Neuroinflammatory Mechanisms Linking Chronic Stress to Motivational Deficits Study Protocol, including Statistical Analysis Plan

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Master Protocol Document

Title	Neuroinflammatory Mechanisms Linking Chronic Stress to Motivational Deficits
Sub-Title	The Brain and Stress Study
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Statement of Compliance

This study will be conducted as specified in the protocol and in accordance with the *International Conference on Harmonisation Guidelines for Good Clinical Practice* (ICH E6) and the *Code of Federal Regulations on the Protection of Human Subjects* (45 CFR Part 46).

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the *Institutional Review Board* (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent, using a previously approved consent form.

If required by the IRB, the master protocol document, informed consent form(s), recruitment materials, and all subject materials will be submitted to the *Scientific Review Committee* (SRC) prior to IRB review (research.unc.edu/clinical-trials/src).

The statistical analysis plans will be consistent with guidance in CONSORT Statement [1] or STROBE Statement [2], ICMJE recommendations [3], the 2016 and 2019 statements of the American Statistical Association [4,5], and recommendations in Nature [6,7].*

All personnel involved in the conduct of this study have completed human subjects protection training.

* [1] www.consort-statement.org
[2] www.strobe-statement.org
[3] www.icmje.org
[4] Wasserstein RL, et al. (2016), The ASA's Statement on p-Values, ***The American Statistician***, 70:2, 129-133
[5] Wasserstein RL, et al. (2019), Moving to a World Beyond p < 0.05, ***The American Statistician***, 73:sup1, 1-19
[6] Amrhein, et al. (2019) Scientists rise up against statistical significance, ***Nature*** 567, 305-307
[7] Editorial (2019) It's time to talk about ditching statistical significance: Looking beyond a much used and abused measure would make science harder, but better. ***Nature*** 567, 283-283.

Table of Abbreviations

AE/SAE	Adverse Events/ Serious Adverse Events
CI	Confidence Intervals
CNS	Central Nervous System
HIV	Human Immunodeficiency Virus
HRF	Hemodynamic Response Function
IDS	Investigational Drug Service
NSAID	Non-steroidal anti-inflammatory drugs
OHRE	Office of Human Research Ethics
PET	Positron Emission Tomography
PI	Principal Investigator
SE	Standard Error
SHAPS	Snaith Hamilton Pleasure Scale
TBI	Traumatic Brain Injury

1. Protocol Synopsis

Title	Neuroinflammatory Mechanisms Linking Chronic Stress to Motivational Deficits
Study Description	Motivational deficits such as anhedonia are core to several psychiatric disorders and underlie significant functional impairment. This double-blind, placebo-controlled crossover trial of minocycline, an anti-[neuro]inflammatory agent, examines links between chronic stress and responses to a reward-related motivation task. It will evaluate the effects of pharmacologically attenuating neuroinflammation on behavioral responses to a reward-related motivation task in individuals experiencing unemployment. Understanding the effects of neuroinflammation on reward function among individuals experiencing chronic stress represents a critical first step in identifying novel neuroimmune targets for future clinical trials.
Specific Aims **(objectives)**	**Aim 1:** To examine differences in performance on a pure motivation task following minocycline (vs placebo). **Aim 2:** To examine differences on self-report measures of motivation following minocycline (vs placebo).
Target Population	**Inclusion criteria:** • 25-60 years old • Unemployed (working less than 20 hours per week) • Seeking employment • Having trouble finding job (i.e., actively seeking and applying for jobs but not successful in landing a job) • Reports greater than 5 points on Job Stress Items • Regular access to a mobile phone **Exclusion Criteria:** • Currently enrolled as a student • Prescribed medication for the following physical illnesses: diabetes, cardiovascular diseases, high blood pressure, autoimmune disease, neurological conditions (e.g., TBI, stroke) • Diagnosis of a severe mental illness (i.e., disorders evincing psychotic symptoms, severe major depression, bipolar disorder) • Pregnant or breastfeeding • Daily psychotropic medications including mood stabilizers, stimulants, antipsychotics. • Daily antidepressants use that has been recently added or modified in fewer than 3 months. • Drug or alcohol dependence • Known allergies or hypersensitivities to tetracycline antibiotics, aspirin or other NSAIDs • Current antibiotic use • Regular use of steroidal or non-steroidal anti-inflammatory medications (i.e., 2 or more times a week)
Numbers of Enrollees	A total of N = 54 eligible individuals will be enrolled. We anticipate that at least n = 50 of the enrollees will complete all aspects of the protocol and have complete data.

Interventions	In this crossover design, all participants will take a 5-day course of 200mg of minocycline and 5 days of a placebo-control pill. Sessions will be separated by a minimum washout period of 14 days.
Outcome Measures	**For Aim 1.** Mean difference in accuracy and reaction time to Progressive Reward Task during reward receipt after minocycline course and placebo course. **For Aim 2.** Mean difference in cognitive performance after minocycline course versus placebo course
Statistical Analysis Plans for Each Aim	**Aim 1 Plans.** A paired samples t-test will be conducted to assess whether taking minocycline was associated with significantly better performance on the PRT as compared to when participants are taking a placebo. **Aim 2 Plans.** A paired samples t-test will be conducted to assess whether taking minocycline was associated with differences on two self-report measures of motivation following minocycline (vs placebo). All outcome variables of interest are continuous measures. All statistical estimates of population parameters and corresponding confidence intervals (CIs) will be computed to convey levels of precision.
Study Duration	The entire study is expected to last 12 months, depending on how quickly a participant can be scheduled for the study sessions. Ideally, both sessions will be completed at least 32 days after the initial phone screen.
Participation Duration	Participation includes: 1) a screening session for assessing inclusion/exclusion eligibility, 2) a baseline onboarding visit and receipt of arm 1 of intervention (~1.5 hr) 3) 5-day course of minocycline (or placebo depending on randomization) 4) behavioral study session 1 with computer tasks (~1.5 hours) 5) 5-day course of placebo (or minocycline depending on randomization) 6) behavioral study session 2 with computer tasks (~1.5 hours) Minimum enrollment period for participants is 32 days.

