Effects of Inflammation on Reward Processes in Women of Early and Late Adulthood

ABSTRACT

Dysregulation in the reward system predicts onset of depression[1], persists during depression remission[2], and underlies anhedonia[3], a key diagnostic symptom associated with greater depression severity[4] and poor treatment response[5]. Compelling evidence implicates inflammation as a biological facilitator of reward dysregulation. Animal models have consistently shown that inflammation reduces preference and willingness to work for rewarding food[6,7], with similar patterns evident for monetary reward in humans [8-10]. However, inflammation appears to have divergent effects on social reward, eliciting both general social withdrawal[11,12] and increased affiliative behavior for close others[13,14]. To date, no studies have directly compared effects of inflammation on these social (i.e., general and close) and non-social reward processes. Further, key contextual factors, such as age, have received little attention. In particular, the transition to late adulthood in women is marked by substantial psychobiological changes, including increased systemic inflammation, shifts in socioemotional goals and preferences[15], and most notably, lower rates of depression. Indeed, depression prevalence is elevated in women throughout adulthood, but drops precipitously after age 65[16,17]. Thus, the overarching aim of the current study is to evaluate effects of an inflammatory stimulus on social and non-social reward processing in women of early and late adulthood. Forty healthy premenopausal women (ages 30-40) will be recruited to participate in a double-blind randomized placebo-controlled study evaluating effects of an inflammatory challenge (i.e., endotoxin) relative to placebo control on reward processing. The study protocol will parallel an ongoing R01 assessing affective consequences of induced inflammation in older adults, affording a unique opportunity to examine inflammation and reward during two key developmental stages. To this end, 40 healthy women in late adulthood from the ongoing trial (age 65+; 20 endotoxin, 20 control) will be included for a total sample size of 80. We hypothesize distinct effects of inflammation as a function of both reward type and age. Specifically, as compared to placebo, endotoxin will *decrease* non-social and "general" social reward response but *increase* "close" social reward response. These effects are expected to be weaker among women in late compared to early adulthood, due to a hypothesized age-related decrease in affective and behavioral sensitivity to inflammation. Results from this study will advance the study of depression pathogenesis, which can in turn enhance intervention and prevention approaches. Assessment of these processes among women in different developmental life stages will further inform our understanding of variations in depression presentation and prevalence across the adult lifespan.

LAY SUMMARY

Depression is prevalent, devastating, and the leading cause of disability worldwide. Compelling evidence implicates inflammation in depression pathogenesis, but potential intermediary mechanisms, such as dysregulated reward processing, are poorly understood. This study will examine effects of inflammation on social and non-social reward processing in younger and older women. Characterizing how inflammation shapes reward processes, and how this may differ by age, will help us better understand how depression develops and identify critical points for intervention.

SPECIFIC AIMS

Effects of Inflammation on Reward Processes in Women of Early and Late Adulthood

Dysregulation in the reward system predicts onset of depression[1], persists during depression remission[2], and underlies anhedonia, a key diagnostic symptom associated with greater depression severity and poor treatment response. Compelling evidence implicates inflammation as a biological facilitator of reward dysregulation, which can manifest as impairments in reward sensitivity, motivation, and/or learning. Animal models have consistently shown that inflammation reduces reward sensitivity (sucrose preference) and reward motivation (willingness to work for sucrose), with similar patterns evident for monetary reward in some, but not all, human studies[8-10]. Inflammation has also been shown to reduce social reward processing, as indicated by social withdrawal[11], feelings of social anhedonia/disconnection[12], and decreased sensitivity to positive social stimuli[18]. At the same time, inflammation may increase motivation to affiliate with close others[13,14], suggesting a facilitative effect that depends on the nature of the social reward. Thus, there is a need to more fully characterize how inflammation shapes the reward system. Indeed, no studies have directly compared effects of inflammation on social (general and close) and non-social reward processes.

Similarly, key contextual factors, such as age, have received little attention. Experimental studies on inflammation and reward have largely focused on younger adults, but these processes may differ in older adults. The transition to late adulthood, particularly in women, is marked by substantial psychobiological changes, including alterations in dopaminergic function[19], menopause, and shifts in socioemotional goals (e.g., preference for close rather than expansive social networks[15]). Most notably, late adulthood is characterized by both *increased* systemic inflammation[20] and *decreased* depression prevalence[16]. Indeed, while elevated throughout adulthood, rates of depression in women drop precipitously after age 65[17]. Our hypothesis is that this "aging paradox" may result from age-related decreases in affective and behavioral sensitivity to inflammation.

The overarching aim of the current study is to evaluate effects of inflammation on multiple dimensions of reward processing in younger and older women, and to probe how these effects may differ as a function of reward type (general/close social, non-social). To this end, 40 healthy women in late adulthood will be selected from an ongoing randomized placebo-controlled study evaluating effects of an inflammatory challenge (i.e., endotoxin) on affect and behavior. An additional 40 healthy premenopausal women (ages 30-40), comparable in terms of BMI, education level, and ethnicity, will be recruited to participate in a parallel randomized controlled trial. Both studies include a comprehensive, vertically integrated assessment of reward processing, including use of standardized behavioral reward tasks that capture multiple reward dimensions and types, non-invasive assessment of dopaminergic activity, and self-report experiences. As a step towards greater ecological validity, daily diary ratings of reward anticipation and enjoyment for a wide range of experiences will be collected one week prior and one week after the experimental session.

AIM 1: Evaluate effects of inflammation on non-social reward as a function of age.

Hypothesis 1: As compared to placebo, endotoxin will decrease non-social reward responses; these effects will be greater among women in early compared to late adulthood.

AIM 2: Evaluate effects of inflammation on general and close social reward as a function of age.

Hypothesis 2: As compared to placebo, endotoxin will *decrease* "general" social reward responses; these effects will be greater among women in early compared to late adulthood.

Hypothesis 3: As compared to placebo, endotoxin will *increase* "close" social reward responses across reward dimensions; these effects will be greater among women in early compared to late adulthood.

Exploratory Aim: Examine changes in dopaminergic activity as a mechanism linking effects of inflammation on non-social reward processing as a function of age.

