 60 minutes (sample #4) and 90 minutes (sample #5) following termination of the TSST. In between blood samplings, participants will be given neutral reading material. Following collection of the final blood sample, the nurse will remove the catheter and check the participant's vital signs. The nurse will then be dismissed back to the CTRC.

For the remaining 60 minutes of the session, participants will complete four computerized tasks designed to measure other psychological processes (besides stress) thought to be influenced by sympathetic nervous system activation. A list of tasks and questionnaires is included below.

In addition, at the completion of the study, participants will be fully debriefed. Contact information for the PI, Study Physician, and IRB is provided on the consent form and debriefing form (which participants can keep) if they wish to address any concerns they had.

4.2 Follow-up procedures

There will be no scheduled follow-up; however, participants will be given the contact information of the PI and study physician and instructed to contact us if they experience any side effects or discomfort following the study.

4.3 Subject Completion/ Withdrawal procedures

If a participant wishes to discontinue their participation in the study before it ends, they will receive $25 for each hour that they participated. Upon completion of the study, they will receive $125.

5. STUDY EVALUATIONS AND MEASUREMENTS (how measurements will be made)

Details of Heartbeat Detection Task: We will use the well-validated Heartbeat Detection Task (Barrett et al., 2004; Kleckner et al., 2016) to provide an index of participants' interoceptive sensitivity (i.e., how aware they are of physiological changes in the body). To do so, we will first prepare the participant for psychophysiology data collection. Participants are given the chance to go to the restroom if need be and get comfortable before we attach the sensors. Female research assistants will be assigned to female participants, while both male and female research assistants will be assigned to male participants, in an effort to reduce participant discomfort with the procedure of attaching the sensors. Only three sensors will be attached for this task as is standard for basic EKG measurement. One sensor will go on the participant's right collarbone, and two more sensors right below their ribs. Once sensors have been attached, we will collect 5 minutes of baseline data while the participant sits and relaxes. This baseline is important for us to help generate participant-specific trials for the heartbeat detection task itself. Next, participants will either complete the heartbeat detection task or our questionnaire measures of conceptual knowledge. These two tasks are counterbalanced because we suspect that the activation of interoceptive concept knowledge could temporarily elevate interoceptive sensitivity--and likewise, interoceptive sensitivity could activate interoceptive concept knowledge. Therefore, counterbalancing each task should help reduce order effects. For the heartbeat detection task, we use MATLAB to automatically generate a person-specific heartbeat task based on their baseline cardiac data. Participants put on headphones and follow the on-screen instructions provided to them. They are instructed to listen for a series of 3 beeps in their headphones then must indicate on the computer whether these beeps occurred *between* their current heartbeats or *during* their current heartbeat. Participants also rate how confident they are in their response. Participants have 2 practice trials with the research assistant to ensure they understand the task, and then complete the 60 target trials on their own in the private, quiet lab room. Accuracy is computed by comparing participant's hits and misses, and determining if they were accurate above chance. After both the heartbeat detection and conceptual knowledge tasks are done, the experimenter will help the participant remove the psychophysiology sensors. The sensors must stay on across both tasks since they are counterbalanced and we wish to keep all psychological factors as equal as possible.

Details of the Trier Social Stress Task (TSST). The TSST is a commonly-used acute stress test to measure psychological and biological responses to stress in the laboratory. In this task, participants will be asked to prepare for and give a 10-minute speech and complete a difficult mental arithmetic task as they are evaluated by trained interviewers. The nature of the task is stressful and participants may experience varying levels of stress, anxiety, and possibly embarrassment. The consent form at the beginning of the study describes that they will take part in a "role play" during a cognitive performance task. We describe the task in this vague way in the initial consent because we want to ensure that the baseline physiology data is clean and not impacted by participants' anticipatory stress about the upcoming task. Additionally, prior to the participant's participation in the TSST, participants will be informed in greater detail about the procedure for TSST, and they will be asked if they would like to continue. If they do continue, but wish to stop at any time, they will be reminded that they can.

After being informed of the procedures and confirming participants would like to continue, the experimenter will introduce two more research assistants who will act as the interviewers/judges. Participants will have 2 minutes to prepare for the speech, in which they will be asked to answer why they are the perfect candidate for their dream job. Participants will then give a 10-minute speech in front of the two interviewers

and a video camera. After the 10-minute speech, participants will complete a verbal math task for 5 minutes. While participants are completing the tasks, interviewers will remain neutral (give neither positive nor negative feedback). The experimenter leading Session II will also be present throughout, to help with timing the tasks, etc.