2. Introduction: Background and Rationale

2.1. Background Information

Motivational deficits such as anhedonia are common to several psychiatric conditions, including depression[1] and schizophrenia [2]. Moreover, these deficits are notoriously difficult to treat and predict poorer treatment response [3], greater chronicity of illness [4], significant functional impairment [2], and suicidality [5]. The lack of efficacy for improving motivational deficits in current psychiatric treatments is mainly due to a lack of understanding of the mechanisms that give rise to these transdiagnostic symptoms. One significant risk factor that promotes decreases in motivation is chronic stress [6]. In fact, many preclinical models of anhedonia rely on chronic stress paradigms to induce reductions in motivation and elicit depression-like symptoms [7]. This preclinical work has demonstrated that a critical pathophysiological model linking chronic stress to deficits in motivation is via neuroinflammatory processes [8]. Considering these findings, there is a paucity of work examining the mechanisms linking stress, neuroinflammation, and the neurobehavioral underpinnings of motivation in humans. Translational research aiming to replicate and extend preclinical research in a clinical sample is critical for elucidating the brain mechanisms underlying the reward-related motivational deficits implicated across several disorders. Thus, this project incorporates an experimental therapeutics approach and computational neuroscience techniques to generate information about the neuroinflammatory mechanisms contributing to stress-related motivational deficits. This mechanistic clinical trial will utilize minocycline to reduce inflammation in the brain in a sample of chronically stressed individuals to examine molecular to neural alterations implicated in motivational deficits. Results from the proposed study will shed light on neuroimmune targets for future prevention and intervention efforts.

Evidence linking chronic stress to anhedonia via neuroinflammatory processes. Although less work has examined the effects of stress on anhedonia in humans, evidence across rodents and humans converges to suggest that chronic stressors contribute to the development of motivational deficits and a lack of responsiveness to rewards[6,9]. In rodents, chronic stress causes anhedonia-like behavioral changes[10] (e.g., prolonged immobility, reduced preference for sweetness, and reduced exploratory activity); and in humans, acute and chronic stressors are associated with motivational deficits (e.g., motor slowing [11], reductions in pleasure[12], and reduced ability for participants' to modulate behavior as a function of rewards [13,14]) as well. Additionally, several studies have found that stress-induced remodeling occurs in the corticostriatal circuit (i.e., medial prefrontal cortex, basal ganglia) to attenuate the reward-seeking and goal-directed behaviors involved in motivation[15–18]. However, despite the evidence linking stress to motivational deficits, more research is needed to understand the stress-induced molecular mechanisms driving corticostriatal alterations in humans.

While multiple neurobiological pathways are likely relevant for understanding the influence of chronic stress on motivational processes, preclinical work identifies neuroinflammation as a key molecular mechanism underlying anhedonia. Indeed, chronic stress leads to changes in the central nervous system (CNS) innate immune cells[19], including microglia, astrocytes, and other CNS-infiltrating macrophages. As the brain's resident immune cells, microglia are studied as markers of neuroinflammation[20,21] and are responsible for actively surveying the environment while also maintaining homeostasis to ensure a healthy nervous system. Functionally, neuroimmune cells such as microglia are critical for defending against infectious and stress derived-agents as well as purging damaged or unnecessary neurons and synapses[19]. Consequently, chronic stressors can damage the CNS by modifying microglial threshold of activation, ultimately giving rise to a maladaptive brain immune response[22,23].

The clinical work examining the role of neuroinflammatory processes underlying anhedonia is in its infancy. Hence, an understanding of the neuroinflammatory disruptions to network functioning and communication that may contribute to anhedonia in humans is severely lacking. Nevertheless, there is

some evidence that neuroinflammation is an issue in patients experiencing conditions characterized by motivational deficits. Specifically, post-mortem and PET imaging studies have found that neuroinflammation is elevated in patients with depression and schizophrenia [24–30]. While these studies provide a critical first step in demonstrating the association between neuroinflammation and motivational deficits in patient populations, studies that examine the links between precursors and consequences of neuroinflammation in these living patient populations are necessary.

Although a link between chronic stress and microglia has been established, what lacks understanding is the relationship between alterations in these neuroimmune cells and neural functioning relevant to motivated behavior in humans. In other words, how exactly does chronic stress engender motivational deficits through alterations in neuroinflammatory processes? Limited preclinical evidence demonstrates that alterations in microglial cell function due to chronic stress may be associated with structural and functional changes in the brain [32] and regions within the corticostriatal circuit [33]. Most relevant to the current proposal, experimental evidence in rodents document that neuroinflammatory processes driven by microglia can result in motivational deficits via alterations in neuron-to-glia communication [34–36], neurogenesis [37–39], neuroplasticity [40–43], long term potentiation [44], and neurotransmitter systems [45–47]. For instance, microglial activation has been associated with the selective degeneration of dopamine-producing nerve cells [48]. This is interesting considering that dopamine has been implicated as a critical mediator between neuroinflammation and social behavior [49]. Certainly, microglia produce disturbances in striatal dopamine function and reward-related behavior [50–52]. These findings indicate that more clarity regarding the neuroimmune to neuron interface can be critical for understanding the neurobiology of motivational deficits. A clinical extension of this work, then, should examine how altering levels of neuroinflammation in humans may influence corticostriatal activity while engaging in a motivation task.

What is minocycline? Because neuroinflammation, especially microglia, is implicated in anhedonia, pharmacological strategies to suppress microglial activity or blunt neuroinflammatory processes have been explored as therapies for depression and schizophrenia. One such treatment is minocycline, a tetracycline antibiotic commonly used to study neuroinflammatory mechanisms in preclinical work [53–56]. Specifically, minocycline has been found to attenuate neuroinflammation's deleterious effects on neurogenesis [57], long-term potentiation [58], and neuronal survival [59]. Minocycline's anti-inflammatory mechanism of action is thought to involve an ability to inhibit microglia functioning [53,54]. Unlike most anti-inflammatory agents, minocycline's lipid-solubility allows for its penetration into the CNS [60]. Indeed, minocycline has been shown to only reduce pro-inflammatory levels of the cytokine IL-1β in the brain and not plasma levels of the same cytokine in the periphery [61], validating that minocycline specifically alters neuroinflammatory processes. This relationship was further substantiated in a mechanistic study, which showed that minocycline reduced experimentally induced neuroinflammation and anhedonia-like behaviors in rodents by limiting microglial functioning [61]. The authors also reported that minocycline consistently attenuated the microglial expression of pro-inflammatory response genes across four independent experiments. Although minocycline trials in clinical samples are significantly limited, there is some evidence that minocycline also attenuates microglial functioning in humans[62] and has been shown to reduce negative symptoms scores (including anhedonia) among individuals with schizophrenia [63,64]. Thus, minocycline would be a candidate drug to map neuroinflammatory effects on neural functioning. The proposed minocycline mechanistic trial would be an initial yet necessary step in understanding the molecular and neural alterations underlying motivational deficits. The results obtained here may have implications for identifying the circuit elements that can be modulated to reverse or compensate for neuroinflammation induced alterations. Notably, while minocycline has been used in the preclinical literature to study mechanisms involved in neuroinflammation, there are several other biological actions that minocycline can exert, including anti-apoptotic and antioxidant properties[65].