Hypothesis 4: As compared to placebo, endotoxin will decrease resting eye blink rate (EBR); EBR will be correlated with learning and motivation for non-social reward; these effects will be greater among women in early compared to late adulthood.

This study builds upon a nuanced and complex literature to systematically characterize the effects of inflammation on non-social and social reward across reward dimensions and in two key developmental stages. By leveraging the existing R01 with older adults, this study is a first step towards a lifespan approach of how inflammation may shape reward processes. Given that alterations in inflammation and reward processing have been shown to precede depression, this line of work may ultimately contribute to the development of biobehavioral risk profiles for depression onset and recurrence. Further, a greater understanding of individual differences in reward dysregulation will also inform treatment and prevention approaches.

RESEARCH STRATEGY

A. SIGNIFICANCE

There is a clinical need to identify how inflammation may alter the reward system

Dysregulated reward processing and the associated diagnostic symptom anhedonia are important and underserved clinical targets. Anhedonia is pernicious and inadequately treated, likely because it involves different neurobiological mechanisms than symptoms that have been more well-studied, such as low mood[21]. In addition, the treatment of anhedonia as a unitary, rather than multidimensional, construct may have clouded prior assessment of reward dysregulation[22,23]. A critical unanswered question is how inflammation, which predicts depression onset and can elicit depressive symptoms in experimental models[24], shapes the reward system and its component dimensions. While elevated peripheral inflammation has been linked to disruptions in reward processing, a consistent picture of the direction and extent of these effects has not yet emerged. Further, it is not known whether inflammation has differential effects on different types of reward and reward dimensions. By incorporating multiple tasks that capture these dimensions (e.g., motivation, sensitivity, learning) and reward types (close/general social and non-social) this study is uniquely positioned to inform this question.

Results from the current proposal will have important clinical implications, as identifying the specific alterations in reward processing sensitive to effects of inflammation will inform intervention approaches. Psychopathology can present with different forms of reward dysregulation. For example, both depression and schizophrenia are associated with reduced reward motivation, but depression is in some cases also associated with reduced reward sensitivity. Different psychosocial therapies are likely warranted depending on the dimensions affected. Similarly, pharmacological treatment may differ based on different neurochemical underpinnings of reward dimensions (e.g., dopaminergic, serotonergic, opioid). Therefore, delineating the relationship between inflammation and reward dimensions represents an important step towards targeted and specific treatment and prevention of depression.

It is also important to understand how inflammation alters the reward system because this system may underlie higher order positive psychological processes that have been shown to serve as psychosocial resources and buffer effects of acute and chronic stress (e.g., optimism, positive affect, eudaimonic well-being). Inflammation has been linked to these constructs, but whether this is mediated by the reward system is not yet clear. The current proposal will include a battery of relevant psychosocial measures that will allow a comprehensive examination of these constructs in relation to behavioral indices of reward processing and inflammation.

Examining how inflammation alters the reward system will inform theory

To the extent depression is related to sickness behavior and inflammation, it is of theoretical interest to characterize how reward dimensions are differentially affected. For example, a compelling hypothesis in the sickness behavior literature is that in the context of acute inflammation, sensitivity to close social reward may be enhanced, ostensibly to recruit others for help, while sensitivity to more general social reward is decreased, to facilitate social withdrawal and time for healing[2524]. However, this pattern has only been observed in a few studies [14,26], and requires a more direct test. Further, it is not clear whether social withdrawal, which we conceptualize here as “general” social reward, is blunted by inflammation, or experiences qualitative shifts. For example, reward devaluation theory[27] suggests that reward can become threatening in the context of depression. The social withdrawal evident in inflammation could be driven by reward blunting, by heightened threat[28], or both.

Additional theories outside of sickness behavior are also of relevance to the current proposal. For example, stress-induced anhedonia has been posited to play a central role in the emergence of depression[22]. As such, dysregulated reward processing is conceptualized not only a symptom, but also a trait vulnerability factor that may be one mechanism linking stress to depression. The inclusion of baseline measures of reward will allow us to test whether individuals with lower reward sensitivity, motivation or learning are more affectively sensitive to the inflammatory stimulus. Another example is Social Signal Transduction theory[24], which proposes stress-induced inflammation as a critical pathway to depression and related health problems. Work from our lab has shown that acute psychosocial stress can induce delayed alterations in reward processing that are attributable to the inflammatory response to stress; the current proposal will isolate and test the inflammation-reward pathway without any potential confounding effects of stress (e.g., compensatory affective responses). Thus, this proposal is one component of a larger theoretical framework we are developing and testing that links stress, inflammation and alterations in reward to depression.

Inflammation may alter the reward system differently for younger and older adults

Age may be particularly important in understanding the nuanced effects of inflammation on social versus non-social reward processing. Following administration of endotoxin, individuals report social anhedonia and feelings of social disconnection, consistent with social withdrawal as a core sickness behavior[12]. However, Inagaki and colleagues (2015) found that individuals who received endotoxin exhibited greater neural reactivity in reward-related regions while viewing a photo of a close other versus a stranger, while no difference was evident in the control group. Endotoxin participants also self-reported a greater desire to be around the close other, which suggests that elevations in inflammation may contribute to increased approach motivation to close others while, at the same time, facilitating general social withdrawal. No studies have tested this hypothesis in younger and older adults, though preferences for social connection show age-related changes[15]. For example, older adults tend to have smaller but more intimate social circles, while younger adults have a wider variety of acquaintances and friendships. Theory suggests this may arise due to differences in goals for older adults (e.g., maintaining emotional well-being) versus younger adults (e.g., expanding social networks[29]). Given this, it is possible that a positive relationship between elevated inflammation and increased desire for close others would be less evident among older adults, who have a preexisting proclivity for close social orientation; by contrast, younger women may show a more dramatic shift. Alternatively, it is possible that aging is accompanied by increased feelings of vulnerability during illness, which could render older adults more likely to seek close others. This study will be able to evaluate these competing hypotheses.

Dopaminergic function may be selectively associated with non-social reward processes, and these effects may differ as a function of age.