Biomarker Information:

Saliva Samples. We will take 4 saliva samples during the study via the passive drool method. Passive drool is a saliva collection method for collecting whole saliva. Passive drool is considered by many researchers to be the gold standard when collecting saliva samples for biological testing, because it provides the purest sample possible and allows researchers to "biobank" samples for testing. Participants allow saliva to pool in mouth, then, with head tilted forward, gently force saliva through a small straw and into the vial. Participants will be aided by trained research assistants wearing rubber gloves. About 2ml of saliva will be collected at each sampling time. Saliva samples will be assayed for levels of the stress hormone, cortisol, as well as another HPA axis byproducts including ACTH and testosterone.

Blood Samples. We will draw 10 mL of blood into vacutainer EDTA tubes at each time point, which will be stored on ice for the duration of the study, and then centrifuged to isolate plasma and stored in 1 mL aliquots in cryovials in a -80 degree freezer until the study is complete. Samples will then be assayed for levels of pro-inflammatory cytokines (i.e., interleukin-6, tumor necrosis factor alpha, interleukin-1) using multiplex bead-based assays.

Psychophysiology. We will collect electrocardiogram and impedance cardiography data from participants to examine how propranolol and exposure to the TSST influence autonomic physiology.

Questionnaires (for exploratory moderator analyese):

Demographics.

We ask participants' age, gender, race/ethnicity, weight/height (to calculate BMI), perceived SES, and also about participants' comfort with giving speeches / being the center of attention.

Health Behavioral Questionnaire (Muscatell, 2016). This asks a series of questions to make sure that participants followed instructions prior to the session and are still eligible to participate. It includes items "Which medications have you taken in the last 48 hours?"

UCLA Loneliness Scale: (Russell, D., Peplau, L.A., and Ferguson, M.L 1978) This is a commonly used, 10 item, measure of loneliness.

The PHQ-9: (Spitzer, 1999) This a multipurpose, validated instrument for screening, diagnosing, monitoring and measuring the severity of depression.

Perceived Stress Scale: (Cohen, 1994) This is a widely used psychological instrument for measuring the perception of stress; i.e the degree to which situations in one's life are appraised as stressful.

Social Provisions Scale (Cutrona, C. E. and Russell, D., 1987). This examines the degree to which respondent's social relationships provide various dimensions of social support.

Fear of Evaluation Scale **(**Leary 1983). This is a is a 30-item, self-rated scale used to measure social anxiety.

State-Trait Anxiety Inventory (Spielberger 1983) this inventory has 40 self-report items pertaining to anxiety affect.

The MacArthur Scale of Subjective Social Status: (Adler, 1984) This is a commonly used measure subjective social status. In an easy pictorial format, it presents a "social ladder" and asks individuals to place an "X" on the rung on which they feel they stand. There are two versions of the ladder, one linked to traditional SES indicators and the second linked to standing in one's community, in this case UNC. We will give them both. It is useful to be able to make comparisons between objective and subjective SES.

Parent SES: This is a six item questionnaire to assess the socioeconomic status of the participant and their family. It will be used to make comparisons between objective and subjective SES.

PANAS-circumplex questionnaire. Participants rate which emotions they felt during the TSST and also how they are feeling currently. Participants rate how intensely they felt those emotions using a Likert

scale. Additionally, participants report how clear their emotions were during the TSST. This is our measure of discrete emotion, perceived emotional intensity, and perceived emotional clarity.

Somatic Sensations questionnaire. Participants rate which somatic sensations they felt during the TSST and also rate how clearly / distinctly they experienced those bodily changes. Participants use Likert scales for their ratings. We will also assess the participants' feelings about the interviewers using standardized measures.

Challenge threat appraisals: Blascovich, J. & Mendes, W. B. (2000). This questionnaire taps into the degree to which the participant views new experiences as a potential challenge or threat.

6. STATISTICAL CONSIDERATION

Dr. Muscatell and her trainees will be responsible for statistical computations and data analysis. She will consult with colleagues in the Quantitative Psychology area of the Department of Psychology and Neuroscience as needed. All hypothesis tests will be evaluated using 95% confidence intervals, corresponding to a *p* value or .05. Only tests that indicate a *p* value of .05 or less will be considered statistically significant; tests above this *p* value will not be interpreted.

6.1 Primary Endpoint- Statistical Methods

Primary Objective 1. To examine if blocking sympathetic nervous system activity via the beta-blocker propranolol will attenuate inflammatory responses to stress.

Analysis Plan. To address this aim, we will first examine if there was a change in inflammation simply as an effect of taking the propranolol (before stress induction). As such, we will conduct a multilevel model (MLM) examining levels of inflammation (IL-6), with time (BL vs. pre-TSST) as a within-subjects factor and condition (propranolol vs. placebo) as a between-subjects factor. If the result of this test shows a significant interaction, then we will only include the pre-TSST time point in subsequent models. If non-significant, we will use both baseline time points in subsequent analyses. Next, we will conduct an MLM examining levels of inflammation, with time (BL and/or pre-TSST, 30-, 60-, 90-min post-TSST) as a within-subjects factor and condition (propranolol vs. placebo) as a between-subjects factor. We hypothesize that individuals in the placebo condition will show an increase in levels of inflammatory markers from pre- to post-TSST, while those in the propranolol condition will show significantly lower levels of inflammation over time compared to the placebo condition (i.e., no stress-related increase in inflammatory markers).