Unemployment as a translational model of chronic stress. Conceptualizing unemployment stress as a translational examination of chronic stress can help to bridge preclinical and clinical understandings of

inflammation and motivation. In animal models, chronic unpredictable stress paradigms have been pivotal for understanding inflammation-related dysregulation underlying anhedonia, depression, and general psychophysiological health. Because these stress paradigms are unethical to administer to clinical samples, the reproducibility and translation of preclinical work in this area limit the findings' external validity. However, unemployment stress is a natural, ecologically valid model of chronic unpredictable stress such that unemployment stress can beget psychological distress over extended periods[66,67], increase exposure to unpredictable and uncontrollable events[68,69], and has been linked with long-term physiological effects[70-73]. Additionally, unemployment stress has been associated with alterations in immune functioning such that those experiencing longer durations of unemployment are more likely to exhibit higher levels of immune system activation[74]. For example, in one study, those experiencing unemployment were five times more likely to have elevated levels of inflammation[75].

Summary and Significance of the Proposal. This proposal seeks to conduct translational work that extends rich preclinical findings to the clinical domain to validate whether neuroinflammatory dysregulation is strongly tied to anhedonia. This project addresses critical gaps in the scientific literature by recruiting a chronically stressed sample of individuals—employment seeking individuals who report significant stress-- and will use an experimental therapeutics approach to attenuate neuroinflammation and assess neural activity underlying motivational deficits.

2.2. Scientific Rationale

One major obstacle in understanding how neuroinflammation influences human corticostriatal activity involves technological challenges such that conventional approaches are invasive, expensive, and/or lacking specificity. Although static levels of neuroinflammation in humans have been measured, capturing neural dynamics during behavioral tasks has been difficult to image in vivo. This obstacle limits the ability to develop a more precise understanding of how neuroinflammation causes circuit-level dysregulation in humans. The proposed project will employ a mechanistic clinical trial of the anti-[neuro]inflammatory agent, minocycline, to address these limitations. In animal models, minocycline has attenuated the deleterious effects of neuroinflammation on neurogenesis, long-term potentiation, and neuronal survival. This study will extend research to humans to examine whether links between neuroinflammation and neural responses to a reward-related motivation task differ among chronically stressed individuals taking minocycline and the placebo control. The proposed project will provide the first evidence of neural alterations that have relevance for motivational deficits due to neuroinflammation.

3. Specific Aims

Aim 1: To examine differences in performance on a pure motivation task following minocycline (vs placebo). We hypothesize that objective performance on a motivation task will be better when individuals are taking minocycline compared to performance after taking placebo.

Aim 2: To examine differences in performance on a task measuring the influence of motivation on cognitive performance following minocycline (vs placebo). We hypothesize that objective cognitive performance will be better after individuals are taking minocycline compared to performance after taking placebo.

4. Investigational Plan

Study Design: This study is a randomized, double-blind, placebo-controlled, crossover trial.

Brief overview of study events: Potential participants will complete eligibility screening for the study. Participants meeting eligibility criteria will take a 5-day course of minocycline (or placebo) and then complete a reward processing task. They will also complete a self-reported motivational deficits scale (i.e., Snaith Hamilton Pleasure scale[76]). Participants will then complete a 14-day washout period before beginning the other arm of the trial. Again, participants will complete a 5-day course and then complete a reward processing task as well as the Snaith Hamilton Pleasure scale. Participants will be monitored for any adverse events throughout study participation. See Figure 1 for visualization of study procedures.

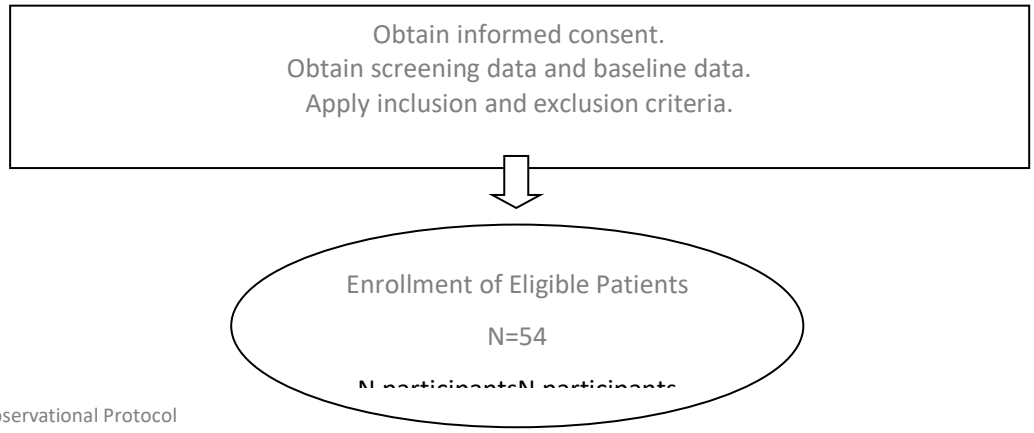
Study Duration: 12 months

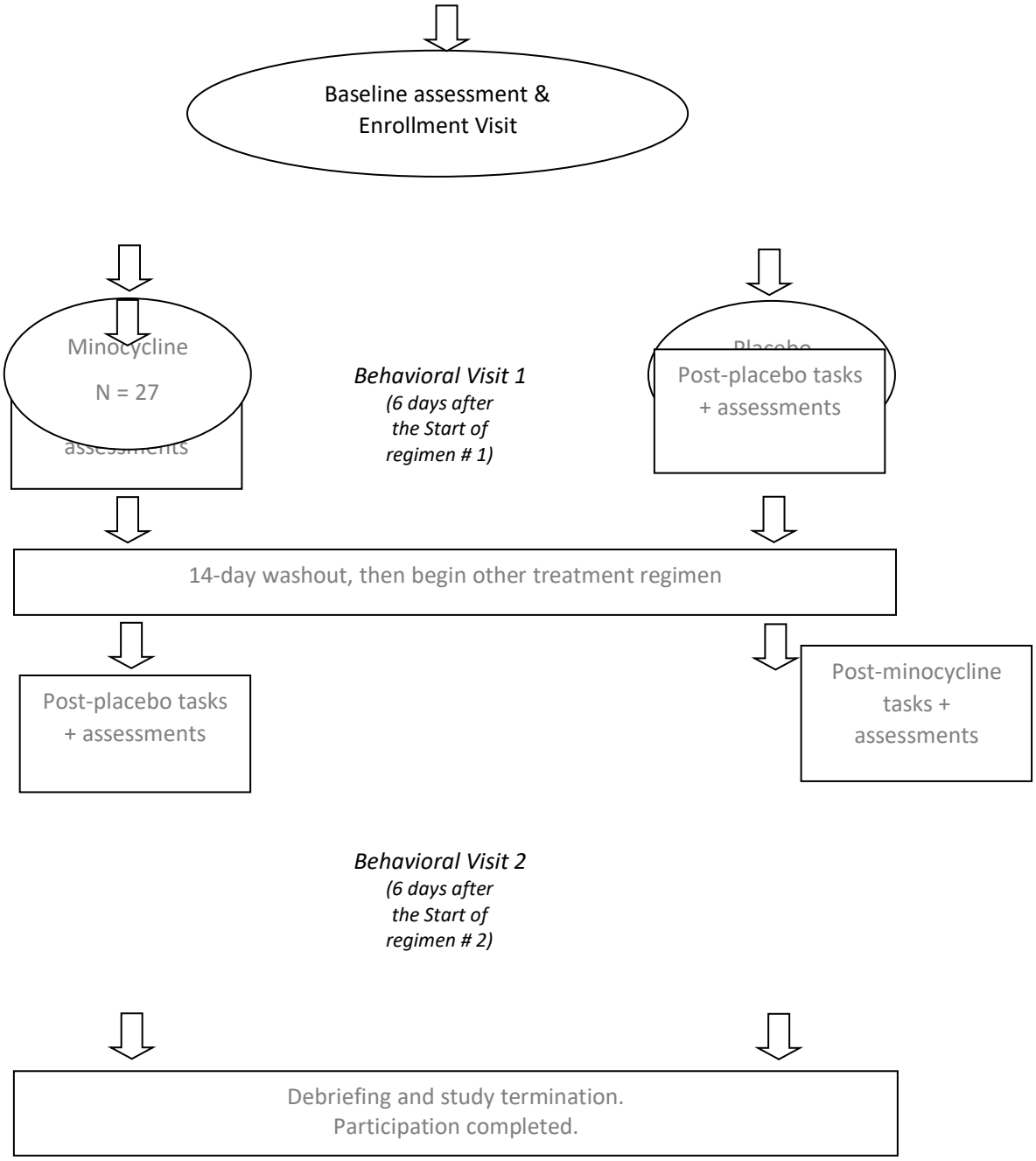
Target Number of Participants: 54 chronically stressed individuals