As a physiological marker of dopaminergic activity, the current proposal includes assessment of resting state eye blink rate (EBR)[30], a method that is simple, short, non-invasive and has not been previously used in an experimental endotoxin study. EBR is used as a marker of striatal dopaminergic (D2 receptor) activity based on several lines of evidence. EBR is lower among individuals with neurological disorders that involve reduced striatal dopamine levels (e.g., Parkinson's disease) and higher among individuals with disorders characterized by greater striatal dopamine (e.g., schizophrenia)[30]. In both non-human animals and humans, dopamine agonists increase EBR, while dopamine antagonists decrease EBR[30]. Finally, in a study with monkeys, EBR correlated with D2 receptor availability in reward-related regions like the ventral striatum, and both EBR and D2 receptor availability were positively correlated with sensitivity to positive feedback during a learning task.[31]

To date, studies have not tested whether EBR is altered after an inflammatory stimulus. The assessment of dopaminergic function in the current study is novel, and if successful will allow us to interrogate the effect of inflammation on reward with greater granularity. Specifically, alterations in dopaminergic function have been posited to play a key role in mediating effects of inflammation on reward motivation[32]; here we can test whether EBR correlates specifically with tasks assessing motivation rather than sensitivity or learning. Further, EBR has been shown to predict increased motivation to obtain rewards presented subliminally, but not supraliminally[33]. This suggests EBR is associated with activity in the mesolimbic system, rather than higher order cortical areas. In the current study, we will examine whether EBR/dopaminergic function is more closely associated with tasks incentivized by non-social reward (e.g., money) compared to general and close social reward. Finally, the relationship between age and resting eye blink rate (EBR) is not clear, with several studies finding stability across the adult lifespan, although a few report age-related decline[30]. This will be the first study to test for age differences in effects of induced inflammation on EBR and associated performance on reward tasks.

The expected impact of the proposal

This comprehensive evaluation of reward dimensions and types, using a randomized controlled design and assessment of dopaminergic function, represents a major advance in our ongoing efforts to characterize how inflammation can shape the multidimensional reward system. By studying two key developmental stages for adult women, this work is also a first step towards a more nuanced lifespan approach. A demonstration of divergent effects of inflammation on reward processing as a function of stimuli type and age (hypotheses 1 and 2) could inform treatment approaches and contribute to the development of biobehavioral risk profiles for depression onset and recurrence. For example, if inflammation alters non-social and general social reward in younger women, it would be worthwhile to investigate whether younger women are more susceptible than older women to the affective consequences of diseases or treatments with an inflammatory basis (e.g. cancer)

and could benefit from prophylactic anti-inflammatory pharmacological treatment.

Similarly, there are several clinical implications that would warrant further study if endotoxin increases close social reward response in younger but not older women. It is possible that older women are rendered more vulnerable than younger women in the context of elevated inflammation because the increased motivation to seek close others confers substantive advantage (i.e., instrumental and emotional social support). Alternatively, an enhanced desire to be around close others could, at least in the context of depression, be problematic for interpersonal relationships among younger women; such a phenomenon would benefit from greater patient awareness and careful therapeutic management. Finally, if older women are less affectively sensitive to inflammation, it would be important to ascertain what biological processes do confer risk to this group (e.g., neuroendocrine dysregulation). Likewise, identifying the processes that render younger women more affectively sensitive (e.g., psychological/catastrophizing, hormone profiles) would also be important to determine. Thus, the potential application of our work to psychosocial and pharmacological treatment and intervention approaches represents an important step towards precision medicine.

B. INNOVATION

Sophisticated assessment of reward on multiple levels of analysis is novel and will contribute to the literature by providing a comprehensive assessment of effects of inflammation on the reward system

The current study is informed by the most recent work on reward processing and its multidimensional nature, and aims to assess reward on multiple levels. Among others, these include an affective forecasting task, daily diary methodology, a subliminal reward motivation task, and assessment of dopaminergic function. The affective forecasting task is derived from affective science and has not yet been used in this context. It provides an assessment of the anticipation of pleasure for close, general and non-social scenarios. The current proposal also includes a daily diary component to test for changes in anticipatory and consummatory reward processing from the week prior to the week after the laboratory session. Precedence for expecting residual effects of the induced inflammatory response comes from recent work conducted in our lab, in which we found that mild increases in IL-6 following the influenza vaccine were associated with increased mood disturbance across the subsequent week.

The daily diary approach was recently used to assess the anticipatory and consummatory pleasure response to daily activities, and we will build upon this work by examining close social, general social, and non-social daily activities. Of note, anticipatory/consummatory reward have proven difficult to measure with existing behavioral paradigms. For example, differences in neural reactivity to phases of reward during the Monetary Incentive Delay Task are often not reflected by differences in reaction time. Daily diaries may be more sensitive than behavioral tasks, and will allow us to incorporate assessment of a variety of rewarding experiences, including close social connection, physical urges/sensations, aesthetic appreciation, and work engagement. A further novel component is the assessment of motivation behind anticipatory reward; specifically, whether certain activities are wanted because they bring pleasure, meaning, or both. This will be among the first studies that incorporate a comprehensive and ecologically valid assessment of self-reported reward processes.

We adopt a novel task from the social psychology literature to assess social reward motivation with subliminal cues. Research on subliminal/unconscious reward processing indicates that subliminal presentation of reward cues can alter subsequent effort on cognitive tasks. In several studies, participants have been shown to perform better (e.g., faster reaction time) after subliminal presentation of a higher versus lower monetary reward. The current study proposes to adapt this task to assess the effects of subliminal presentation of different types of reward on subsequent performance. This task will be designed to assess alterations in sensitivity to non-social rewards (e.g., presentation of high value money) and social rewards (e.g., presentation of positive faces), in comparison to no reward (e.g., presentation of neutral faces or low value money). Thus, this task has the advantage of 1) assessing non-social and social reward and 2) assessing basic motivation without the complex cognitive processing and probability assessment that characterizes tasks like the EEfRT.