All outcomes will first be examined using histogram and scatterplot for assumptions of normality and homoscedasticity. Any variables that need to be log-transformed (e.g., cortisol, sAA, IL-6) will be before analyses are run. Across all models, we will evaluate if we need to control for sex, BMI, and objective SES.

Prior to analyses, we will also examine each outcome for outliers existing +/- 3 SDs from the mean within each time point within each condition. Outliers are excluded only within the time point where they were problematic but retained elsewhere to reduce data loss.

All data will be analyzed in R using the lme4 package. As time points are nested within individuals, we take a multilevel modelling approach with the inclusion of a random intercept to model individual differences in each outcome.

6.2 Secondary Endpoints- Statistical Methods

Analysis Plan. To address secondary objectives, we will follow the identical procedure outlined above, but will swap in the relevant outcome as the dependent variable (i.e., cortisol, salivary alpha amylase, PEP, RSA, psychological responses). We will first examine if there was a change in the outcome simply as an effect of taking the propranolol (before stress induction). As such, we will conduct a multilevel model (MLM) examining levels of the outcome, with time (BL vs. pre-TSST) as a within-subjects factor and condition (propranolol vs. placebo) as a between-subjects factor. If the result of this test shows a significant interaction, then we will only include the pre-TSST time point in subsequent models. If non-significant, we will use both baseline time points in subsequent analyses. Next, we will conduct an MLM examining levels

of each relevant outcome, with time (BL and/or pre-TSST, post-TSST time-points) as a within-subjects factor and condition (propranolol vs. placebo) as a between-subjects factor.

6.4 Sample Size and Power

We are planning to enroll 45 participants per condition in our study, for a total of 90 participants. The sample size was selected based on a thorough literature review of prior studies that have examined the effects of propranolol on psychological tasks in healthy participants (similar to those in the present study) revealed the average sample size of each condition to be 21 participants (22 in the propranolol condition, 21 in the placebo condition). Thus, by randomizing 45 participants to each condition, we are sufficiently powered to achieve our aims. We have included more participants than prior work in this area because we are interested in examining *moderators* of our main effects of interest, and such analyses require greater sample size to be sufficiently-powered. We also considered availability of possible eligible participants when making this decision; we do not anticipate significant difficulty in finding participants who meet our inclusion/exclusion criteria, given that they are relatively open and should be relatively prevalent in our target recruitment population (i.e., primarily healthy, undergraduate students). Finally, we also considered practical constraints in terms of funds and time available to complete the study. As the project will be funded off of Dr. Muscatell's laboratory start-up funds, we do not currently have grant funds to enroll additional participants. We are also hoping to complete the project within one year so that we can use the data generated by the project as preliminary data for a grant to be submitted to NIH to run a similar study including a neuroimaging (fMRI) component.

7. STUDY INTERVENTION (drug, device or other intervention details)

Description. Propranolol blocks the effect of epinephrine and norepinephrine by competitively binding to on both β_1- and β_2-adrenergic receptors in both the peripheral and central nervous system. It was originally used to treat hypertension, approved by the FDA in 1965. Since then it has been FDA approved for a variety of other indications, including migraine prevention, and essential tremor. It is even approved for certain pediatric conditions. Propranolol is one of the historically most commonly-used prescription medications, and it is generally viewed as a safe, nontoxic medication in the daily dosage range of up to 640 mg, divided into 2-4 doses per day.

Receipt/Storage. The project manager will pick up the medication at IDS the day prior to the participants second session. All medication tablets will be stored in a room temperature locked cabinet in the Muscatell Lab in Howell Hall at UNC. Only the project coordinator and the PI will have a key for this cabinet.

Packaging/Labeling. Medication will be packaged and labeled in accordance with IDS protocol.

Dosing. Each participant will receive 40mg of propranolol, or a tablet of equivalent size of a placebo.

Treatment compliance and Adherence. This is a one-time dosage, so not applicable.

Drug Return/Destruction. If a participant does not show up for their appointment, we will return the drug to IDS and they will destroy it in accordance with their protocol.

8. STUDY INTERVENTION ADMINISTRATION (if applicable)

This is a double blind, randomized clinical trial. IDS will randomly assign patients to the experimental group (propranolol) or the placebo group. Research staff who have direct contact with the participant will be blind to the condition of the participant. The psychiatrist who is monitoring safety and possible adverse events while the participants are on propranolol or placebo will be unblinded during the study and therefore able to identify which pill was taken if there are any significant adverse effects felt by the participant after taking the pill. The psychiatrist will not have direct contact with participants during the experimental visits unless the participant is experiencing adverse side-effects. Thus, no study team member who interacts with participants on the propranolol/placebo Session II visit will know whether a participant is on placebo or propranolol. After data collection for the study is completed, the pharmacist who made order assignments will reveal which group participants were in (propranolol vs. placebo) so appropriate analyses can be conducted.