Minocycline Dosage: A 200mg/day dosage was selected based on prior studies examining the efficacy of minocycline for psychiatric symptoms [78-82]. As described in the prescribing information leaflet (see Appendix 1), the serum half-life in normal participants ranged from 11-22 hours (M=15.5 hours). It takes 4 days for minocycline to achieve a steady state and approximately 4 days for it to be eliminated. Because a 5-day course of 200 mg/day dose has been found to have neuroprotective effects in response to brain injury (i.e., stroke)[83-86], we plan to assess whether a similar 5-day course can potentially decrease neuroinflammation and ameliorate motivational deficits among individuals with chronic stress. To follow standards for other neuroactive agent crossover trials, a 14-day washout period will be implemented. Because we expect that the washout period is sufficiently long to eliminate the potential for carryover effects, we assume that there will be no carryover effects with our design.

Timing of Visits: Participants will first complete a screening session for assessing inclusion/exclusion eligibility and can occur at -30 to -1 days. At day 0, participants will complete a baseline onboarding visit (~1.5 hr) and receive the first arm 1 of intervention. The 5-day course of minocycline (or placebo depending on randomization) will begin between day 1 and up to day 30 of enrollment. This will be decided based on participant availability. Behavioral session 1 will be scheduled 6 days after beginning the treatment (~day 6 to day 36). This session is approximately 1.5 hours and participants will receive the second treatment during this visit. Following the visit, a 2-week washout period will commence. The second behavioral visit will determine when the second treatment will begin and will not occur any earlier than 20 days after behavioral session 1. This 20+ day window accounts for 2 weeks of washout and 5 days of the second treatment. The 5-day course of placebo (or minocycline depending on randomization) can begin as early as day 26 or as late as is needed. Behavioral session 2 will be scheduled to occur 6 days after beginning the second treatment (day 32 or later). After the second visit, participants will be debriefed and receive the remaining payment. The minimum enrollment period for participants is 32 days.

Figure 1. Crossover randomized trial schematic.




Baseline assessment &
Enrollment Visit
Minocycline
N = 27
Post-placebo tasks
+ assessments
Behavioral Visit 1
(6 days after
the Start of
regimen # 1)
14-day washout, then begin other treatment regimen
Post-placebo tasks
+ assessments
Post-minocycline
tasks +
assessments
Behavioral Visit 2
(6 days after
the Start of
regimen # 2)
Debriefing and study termination.
Participation completed.

5. Study Participants

5.1. Numbers of Participants

A total of N = 54 eligible individuals will be enrolled. We anticipate that at least n = 50 of the enrollees will complete all aspects of the protocol and have complete data.

5.2. Eligibility Criteria

The following criteria were selected to minimize confounding effects and optimize signal detection for effects of chronic stress in this preliminary study. Individuals will be excluded from the study if they endorse any of the following, which are known to influence inflammation [87] or interfere/confound with minocycline effects [88]: regular nicotine or recreational drug use; neurological conditions (e.g., history of stroke, TBI); chronic diseases or infections that significantly impact inflammatory markers (i.e., diabetes, cardiovascular diseases, high blood pressure, inflammatory bowel diseases, autoimmune disease); known allergies or hypersensitivities to tetracycline antibiotics, aspirin or other NSAIDs; antibiotics; regular use of steroidal or non-steroidal anti-inflammatory medications; and have any liver or kidney problems. Individuals will also be excluded if they are pregnant or breastfeeding or meet diagnostic criteria for a severe mental illness (i.e., disorders evincing psychotic symptoms, severe major depression, bipolar disorder). Finally, adults aged 25-60 will be eligible to participate.

Several decisions regarding the stress and unemployment inclusion criteria were defined based on a prior study that examined links between inflammation and neural activity among a chronically stressed sample of unemployed individuals[92]. For that study, researchers included participants who were:

- Unemployed (working less than 20 hours per week)
- Seeking employment
- Having trouble finding job
- Stressed: greater than 5 points on Job Stress Items

The Job Seeking Stress Items were adapted to screen participants for reports of stress due to unemployment in the Creswell et al. study[92]. The items are described in further detail in section 6.2. Finally, given recommendations and best practices for stress measurement[93,94], a situation should be ongoing for at least six months to be considered a chronic stressor.