Given that inflammation can influence dopaminergic function, which underlies reward motivation and learning, this proposal includes an assessment of **resting state eye blink rate (EBR).** EBR is used as an indicator of striatal dopaminergic (D2 receptor) activity and correlates with performance on reward motivation and learning tasks. Alterations in dopaminergic function activity have been proposed to play a key role in mediating effects of inflammation on reward motivation, and EBR has been shown to decrease with greater age. However, EBR has not been assessed following endotoxin administration. With the addition of this brief and simple measure, we will test for changes in central dopaminergic activity following endotoxin, and examine

whether these changes are correlated with reward task performance among women in early and late adulthood.

Focus on age represents a major advance from current research
This will be the first study to characterize potential age differences in the effects of inflammation on reward processing. These processes evidence changes across the lifespan, so it is possible that inflammation may have different effects depending on age. Understanding these differences has important implications for developing targeted and effective interventions to improve well-being and reduce distress in younger and older adults.

C. APPROACH

C.1. Preliminary studies

C.1.1. Increases in inflammation following influenza vaccine are associated with changes in reward processing

Aim: The aim of this observational study was to assess whether increases in the pro-inflammatory cytokine interleukin-6 (IL-6) would increase pre- to post-influenza vaccine, and whether these changes would correlate with changes in performance on reward tasks assessing motivation (monetary reward), learning (monetary reward) and sensitivity (monetary and general social reward).
Methods: 41 healthy UCLA undergraduate students participated in this pre-post within-subjects study. Participants completed baseline assessment of reward tasks, a week of daily diary assessment, and were then scheduled to receive the annual influenza vaccine. Participants provided blood samples prior and 24-29 hours after the vaccine. Participants completed behavioral reward tasks 24-29 hours after the vaccine; this 24-29 hour period was based on prior research showing an IL-6 peak one day post-influenza vaccine.
Results: Levels of IL-6 increased significantly from pre-to post-vaccine, but performance on the reward tasks did not significantly differ from pre-to post-vaccine. However, as hypothesized, increases in IL-6 were correlated with decreases in reward motivation for monetary reward, and decreases in attentional bias to positive faces (an index of sensitivity to general social reward). Changes in sensitivity to monetary reward, as operationalized through parameters in the reward motivation task, were not associated with changes in IL-6. Increases in IL-6 correlated with increases in reward responsiveness on a standardized learning task, although sample size for computational analyses was not sufficient to identify whether this was driven by increases in learning or sensitivity.
Conclusion: Consistent with hypotheses, mild increases in IL-6 following influenza vaccination correlated with decreases in motivation for monetary reward, and decreases in sensitivity to general social stimuli. Contrary to hypotheses, increases in IL-6 were not correlated with change in sensitivity for monetary reward and were associated with increases in reward responsiveness on a reward learning task.
These data suggest an association between the pro-inflammatory cytokine IL-6 and performance on reward tasks in multiple reward dimensions for both non-social and general social reward.

C.1.2. Stress-induced inflammation reduces sensitivity to general social stimuli, but increases motivation and learning for monetary reward

Aim: The aim of this randomized controlled study was to assess whether increases in IL-6 following stress mediated delayed effects of an acute psychosocial stressor on reward processing using standardized behavioral measures of reward motivation, learning and sensitivity.
Methods: 54 healthy female undergraduate students completed a baseline visit assessing reward processing, and were then scheduled for an experimental session at the UCLA CTRC. They were randomly assigned to experience stress (Trier Social Stress Task) or no-stress (Placebo Trier) and completed reward tasks 90-120 minutes post-stressor, at which point the inflammatory response to stress peaks.
Results: Participants in the stress condition had a greater increase in IL-6 from baseline to 120 minutes post-stress than participants in the control group. Mediation analyses demonstrated that increases in IL-6 mediated the effect of stress on increased motivation for monetary reward in the context of low probability, and decreases in sensitivity to general social reward. No effects were observed for sensitivity to monetary reward.
Conclusion: Consistent with our prior work, increases in IL-6 were associated with decreases in sensitivity to general social reward. Effects on reward motivation conflict with our prior work, but this facilitative pattern has been observed in other studies using endotoxin as the inflammatory stimulus.
These data suggest a causal role of inflammation in alterations in reward processing following stress. Inflammation following stress may increase reward processing for monetary reward, but decrease processing for general social reward.

C.1.3. Decreases in inflammation following a stress-reduction intervention are associated with increased neural reactivity in the ventral striatum to positive non-social, but not social reward stimuli.
Aim: The aim of this within-subjects pre-post study was to assess whether decreases in IL-6 following stress reduction (mindfulness meditation) were associated with pre-post intervention increases in neural sensitivity to social and non-social reward in a sample of younger breast cancer survivors.
Methods: 22 women with a history of early stage breast cancer who had no current signs of disease completed a baseline assessment of neural sensitivity to presentation of positive social images and positive non-social images (e.g., nature scenes) compared to neutral images. After completing a 6-week mindfulness meditation intervention the women completed the task a second time.
Results: The intervention reduced psychological distress and increased psychological well-being. There was a significant increase in reactivity in the right ventral striatum in response to positive non-social versus neutral images. There were no significant pre-post changes in IL-6, and reactivity to positive social images showed a non-significant decline. Most importantly, decreases in IL-6 correlated with increases in the ventral striatum response to positive non-social images.
Conclusion:
These data suggest a link between decreases in IL-6 and increases in sensitivity to non-social rewarding stimuli.

C.2. Research Design and Methods

C.2.1. Overview of Study Design: This double-blind randomized placebo-controlled study will allocate 40 healthy premenopausal adult women (1:1) to receive endotoxin or saline infusion during a full day experimental session. Women will first complete a baseline visit, then one week of daily diary assessment prior to the experimental session. Daily diary assessment will resume for one week after the experimental session. At the baseline visit, participants will complete behavioral reward tasks and questionnaires, receive instructions on completing the daily diaries, and be scheduled for the experimental session at the UCLA Clinical and Translational Research Center (CTRC). Participants will complete behavioral tasks and questionnaires and provide blood samples for assessment of inflammatory markers during the experimental session.