9. SAFETY MANAGEMENT

The 40mg dose of propranolol to be given in this study is expected to be well-tolerated in this young, healthy population. The greatest risk to participants taking this medication is allergic reaction, and we will assess all medication allergies prior to study enrollment. Propranolol rarely causes side effects, including: hypotension, insomnia, fatigue, light-headedness, nausea, brochospasm, dizziness, headache, hyperglycemia, and muscle weakness. Each of these side effects is uncommon at the low, single dosage being utilized in these studies. Moreover, these side effects are not only rare, but also quite mild and rarely prolonged (i.e. < 1-2 hours) when they do occur. If any of these side effects do occur, the subjects will be given a comfortable place to lie down until the symptoms pass, and will be monitored closely by the nurses and research staff.

The study MD will be on-call to assess any side effects and prescribe appropriate management as needed, and a CTRC nurse will be present throughout the majority of study procedures. Given that we do not anticipate any serious side effects to occur and that there is currently no data to suggest that an acute dose of propranolol leads to serious side effects in healthy populations, we believe the facilities at Howell Hall are sufficient to manage the milder, transient side effects that may occur in response to taking propranolol.

The MD who is monitoring safety and possible adverse events while the participants are on propranolol or placebo will be un-blinded during the study and therefore able to identify which pill was taken if there are any significant adverse effects felt by the participant after taking the pill. The psychiatrist will not have direct contact with participants during the experimental visits unless the participant is experiencing adverse side-effects. Although they are not anticipated, any adverse events will be immediately reported to the UNC IRB.

10. DATA COLLECTION AND MANAGMENT

The study coordinator, Emma Armstrong-Carter, will be responsible for data management, with supervision from the PI. Data entry will be captured and managed using the REDCap software provided by the NC TraCS Institute, which is HIPPA compliant and provides a state-of-the-art system for data entry and management, especially for a study such as that proposed in the present protocol that includes measures of multiple endpoints. All Information and data collected for the purpose of this research study will be kept confidential as required by law. Telephone numbers and e-mail addresses will be used for scheduling study sessions. These identifiers will be stored separately from any data. All copies of test records and results will be kept in computer files that require a password to open. All information will be accessible only to authorized personnel. Codes will be used on all data sheets in place of names. No subjects will be identified in any report or publication about this study. A single file will link the identifying information to participant's subject ID code and that will only be stored on the Lab Manager's computer and it will be password protected. Only the Lab Manager and the PI will have access to the password for this file.

Data quality will be ensured with precise documentation throughout Session II. Specifically, both the CTRC nurses and the study coordinator will have "flow sheets" created by the PI that specify when samples should be taken, when the medication should be administered, and when the questionnaires and computer tasks should take place. The study coordinator and nurse will document the precise time that samples are taken during the session, recording reasons for any deviation from the planned timing on the flow sheet. They will also record when tasks and questionnaires are administered, and any other details relevant to the session. Following the session, all timing information and details about the session will be transferred to REDCAp and linked to the rest of the participant's data. Questionnaire measures will be administered in REDCap, and while participants can elect to skip any question they do not feel comfortable answering, a prompt will appear on screen notifying participants if they have skipped a question, to ensure that they have intentionally not answered the question rather than accidentally leaving it blank.

11. RECRUITMENT STRATEGY

Participants will be recruited through posting of IRB-approved flyers around UNC and the neighboring areas (Franklin Street, Carrboro, etc.), announcements made in classes at UNC, and email list-serves at UNC. All

recruitment-related materials will be approved by the IRB, including flyers, e-mails, and announcements scripts. We do not anticipate having any difficulty recruiting the projected number of subjects identified.

12. CONSENT PROCESS

All subjects will participate in the informed consent process. Participants will be familiarized with the protocol by the PI or qualified study personnel, including its risks and benefits, and informed consent will be documented according to the regulations governing human subject research at the University of North Carolina, Chapel Hill, which meet the standards of the NIH.

Upon arrival to participate in Session I, participants will read and have explained to them the informed consent document. Informed consent is obtained in a comfortable, private area in Howell Hall designed for this purpose. Participants' questions about the protocol will be thoroughly answered prior to obtaining consent, with no time limit to this procedure. Upon consent, the subject is free to withdraw that consent at any time, which will be emphasized to the subject. Consent will be obtained by approved study staff.

13. PLANS FOR PUBLICATION

We plan to submit the results from this study to peer-reviewed psychology and neuroscience journals.

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