5.2.1. Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- 25-60 years old
- Unemployed (working less than 20 hours per week)
- Seeking employment
- Having trouble finding job (i.e., actively seeking and applying for jobs but not successful in landing a job)
- Reports greater than 5 points on Job Stress Items
- Regular access to a mobile phone

Having trouble finding a job includes actively seeking jobs and not being successful. Active search methods are defined as those that have the potential to result in a job offer without any further action on the part of the job seeker. Examples of active job search methods include:

- contacting an employer directly about a job
- having a job interview
- submitting a resume or application to an employer or to a job website

- using a public or private employment agency, job service, placement firm, or university employment center
- contacting a job recruiter
- seeking assistance from friends, relatives, or via social networks; for example, asking friends and family for job leads or indicating one's job seeking status on social media
- placing or answering a job advertisement
- checking union or professional registers

5.2.1. Exclusion Criteria

Any individual who meets one or more of the following criteria will be excluded from participation:

- Currently enrolled as a student
- Prescribed medication for the following physical illnesses: diabetes, cardiovascular diseases, high blood pressure, autoimmune disease, neurological conditions (e.g., TBI, stroke)
- Diagnosis of a severe mental illness (i.e., disorders evincing psychotic symptoms, severe major depression, bipolar disorder)
- Pregnant or breastfeeding
- Daily psychotropic medications including mood stabilizers, stimulants, antipsychotics.
- Daily antidepressants use that has been recently added or modified in fewer than 3 months.
- Drug or alcohol dependence
- Known allergies or hypersensitivities to tetracycline antibiotics, aspirin or other NSAIDs
- Current antibiotic use
- Regular use of steroidal or non-steroidal anti-inflammatory medications (i.e., 2 or more times a week)

5.3. Randomization and Blinding

The UNC Investigational Drug Service (IDS) will randomly assign participants to start the experimental regimen (minocycline) or the placebo regimen. A randomized block approach by gender will be implemented such that there are equal numbers of male and female participants in each arm of the study. 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 minocycline 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 course of medication. 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 during visits will know whether a participant is on placebo or minocycline. After data collection for the study is completed, the pharmacist who made order assignments will reveal when participants were in the minocycline vs. placebo phase so appropriate analyses can be conducted.

In order to maintain randomization and blinding for participants, the minocycline and placebo pills will be enclosed in the same capsules as to preclude any noticeable differences between the pills. The pills will be placed in the same containers and be administered to the participants following the same procedures during both conditions.

5.4. Recruitment and Retention

Participants will be recruited via online registries of research studies at UNC (e.g., Join the Conquest; emails to UNC staff listservs), flyers posted in the Chapel Hill, Durham, and Raleigh communities (e.g., community centers, grocery stores), targeted ads on social media (e.g., Facebook), and ads on buses and in local publications. To ensure participation from individuals experiencing stress due to unemployment, we will offer vouchers to cover transportation to UNC for the study session, free on-site

parking, and evening/weekend sessions in an effort to lower barriers to participation. To facilitate retention, we will provide bonus compensation to those who complete the follow-up assessments.

6. Schedule of Activities and Procedures

6.1. Table of Events

Table 3. Schedule of activities and procedures

Procedure		Visit 0 screening	Visit 1 baseline	Pre-V2	Visit 2	Pre-V3	Visit 3	Debrief	Early Stop
Recruit a Sample of Patients	Informed consent		X						
	Eligibility assessments	X	X	X		X			
	Enrollment and randomization		X						
Treatment	Administer regimens			X		X			
	Measure Compliance			X	X	X	X		X
Safety Monitoring	Review concomitant meds		X	X	X	X	X		
	Medical history		X		X		X		
	Psychological surveys	X	X	X	X	X	X		
	Assessment of AEs		X		X		X	X	X
Reported Outcomes	Motivation Scale		X	X	X	X			X

6.2. Screening

Prospective participants who email the Social Neuroscience and Health Lab regarding participation will first complete a pre-screening questionnaire that collects information about the individuals. The prescreen questionnaire will be administered via Qualtrics and will ask about age, gender identity, pregnancy status, employment status, job seeking stress screening items, and gather name and contact info.

Participants will be eligible for inclusion if they indicate that:

- 25-60 years old
- Unemployed (working less than 20 hours per week)
- Seeking employment
- Having trouble finding job (i.e., actively seeking and applying for jobs but not successful in landing a job)
- Reports greater than 5 points on Job Stress Items
- Regular access to a mobile phone

Job Seeking Stress Screening Items[92]: Participants will be asked the following questions and be asked to rate them on a scale of 0 (never) to 4 (very often). To be eligible for participation, participants will need to score greater than 5 points on the scale. Items 2 and 3 are reversed scored.

1. In the last month, how often have you felt that you were unable to control the important things in your life?
2. In the last month, how often have you felt confident about your ability to handle your job-related problems? This could include your efforts at finding a job or paying your bills.
3. In the last month, how often have you felt that things were going your way in finding a job?
4. In the last month, how often have you felt difficulties were piling up too high that you could not overcome them?

After completing the prescreen questions, participants will then be screened via telephone by a trained research assistant in the lab to assess whether they meet additional eligibility requirements. On this call, participants will be asked about exclusion criteria including:

- Currently enrolled as a student
- Prescribed medication for the following physical illnesses: diabetes, cardiovascular diseases, high blood pressure, autoimmune disease, neurological conditions (e.g., TBI, stroke)
- Diagnosis of a severe mental illness (i.e., disorders evincing psychotic symptoms, severe major depression, bipolar disorder)
- Pregnant or breastfeeding
- Daily psychotropic medications including mood stabilizers, stimulants, antipsychotics.
- Daily antidepressants use that has been recently added or modified in fewer than 3 months.
- Drug or alcohol dependence
- Known allergies or hypersensitivities to tetracycline antibiotics, aspirin or other NSAIDs
- Current antibiotic use
- Regular use of steroidal or non-steroidal anti-inflammatory medications (i.e., 2 or more times a week)

Additionally, a Mini-International Neuropsychiatric Interview (M.I.N.I.)[95] will be administered to assess whether participants meet the criteria for a severe mental illness via phone. The MINI is a brief diagnostic structured interview for major psychiatric disorders and takes approximately 15 minutes to administer. The researcher will complete the MINI screening questions first. If the participant responds positively to any of the screening prompts, follow-up questions will be asked to assess the possibility of a diagnosis. Participants who meet eligibility for severe mental illnesses will be excluded.

If eligible, participants will learn more information about the study and be asked whether they would be interested in participating. If so, they will be scheduled for all the following study visits.

Eligible participants will be scheduled for the Baseline in-person visit. There, the informed consent process will begin, and confirmatory tests/evaluations will be performed to verify eligibility criteria. During this visit:

- The study coordinator will review the consent forms and answer any questions the participant may have
- Participant will be given time to review materials and then sign the consent form
- Participants will meet with the study physician to complete a brief health screening, collect vital signs information, and to carefully discuss the risks/benefits of participating in the study
- Complete questionnaires about employment stress and their levels of motivation
- Begin enrollment procedures outlined below
- Participant will be thanked for their time and reminded of their next in-person appointment

The names, phone numbers and emails of participants who do not meet eligibility criteria or who decline participation will be retained on a secure password protected network for the study term (5 years) to ensure that the same participant is not contacted more than once regarding participation. All other identifiable information about these individuals will be deleted as soon as they decline participation. Participants who endorse one or more exclusionary criteria will also be asked if they would like to be contacted for future studies in our lab.