C.2.2. Subjects: We will first select 40 healthy women in late adulthood (age 65+) from the ongoing SHARE-D study to guide recruitment of the younger group to ensure the groups are comparable in terms of BMI, years of education, and ethnicity. We will use the existing infrastructure in place for the SHARE-D study to recruit the younger group. Specifically, potential participants will be identified through the GENESYS Sampling System (Fort Washington, PA), which records telephone numbers and mailing addresses of households across the United States. We will select for households with at least one female person aged 30-40 years and living in the greater Los Angeles area. (Age information is based on known age-related data or a statistical estimate of age, predicted using individual household characteristics and Census demographic information.) This recruitment system is already in place for the parent study (IRB # 16-000583, R01 #1R01AG05194401A1). The PI or authorized research staff will send out the recruitment letters and respond to inquiries by phone. If potential participants do not reach out to the study team after receiving the recruitment letter, we will follow-up with a phone call 2-3 days later. As with the parallel study, compensation for completion of all study components will be $1,000. Unless irrelevant (e.g., age), the current study will use the same inclusion and exclusion criteria as the parallel study.
Inclusion Criteria: Women (biologically female) between the ages of 30 and 40 who are in good general health, premenopausal (as evaluated during eligibility assessments), and able to use an iphone/Android phone to complete daily diaries.
Exclusion Criteria: Males will be excluded to reduce variability and enhance power; past studies have demonstrated sex differences in reward processing,[25] higher prevalence of depressive symptoms and inflammation related disorders in women[11], and greater behavioral sensitivity to an inflammatory stimulus in women versus men[26]. Other exclusion criteria include pregnancy or planning to become pregnant; presence of chronic mental or physical illness, history of allergies, current and regular use of prescription medications, and nightshift work or time zone shifts (>3 hours) within the previous 6 weeks, or previous history of fainting during blood draws.

Detailed exclusion criteria for chronic disease include: 1) presence of co-morbid medical conditions not limited to but including cardiovascular (e.g., history of acute coronary event, stroke) and neurological diseases (e.g., Parkinson's disease), as well as pain disorders; 2) presence of co-morbid inflammatory disorders such as rheumatoid arthritis or other autoimmune disorders; 3) presence of an uncontrolled medical condition that is deemed by the investigators to interfere with the proposed study procedures, or to put the study participant at undue risk; 4) presence of chronic infection, which may elevate proinflammatory cytokines; 5) presence of an

acute infectious illness in the two weeks prior to an experimental session.

Detailed exclusion criteria for psychiatric disorders include: (6) current Axis I psychiatric disorders as determined by the Research Version of the Structured Clinical Interview for DSM-5 (SCID-5-RV), including a current major depressive disorder and substance dependence (a prior history of depression is not an exclusion criterion) (7) lifetime history of suicide attempt or inpatient psychiatric admission; (8) current history of sleep apnea or nocturnal myoclonus, as confirmed by PSG; (9) phase-shift disorder, which will be identified by the SCID and the Duke Structured Interview for Sleep Disorders (DSISD);

Detailed exclusion criteria for medication and substance use include: (10) current and/or past regular use of hormone-containing medications including steroids; (11) current and/or past regular use of non-steroid anti-inflammatory drugs; (12) current and/or past regular use of immune modifying drugs that target specific immune responses such as TNF antagonists; (13) current and/or past regular use of analgesics such as opioids; (14) current and/or past regular use of cardiovascular medications, including antihypertensive, antiarrhythmic, antianginal, and anticoagulant drugs; (15) use of antidepressant medications or other psychotropic medications (none in the last 6 months); (16) current smoking or excessive caffeine use (>600 mg/day) because of the known effects on proinflammatory cytokine levels; (17) evidence of recreational drug use from urine test.

Detailed exclusion criteria for health factors include: (18) BMI > 30 because of the effects of obesity on proinflammatory cytokine activity and also on risk for sleep disordered breathing; or (19) any abnormalities on screening laboratory tests. In addition, participants who, on arrival to the study, show any of the following symptoms will not be allowed to complete the study: (a) blood pressure less than 90/60 or greater than 160/120, (b) pulse less than 50 beats/minute, or (c) temperature greater than 99.5F.

These inclusion and exclusion criteria will be examined in detail and confirmed in the in-person screening session by the study physician (Michael Irwin, MD).

C.2.3. Procedures:

Recruitment and Enrollment: Individuals interested in participating will be asked to contact the research staff through a study email account. Participants will go through a two-step screening and assessment procedure to ensure that they are eligible for participation in the study. The initial screening process will be completed over the phone by research staff or the PI. In the first step, participants will be telephoned and given an overview of the study, including the fact that they may be exposed to a bacterial toxin that could induce mild flu-like symptoms and that they will have multiple blood draws. Participants will be asked if they are still interested. If so, participants will be asked a pre-approved list of questions and will be excluded from the study if they have certain conditions (e.g., auto-immune diseases). Eligible and interested participants will then be scheduled for an in-person screening session (Visit 1). We will ask participants to schedule this session near the last day of their menstrual cycle to minimize variability due to hormonal status. At the beginning of the session, we will go over the study procedures and the potential side effects of endotoxin. Participants will be asked to read over and sign the informed consent. Participants will be told their participation is completely voluntary and that they are free to discontinue their participation at any time. After reading the consent form, each participant will be encouraged to ask any questions that they may have about the procedure, and the study physician (Dr. Irwin) will be available to answer any questions that they have. Each participant will be asked if they are comfortable and that they understand the procedure to their full satisfaction. Under no circumstances will coercion be applied to obtain informed consent, and participants will be thanked for their participation in the study regardless of whether they choose to continue in the main study. In the event that the participant has signed the consent, but wishes to terminate the study at any time before completion they will be allowed to do so. Informed consent will be delivered by the PI or research staff. Consent will be deemed provided if after reading the consent document, the prospective participant signs it. For eligibility assessment, participants will have their height and weight measured, provide a urine sample to check for substance use, and an EKG to check for signs of heart disease. They will complete the Structured Clinical Interview for DSM-V Disorders with trained research staff. Final eligibility will be determined by reviewing results with the supervising study physician.

Baseline Visit 1: Participants will arrive at the UCLA Cousins Center in the late morning to complete informed consent and eligibility assessment, followed by administration of behavioral tasks. Because reward processing may have diurnal variation, we will administer baseline tasks between the hours of 10am and 2pm to match the time of administration during the experimental session. Participants will also complete questionnaires assessing early life adversity and trait personality measures that capture individual differences in reward processing. At the end of the visit, participants will receive instructions for the daily diary assessments. They will also be scheduled for their Visit 2 and Visit 3 session at the UCLA Clinical and Translational Research Center (CTRC).

Daily Diaries: Daily diaries will be completed for 7 days prior to the experimental session and 7 days after the session. Participants will receive prompts by text or email at 5 random points during the day. Completing each prompt will take 1 to 2 minutes. Participants will complete one additional prompt each evening assessing positive and negative mood, daily rumination and sleep quality (estimated time: 3 minutes). To maximize compliance with study procedures and retention, participants will be contacted by phone at least once during each week of daily diary assessments.

Visit 2: Participants will arrive at the UCLA Cousins Center in the late morning to complete tasks and questionnaires. They will also be reminded of requirements for the Visit 3 (e.g., no caffeine).

Experimental Study Session - Endotoxin versus Saline Placebo:

Randomization will be coordinated with CTRC staff so that participants are not assigned a condition until this session, and so that study staff will remain blind to the condition. Upon arrival, participants will have one catheter inserted on their dominant forearm for hourly blood draws to assess IL-6 and one on their non-dominant forearm for continuous saline flush and drug administration. At 90 minutes post arrival, a nurse will administer via intravenous bolus either endotoxin (.8 ng/kg of body weight) or a placebo (same volume of .9% saline). For the remaining session, the participant's vitals will be monitored every half hour, and they will report on sickness symptoms, feelings of social connection, and mood every hour. Participants will complete behavioral tasks in the same order as the older adults in the parallel study to the extent that the tasks overlap. Participants will be reminded of the remaining study procedures, which include another week's worth of daily diaries, at the end of the session.

Safety Monitoring and Discharge from Study: Participants will be discharged from the session 10-12 hours post injection upon approval of the study physician and assurance that physical and psychological symptoms returned to baseline. Participants will receive a safety follow-up phone call 1 and 7 days after the CTRC session.

C.2.4. Reward Processing Tasks (see Table 1)

Motivation for Social and Non-Social Reward

The *Effort Expenditure for Rewards Task (EEfRT)* [34] is an established task in the depression literature that assesses motivation to work for monetary reward at varying degrees of probability of winning and varying degrees of potential reward magnitude. Participants play a 20-minute "button pressing game" in which they choose between an easy task (worth $1.00) and a hard task (worth between $1.24-$4.12). Easy tasks require 30 button presses using the dominant index finger in 7 seconds, while hard task trials require 100 button presses with the pinky finger of the non-dominant hand in 21 seconds. Reduced motivation for reward on the EEfRT is operationalized as a decreased willingness to exert greater effort for monetary reward.

A *subliminal reward motivation*[35] task will be used to assess motivation for non-social and general social reward. The task takes 14 minutes to administer and consists of 70 trials. Trials are preceded by subliminal presentation of one of two types of faces and two types of money stimuli (14 trials each) all drawn from a standardized database. Each trial takes 10 seconds, and involves subliminal presentation (17ms) of one of the face/money types, a 1500ms fixation period, presentation of a mathematical problem (e.g., 3+5+9=16), a response from the participant (i.e., indicating whether the statement is correct or not using the keyboard) and feedback (1500ms). The two faces will be high arousal positive faces, or neutral faces; the two types of money stimuli will pictures of a silver dollar and a dime. We hypothesize greater speed for trials primed by high reward (positive faces) compared to low reward (neutral faces) across groups, and that this difference will be attenuated in the endotoxin versus control group.

Learning for Monetary and Social Reward: The *probabilistic reward task* (PRT)[36] is a 14-minute signal-detection task that objectively measures implicit reward learning and reward sensitivity for monetary reward. It has been used in the depression literature with some consistency. Participants will complete two blocks of 100 trials each. In each trial, participants are asked to identify which of two difficult-to-differentiate stimuli are presented. Both stimuli are presented equally often, but an asymmetric (3:1) reinforcement schedule is used to induce a response bias towards more frequently rewarded stimuli. The magnitude of this response bias changes is used as an index of reward responsiveness, which encompasses learning and sensitivity. Computational modeling is used to disentangle the learning and sensitivity parameters. This study will also use a second version of this task which relies on positive social stimuli (images of positive faces) to induce the response bias, rather than monetary reward. This version of the task has been used in previous studies, and we will administer it several hours after the monetary PRT has been completed.

The *probabilistic selection task*[36] is a 15-minute trial and error (procedural) implicit learning task that assesses reinforcement learning for both positive and negative monetary cues. Prior work suggests that dopaminergic function is associated with learning from both types of cues via different neurobiological

mechanisms. Lower dopamine levels are associated with decreased sensitivity to positive reinforcement, but increased sensitivity to negative reinforcement. A similar trend is expected following the inflammatory stimulus.

Anticipatory Social and Non-Social Reward: The *Affective Forecasting Task*[38] consists of 36 hypothetical future events, 18 negative (e.g., A very close friend tells you that you are being really annoying) and 18 positive (e.g., A family friend comes to town and takes you to a fancy dinner). Participants are instructed to imagine each event happening a month from today, immerse themselves in the experience of the event, and rate how they would feel on a scale from 1 (unhappy) to 7 (very happy). Higher anticipatory reward sensitivity is operationalized as higher ratings on this scale. Events are categorized as close, general, or non-social. Because the original items were developed for an undergraduate population, the current study will modify scenarios as needed to make them age appropriate.

Sensitivity/Consummatory Response to Social and Non-Social Reward: Two common emotion processing tasks, the emotional dot probe and face morphing task, will be used to assess sensitivity to general social reward cues. The 5-minute *emotional dot probe task*[39] will be used to assess attentional bias towards positive versus neutral faces. The 5-minute *emotion identification task*[40] will present participants with pictures of faces portraying different emotions that vary in level of intensity. Pictures will be presented at 10% increments of intensity of the target emotion and participants will be asked to indicate which of the three emotions is present; reduced sensitivity to rewarding social stimuli is indicated by slower identification of happy emotional faces.