6.3. Enrollment

During the baseline screening and enrollment visit, participants and research team will:

- verify inclusion/exclusion criteria,
- review study procedures and schedule and briefly describe the purposes of the study
- provide first course of medication
- explain minocycline and its known side effects
- explain that individuals will be asked about AEs during each study visit and will be monitored
- develop plan and help participant set an alarm for when to take the medication
- explain the text reminder system where they are reminded to take the medication around a specific mealtime and then asked to take a picture of medication in palm to verify
- explain the payment schedule for participation in the study

Before both study visits, inflammation-related drug use and employment status will be reassessed at least 24 hours before the appointment. If the individual has recently taken an antibiotic or anti-inflammatory medication, the visit will be cancelled.

6.4. Study Visit Procedures

During study visits 1 and 2, the schedule of events will be nearly identical. First, participants will provide dried blood spots to measure levels of pro-inflammatory cytokines. Then, participants would complete the Progressive Reward Task. Next, participants will complete a few questionnaires including measures of anhedonia, stress, and an assessment of medication adherence. Finally, participants will be given the next course of medication in preparation for behavioral visit 2.

The following measures will be collected at study visits 1 and 2:

- Resting Heart Rate Variability (HRV) will be collected for 5 minutes while participants complete the following questionnaires. Sensors will be placed to collect autonomic nervous system reactivity.
- The Snaith–Hamilton Pleasure Scale (SHAPS) is a tool to assess symptoms of reduced motivation. The SHAPS uses 14 questions, each rated on a Likert scale of 1-4. The total score on the scale ranges from 14-56, with lower scores reflecting lower motivation. Scores will be compared across conditions to determine whether motivation changes in the minocycline condition as compared to the placebo.
- The Motivation and Pleasure Scale (MAP) will be used to capture self-reported aspects of reduced motivation. The scale uses 18 questions, each rated on a Likert scale of 0-4. The total score on the scale ranges from 0-72, with lower scores reflecting lower motivation. Scores will be compared across conditions to determine whether motivation changes in the minocycline condition as compared to the placebo.
- Job Seeking Stress Items as discussed in section 6.2.
- Given that unemployment is linked to greater levels of stress generally, participants will also complete the Perceived Stress Scale (PSS)[96]. The PSS is the most commonly used and well-validated measure of stress. The PSS is a 10-item scale that asks how often individuals have experienced irritations or lack of control in one's life. Participants completing the PSS will be instructed to respond based on their experiences since the last visit.
- The Patient Health Questionnaire (PHQ-9)[97] will be administered to measure symptoms of depression. The PHQ-9 is a 9-item scale that is used for diagnostic purposes to assess the severity of depression an individual is experiencing. Participants completing the PHQ-9 will be instructed to respond based on their experiences since the last visit. Scores on this scale will be used to assess whether Aims 1 and 2 are moderated by depression severity. This scale will also be used to assess the safety of each participant. See section 10 for more information about safety plans.
- A trained research assistant will administer a Dried Blood Spot (DBS) test. To complete this test, participants' skin will be punctured with a single-use safety lancet, which is the same procedure diabetic individuals use on a daily basis. This may be momentarily uncomfortable or painful for some participants, but we expect that it will be a mild experience.
- The Health Information Form is used to assess current medications, illness, or other covariates that could affect inflammation and thereby eligibility.

- Participants will be verbally instructed on the Progressive Reward Task and able to follow along with written instructions. They will be informed that the goal of the task is to win as much money as possible by correctly identifying the presence of a short versus long mouth on a cartoon face. Further, they are informed that not all correct responses will receive reward feedback. They will then complete a brief practice trial. Each trial of the task begins with a fixation cross in the center of the screen for 500 ms. Then, a mouthless cartoon face will be presented. With a 500 ms delay, either a short mouth (11.5 mm) or a long mouth (13 mm) is presented on the face for 100 ms. The participants must identify whether they saw a short or long mouth by pressing a key on the keyboard. The mouthless cartoon remains present until the participant responds. The short and long mouths are presented equally across the trials. However, the number of reinforcers presented for short VS long mouths is not balanced. The task aims to produce a response bias toward the mouth length that is more often positively reinforced.

At the end of both study visits, the research assistant will contact the study physician to report whether the participants reported any AEs.

6.5. Final Debriefing Visit

After completing both randomized conditions, participants will be asked to stay for a 30-minute debriefing session with the study coordinator. During this visit, participants will be unblinded, learn more about the purpose of the study (i.e., links between stress, inflammation, and motivation), have any concerns or questions participants may have about the study answered and addressed. Participants will also receive final compensation for participation in the study during this time.

6.6. Phone Contacts

Considering the complexity of activities, there will be frequent communication by the research team to the participants to remind them about study visits and to take the medication as directed.

- Study visit reminders will be sent once a day starting 2 days before the study visit via text messaging.
- Medication reminders will be sent once a day, during the medication period, at the participant's earliest mealtime (e.g., breakfast, lunch). If participants elect to be reminded at their next mealtime, they will be texted 4 hours later.
- To verify that participants have taken the medication, participants will be called each evening to ask about compliance and if they experienced any adverse events.

6.7. Assessing Medication Compliance

During the enrollment visits, participants will be asked what time of the day would be best to contact them for daily medication check-ins when the medication period begins. Participants will be given the option to receive daily reminder texts to take the medication and can confirm taking them in response to that text. Whether participants indicate evening, morning, or afternoon, all participants will be contacted by study staff to assess compliance for the previous day and whether the individual experienced any AEs. If a participant is unable to answer or call us back within 24 hours, it will be marked in a log for follow-up on the following visit. The following daily calls will proceed until the visit. To compensate participants for their time completing the daily check-ins, they will be entered in a raffle to win an extra prize at the end of the study.

During study visits, medication compliance during the treatment period will be assessed via a questionnaire in order to decrease pressure to respond in a socially desirable way. The questionnaire prompt will state “Taking pills is difficult for a lot of people. It is not uncommon for people to miss doses from time to time. These items/questions ask you about doses you took and doses you missed. Please try to remember as best you can what actually happened and not what you intended to have happen or what you think that other people want you to report. By answering these questions accurately, you are making a big contribution to this research.”

Participants will be asked to return the pill bottle and extra pills at the end of the study. The extra pills will be returned to IDS for disposal. Together, the pill bottle, questionnaire responses, and daily check-ins will be used to assess medication adherence.