EEfRT: An index of reward sensitivity can be derived from performance on the EEfRT. Specifically, reduced reward sensitivity is operationalized as an attenuated association between increases in the magnitude of monetary reward across trials and increases in hard trial choice (within those hard trials that are chosen).

PRT: Computational modeling will be used to derive a score capturing sensitivity versus learning rate on the PRT for both the monetary and social reward version.

Intersection of Reward Motivation and Sensitivity for Non-Social Reward: A "Cartoon Effort Task"[41] that has been used in samples with depressed adults as well as healthy controls, will be used to assess the degree to which reward motivation and sensitivity coincide. Past work suggests dissociation is a feature of depression. The task uses humorous and nonhumorous single-panel cartoons as reward and non-reward stimuli. Motivation is the amount of effort participants are willing to exert to view funny vs non-funny cartoon. Participants also rate their enjoyment when viewing funny cartoons. The task is 20-30 minutes long, and has been piloted in our lab previously.

Intersection of Reward Motivation and Sensitivity for Social Reward: A *social choice*[42] *and semi-structured social interaction*[43] task will be used to assess motivation and sensitivity for social reward. The social choice task will be administered at baseline and during the experimental session. Participants will be asked to rate their desire to engage in three 10-minute activities on a 1-10 Likert scale. These include a social activity (talking with another person) and two solitary activities (solving word problems, sitting quietly). Participants are told that this preference along with an element of chance will determine which they actually do. At the baseline visit, a neutral option will always be chosen. During the experimental session, the social interaction will always be selected. For this semi-structured social interaction task, participants will be asked to spend 5 minutes talking about an important person in their life to a research assistant trained in reflective listening. The discussion will be recorded and later transcribed and scored for percentage of positive and negative emotional words using Linguistic Inquiry and Word Count Software. Perceptions of the RA will also be assessed (e.g., "S/he was truly interested in me").

Monetary Reward Sensitivity: A 6-minute Balloon Analogue Risk task (BART) will be used to assess sensitivity and impulsivity for monetary reward. The goal during the BART is to earn as many dollar points as possible; participants view a computer screen with a balloon that they can click to pump with air; the bigger the balloon gets, the more money the participant can win (5 cents per pump). However, each pump of air involves risk of the balloon popping, in which case the participant loses money gained for that trial. There are 30 trials and the balloon is set to pop at random points (between 1-128 pumps).

C.2.4.5 Inflammation: Cytokines, signaling pathways, gene expression analyses. For Visit 3, blood samples will be collected at baseline, every half hour for the first two hours, and hourly for the remainder of the 12 h. Samples will immediately processed and stored at -800C. Plasma samples (all timepoints) will be assayed for pro- and anti inflammatory cytokines (IL-1, IL-6, IL-8, TNF, and IL-10) by means of a high sensitivity bead-based multiplex immunoassay (Performance High Sensitivity Human Cytokine, R& D Systems, Minneapolis, MN) and a Bio-Plex 200 (Luminex) Instrument. Baseline levels of CRP will be collected to assess systemic inflammation. Peripheral blood mononuclear cell nuclear extracts (baseline, 30 min, 1 h, 2 h or peak cytokine response) will be quantified for activated NF-κB as ng p65/□g total protein utilizing recombinant p65

(Active Motif, Carlsbad, CA) as the reference standard (range 0.08-5.00ng). At baseline, 2- and 4 hours post-injection, blood samples will be drawn in PaxGene RNA tubes, which preserve RNA integrity. Expression of genes involved in proinflammatory pathways (*IL1B, IL6, IL8, CD83, CCL3, TNFAIP3, and NF-κB/Rel* family) will be assayed by quantitative real-time RT-PCR using established TaqMan Gene Expression Assays. Genome-wide transcriptional profiling will use Illumina HT-12 BeadArrays.

C.2.4.6 Dopaminergic Function

Resting eye blink rate, an indicator of striatal dopaminergic (D2 receptor) activity, will be assessed prior to infusion, and 1.5, 3, 4.5, and 6 hours post-infusion. Eye blinks will be video recorded for a 5-minute period. The participant will be asked to sit quietly, look ahead, and refrain from visually fixating on any objects. The video will be coded by two research assistants (blinded to condition).

C.2.4.7 Daily Diary

Daily diaries will be completed for 7 days prior to the experimental session and 7 days after the session. Participants will receive prompts by text or email that will direct them to the survey site at 5 random points during the day (range of time between prompts = 30 to 180 minutes). Participants will have 1 hour to respond to a single prompt; this is to ensure that participants are given sufficient time to complete an assessment during a working environment (e.g., business meetings) or family obligations (e.g., driving children to school). Data from a second or third submission in the same 30-minute window will be discarded. Completing each prompt will take 2 minutes. Participants first indicate the extent to which they enjoyed 10 types of activities since the last prompt (or since waking) on a 0-100 visual analogue scale. From the same list of activities, participants then rate how much they are currently looking forward to each activity. For each activity, they rate their motivation (hedonic, eudaimonic, or not applicable if their "wanting" is sufficiently low). Each evening, participants will complete one additional prompt assessing positive and negative mood, daily rumination and sleep quality (estimated time: 3 minutes).

C.2.4.8. Emotion and Cognition Assessment

To maintain convergence with the parent R01, the current study will also assess emotion and executive function processes during the experimental session at the CTRC.

C.2.4.8.1. Self and Observer Reports. Participants will self-report negative mood each hour using the Profile of Mood States (POMS), the Montgomery Asberg Depressing Rating Scale and the Depression Adjective Check List. Participants will also report on feelings of social disconnection and loneliness and subjective feelings of social support throughout the session. Research staff (blind to condition) will make ratings of observed depressed mood using items from the Hamilton Depression Rating Scale, which will be adapted to evaluate acute changes in depressive symptoms. Participants will self-report positive mood each hour using the Profile of Mood States (POMS), and items that assess anticipatory and consummatory pleasure and interest in activities and social interaction (e.g., the Snaith Hamilton Pleasure Scale).