7. Statistical Analysis Plans

Statistical procedures will be performed in R. The study was designed to reduce the possibility of period, carryover, and sequence effects and we assume that these effects will not occur with this data. Specifically, we will employ a blocked randomized design to prevent period and sequence effects and allow for the opportunity to observe if the effects will occur. Additionally, we have extended our washout period to decrease the possibility of carryover effects.
Aim 1: To examine differences in performance on a pure motivation task following minocycline (vs placebo).

Aim 1 analysis plan: A paired samples t-test will be conducted to assess whether taking minocycline was associated with a change in response bias on the PRT as compared to when participants are taking a placebo.

Aim 2: To examine differences on self-report measures of motivation following minocycline (vs placebo).

Aim 2 analysis plan: A paired samples t-test will be conducted to assess whether taking minocycline was associated with a change in SHAPS scores as compared to when participants are taking a placebo. Another paired samples t-test will be conducted to assess whether taking minocycline was associated with a change in MAPS scores as compared to when participants are taking a placebo.

8. Sample Size Rationale

We conducted a power analysis to determine the appropriate sample size for this study. Although a prior study exploring the effects of minocycline on a memory task demonstrated a large effect size (Berens et al., 2020), we conducted a power analysis that assumed a small-medium effect size given that associations with our task types have not been explored in the literature before. Power analyses were conducted assuming a small-medium effect size Dz of 0.4 for a paired-sample t-test examining the change in accuracy on both tasks from post-minocycline to post-placebo. Given an effect size of Dz=0.4 for a paired-sample t-test and an alpha of .05, we would need 52 participants to achieve 80% power. As such, we aim to recruit 54 participants to account for any potential issues with participant retention.

9. Data Collection and Management

Questionnaire and Symptom Data Collection: Research material obtained from all of participants will include self-report data and responses on behavioral tasks. These assessments will be conducted via in-

person assessment sessions. Self-report measures will be collected using Qualtrics survey software implemented behind the HIPAA-compliant firewall at UNC-Chapel Hill. Qualtrics is encrypted and requires ONYEN credentials and Duo two-step authentication by authorized study personnel to access. Each session will last between 1 and 2.5 hours and breaks will be provided throughout the session as needed. Responses on self-report and behavioral measures will exist in digital form on computer disk and on secure servers at UNC.

Data Quality and Monitoring: All data are collected by trained bachelor's-level or Master's level study staff. Data quality and safety will be monitored by the PIs and research team members. Any issues will be discussed with the PI, and as needed, the scientific advisory team. The study PI assumes responsibility for ensuring study staff is complying with the investigational plan and IRB regulations, as well as ensuring any changes to the protocol have received IRB approval and have been reported to the sponsor, and that accurate, complete, and timely reports are made to the IRB. A weekly study team meeting will be conducted to discuss research procedures.

- All identifiable data will be recorded in the database using alphanumeric identifiers that are unrelated to any personal identifying information about the participant. All participant data are coded by subject number only on password-protected secure servers of which only the research team has access.
- All sources of electronic data (i.e., computerized symptom data collected via UNC Qualtrics) are saved on a password-protected file on a secure UNC server, to which only the research team members will have access.
- All sources of non-electronic research data will be coded by a unique subject number and stored in a dedicated locked file cabinet. Materials identifying participants by name (e.g., signed consent forms) will be stored in a separate locked file cabinet. The computer file matching participant names with code numbers will be saved in a double password-protected file on a secure UNC server directory only accessible by the PI and key research team. All data will be entered into the dataset using the subject identification number only. Participants are not identified by name in any analysis of these data, or any presentation or publication resulting from the analysis of these data.

10. Safety Monitoring and Management

10.1. Risk / Benefit Assessment

Potential Risks:

- Minocycline: The most common side effects are gastrointestinal (i.e., nausea) and mild central nervous system effects (e.g., vertigo, light-headedness). Like other tetracycline-class antibiotics, minocycline may cause fetal harm when administered to pregnant women. Pregnant woman (measured by self-report during prescreen) will be excluded from participation due to the potential risks of minocycline to a fetus. The use of minocycline during pregnancy may affect tooth development and cause permanent discoloration of teeth. If any tetracycline is used during pregnancy or if the patient becomes pregnant while taking these drugs, the participant will be apprised of the potential hazard to the fetus. Most adverse effects occur early after administration and disappear when the medication is discontinued. Participants will also be advised to use a second method of contraception as minocycline may increase the chances of an unintended pregnancy.
- Questionnaires / Behavioral Measurements: There are minimal risks involved in the collection of questionnaires and behavioral data. Although it is unlikely, it is possible that participants may become bored or frustrated by completing the tasks. Individuals may become distressed while reporting on difficult emotional experiences.

Potential Benefits: Beyond monetary compensation for participation, there are no direct benefits to individuals for participating in the study. However, participants may derive satisfaction, knowing that their participation may help advance knowledge about chronic stress-related neuroinflammation. The

proposed study is similar to other experiments conducted in the lab. The adverse experiences (e.g., dropping out of a study due to discomfort with study procedures) have been minor and rare. Thus, we maintain that participants' risks are minimal and less than the benefit they gain from participating in the study.

10.2. Safety Monitoring

Personnel: The present study does not raise any obvious health concern issues. Nonetheless, during the course of individual study sessions with participants, researchers will frequently verbally check in with participants to make sure they are comfortable and not experiencing any physical or psychological discomfort. Participants can withdraw participation at any point in the study. All research staff will be trained on recognizing and reporting adverse events prior to engaging in research activity. If a serious, unexpected adverse event occurs, the PI will report the event to the UNC IRB in accordance with their regulations. The PI will review the adverse event report and gather other information as needed to investigate the event and determine the need for subsequent action. Any subsequent action will be documented and reported to the UNC IRB. All study team personnel will obtain research training certification before working in the lab as required by NIH. Confidentiality will be protected to the fullest extent permitted by law. All research personnel will have completed CITI training and HIPPA training for researchers. Consent forms will be stored in locked file cabinets only accessible to those on the study team. Computerized data (behavioral task data and questionnaire data entered into the computer) will be stored on secured, password-protected servers behind a UNC-Chapel Hill and lab-specific firewall.

Minocycline: The 200mg dose of minocycline 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. Minocycline rarely causes side effects, especially when used for a short duration like this study. However, like with most antibiotics, participants will be counseled about the possibility of diarrhea and gastrointestinal upset as well as signs of an allergic reaction. They will also be alerted to the possibility of photosensitivity and be encouraged to wear sunscreen. The study physician will be on-call to assess any side effects and direct appropriate management as needed. If a participant begins to notice signs of an allergic reaction when taking the medication at home (e.g., difficulty breathing, swelling) they will be advised to call 911 and seek medical attention immediately. While we will encourage participants to alert the study team to any issues that may arise, we will also contact participants daily to assess potential adverse reactions during the medication duration via phone and surveys. Once contact with the participants is established, the study psychiatrist will follow-up to assess the participants wellbeing and treatment plan. Participants will be contacted daily during the at-home medication period to assess any potential adverse reactions to minocycline. During the daily calls, participants will be asked about any symptoms of allergy, gastrointestinal upset such as nausea and diarrhea, as well as any feelings of dizziness or fatigue.

Questionnaires / Behavioral Measurements: To minimize the risk of a participant becoming uncomfortable, the study team will make every effort to maintain a personally supportive relationship with each participant. Participants will be informed at the start of the study what to expect and how long the study visit will be. All data will be coded with identification numbers and will not contain personally identifying information.

Protocol for Assessing the Emergence of Severe Depression or Suicidality: Participants will be recruited to be at low risk for severe depression and suicide attempt following the baseline assessments with the study psychiatrist. However, the PHQ-9[97] will be administered at all study visits to track risk for psychological crises. If a participant screens positive to question 9 on the questionnaire, selecting anything other than zero for the item "Thoughts that you would be better off dead or of hurting yourself in some way", the study psychiatrist will assess suicide risk with the Ask Suicide-Screening Questions (ASQ). See Appendix 4 for the ASQ. If the psychiatrist identifies potential positive risk, they will be offered information for immediate crisis services including Orange County mobile crisis units and the Emergency Department. The same resources will be shared with participants who score higher than 15 on the PHQ-9, indicating severe depression.

10.3. Adverse Events and Serious Adverse Events Reporting Procedure

An **adverse event (AE)** is generally defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events are to be recorded regardless of their relationship to the study intervention.

A **serious adverse event (SAE)** is generally defined as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly.

The most common side effects from this medicine are gastrointestinal symptoms, dizziness and fatigue. Minocycline may increase sensitivity to sunlight, resulting in more frequent sunburns or the development of rashes following sun exposure. We will recommend that patients apply sunscreen (SPF 15 or greater) before outdoor activities or avoid prolonged exposure to the sun while taking minocycline. Minocycline-associated SAEs are very rare, and the number needed to cause one additional case has been estimated at 1/11,364 from a UK database of over 97,000 patients treated for acne[103]. No neurological side effects such as extra pyramidal symptoms have been reported.

Each participant will be asked about the presence and absence of adverse events at every study visit. The research team will record the event, severity, the start and end date of the event, the relationship to the study (e.g., whether related or unrelated to the study procedures) and the final outcome. Data will be collected using a form (see Appendix 2 for examples). The study physician will be consulted when events are unexpected and/or of greater than mild severity. The study physician will speak to the participant within 24 hours to more thoroughly assess the next steps to determine the best course of action. If deemed necessary, the physician will refer the participant to a follow-up. The occurrence of adverse events will be immediately reported by study staff (i.e., study coordinator, graduate student research assistants, research nurses at the CTRC) to the PI and the study physician. The PI will then report the adverse event to the UNC Office of Human Research Ethics (OHRE). OHRE standard operating procedures specify that the PI must notify information previously unknown to the IRB involving new or increased risk to subjects (i.e., New Safety Information) to OHRE within seven calendar days of the investigator becoming aware of the information. New Safety Information is communicated to OHRE through the online IRB management system, IRBIS. Reports to the IRBIS will include Adverse Events, Serious Adverse Events, and Unanticipated Problems.

The following grading scale will be used to identify the intensity of an Adverse Event:

- Mild: Awareness of a sign or symptom that does not interfere with the participants usual activity or is transient, resolved without treatment and with no sequelae;
- Moderate: Interferes with the participants usual activity, but the patient is still able to function;
- Severe: Events that interrupt a patient's usual daily activity and generally require a systemic drug therapy or other treatment.

All AEs/SAEs will be evaluated as to its expected occurrence, or lack of expected occurrence:

- Expected: An adverse event is expected when the specificity and severity of the event is consistent with the study procedures and Standard of Care, (e.g., the participant fainted in response to having his blood drawn) or underlying disease state, (the participant's depressive symptoms worsened).

- Unexpected: An adverse event is unexpected when the specificity or severity of an adverse event is not consistent with the study procedures or Standards of Care, (e.g., the phlebotomist used the same blood collection needle on multiple participants, resulting in a participant acquiring Hepatitis C), or underlying disease state (e.g., the participant experienced a manic episode during the study). Unexpected, as defined above, refers to an adverse event that has not been observed before.

The following conditions will be used to define the relatedness of any study procedure to an AE/SAE.

- Not related: The PI has determined that the event is not related to any study procedure.
- Possible: The PI has determined that the event possibly has a reasonable relationship to the study procedures.
- Probable: The PI has determined that the event probably has a reasonable relationship to the study procedures. Definite: The PI has determined that the event is related to study procedures.
- Unknown: The PI has determined it is impossible to determine the relationship of the event to the study procedures.

The clinical outcome of the AE/SAE will be characterized as follows:

- Recovered/Resolved: The patient recovered from the AE/SAE.
- Not Recovered/Not resolved: Patient did not recover, and symptoms continue.
- Recovered/Resolved with Sequelae: The patient has recovered but with clinical sequelae from the event.
- Fatal: The event resulted in death. The SAE, Death, and Exit forms must be completed for this outcome.
- Unknown: The clinical outcome of the patient remains unknown at the time of the report, or the patient was lost to follow-up.

All AEs/SAEs will be followed until resolution or until the end of participation in the protocol. Participants may report AEs freely or in response to general questioning or through patient or clinician assessments. If it is determined that an AE has occurred, the PI will be notified.

11. Regulatory, Ethical, and Study Oversight Specifications

11.1. Informed Consent Process

Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without penalty. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted, and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

11.2. Confidentiality and Privacy

We will safeguard against breaches of confidentiality by refraining from collecting personal identifying information and instead coding participant data by assigning alphanumeric ID numbers on digital data and hardcopy forms. The key linking the alphanumeric ID and participant's identity will be maintained on a separate drive that is double password protected and to which only the PI and key study personnel have access. The only exception to alphanumeric ID coding will be for information, including names, telephone numbers, dates, mailing and electronic address, provided on the Contact Form, which will only be used to contact participants to schedule and confirm study visits. Contact Forms will be kept separately from study data under double locks only available to the PI and key research team. Clinical records will be kept confidential with access granted only to those medical and research professionals directly involved with the study. If any scientific paper based on the data collected for this study is published, no information that could be linked to any single participant will be reported. Confidentiality will be protected to the fullest extent permitted by law. All research personnel must complete HIPPA training for researchers and CITI training.

11.3. Key Roles and Study Governance

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12. Appendices

Appendix 1: Prescribing Information Leaflet for Minocycline

Appendix 2: Example Adverse Events Form

Appendix 3: Minocycline Care Notes for Participants

Appendix 4: Suicide Risk Screening Tool

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