C.2.4.8.2. Emotion Regulation and Perception. Participants will complete questionnaires assessing dispositional and situational emotion regulation strategies and attitudes towards emotions. Participants will also complete a 20-30 minute standardized emotion regulation task[44]. The task includes two phases: a reactivity phase and a regulation phase, and assesses the ability to down-regulate negative emotional response to negative images and/or film clips using reappraisal strategies. Participants receive instructions as to how to reappraise (e.g., thinking about the "silver lining" or imagining oneself at a distance from the negative emotion). The dependent variable is the degree to which self-reported emotion changes when reacting to versus reappraising negative stimuli. The task also includes assessment of the ability to up-regulate positive emotion using cognitive strategies (e.g., thinking about a positive image/film clip in such a way that more positive emotion is felt). The stimuli used in the emotion regulation task are drawn from standardized databases and have been used in many previous studies, including studies conducted in our group with younger breast cancer survivors. To assess emotion perception, participants will complete the mind-in-the-eyes task, a standardized task that assesses the ability to identify emotional expressions from images of eyes only.

C.2.4.8.3. Cognition/Executive Function. Three cognitive tasks will be administered by computer to assess three aspects of executive function - Updating, Shifting, and Inhibition[45]. Total task administration time is 30 minutes with each task lasting 10 minutes. The *Spatial 2-Back* task is used to assess Updating, the ability to monitor and replace information in working memory. For each of 120 trials, participants view an array of boxes, 11 white and 1 black. The location of the black box varies across trials. Participants are tasked with using one of two keys to indicate whether the black box is presented in the same location as it was two trials back. Participants do not receive feedback regarding accuracy, and trials proceed automatically if a response is not made within 2000ms. The task includes 20 practice trials. The *Color-Shape* task is used to assess Shifting, or the ability to switch between mental sets. For each of 104 trials, participants are presented with a

circle or square that is of blue or red color. Participants are asked to use 1 of 2 keys to indicate whether the figure is red or blue (52 color trials) or square or triangle (52 shape trials). One key is paired with a color and a shape (e.g., red and square) and one key is paired with the other color and shape (e.g., blue and circle). For each trial, the letter C or S is presented above the figure to indicate color versus shape trials. The order of C/S trials is randomized, and there is no time limit for participant response for each trial. A quiet "ding" occurs following incorrect trials. The task includes 52 practice trials: 26 color followed by 26 shape trials. The *Antisaccade* task is used to assess Inhibition. Participants are tasked with inhibiting a reflexive response towards a visual cue in order to correctly identify a target stimulus presented elsewhere. For each of 72 trials, participants view a centrally positioned fixation point (the letter X) on the computer screen for a variable amount of time (1500-3500ms). A visual cue (a black circle) is then presented on one side of the computer screen for 225ms. The target stimulus (a number from 1 to 9) is presented on the opposite side of the screen for 250, 233, or 200ms before being masked by gray cross-hatching. The participant verbally reports the target number to a trained research assistant or recording device or uses the keyboard to indicate the number. The participant does not receive feedback regarding accuracy, and there is no time limit for participant response for each trial. The task includes a series of practice trials in which the visual cue is not presented with the target stimulus (n = 12) followed by trials in which the visual cue and target stimulus are presented on the same side of the computer screen (n = 24).

C.2.4.8.4. Negative Affective Processes. Participants will complete the *Cyberball Social Exclusion Task*, which assesses self-reported sensitivity to social rejection at 2 hours post-injection only. Participants are told that they will play a virtual ball-tossing game with two other players over the Internet, although the task is actually a preset computer program. In the first round (inclusion),participants will play with the two other players for the entire period. In the second round (exclusion), participants will receive the ball for seven throws and then will be excluded for the rest of the round when the two players will stop throwing the ball to the participant. After the task, participants complete a self-report measure of social distress in response to the social exclusion. As described above, participants will also complete two common emotion processing tasks, the emotional dot probe and face morphing task, which can be used to assess sensitivity to negative social cues. Specifically, the 5-minute *emotional dot probe task*[39] will be used to assess attentional bias towards negative (e.g., sad, angry) versus neutral faces. The 5-minute *emotion identification task*[40] will present participants with pictures of faces portraying different emotions that vary in level of intensity. Pictures will be presented at 10% increments of intensity of the target emotion and participants will be asked to indicate when they detect presence of fear and sadness; increased negative affective sensitivity is indicated by faster dentification of negative emotional faces. Finally, participants will complete a thematic apperception test (TAT), a projective psychological test in which participants view ambiguous pictures of people and are asked to talk aloud about their impressions of the images. The verbal descriptions are audio recorded and later subjected to linguistic inquiry and word count (LIWC) which calculates the degree to which different categories of words are used, including valence.

C.2.4.9. Telephone Follow-up

Participants will receive a follow-up safety phone call the day after and 1 week after the experimental session in which they will be asked if they have experienced any unusual physical symptoms or feelings of depressed mood. If a subject indicates any unusual physical symptoms or feelings of depressed mood, then the interviewer will document those responses and report those results to Dr. Michael Irwin.

C.3. Interpretation of Results and Potential Concerns

Endotoxin elicits sickness symptoms (e.g., nausea, achiness) that will be assessed as potential covariates in statistical analyses. The battery of administered tasks could lead to excessive fatigue and non-compliance. This is not currently a concern in the related study with older adults, but we will monitor participants' subjective response to the tasks, as well as current fatigue, and assess these variables either as potential covariates or as indicators that the experimental session requires modification. While we do not anticipate strong emotional response to any of the tasks, there is the potential for carryover effects without task counterbalancing. However, since the order is the same for the experimental and the control group, such effects should not impact the primary research questions assessing group differences.

C.4. Statistical Analyses

All measured variables will be assessed for distributional qualities and transformed if necessary. Adequacy of random assignment will be tested using t-tests and chi-square tests as appropriate. Consistent with our previous work with the behavioral reward tasks, we will use multiple regression analyses with group (endotoxin vs. placebo; binary predictor), age (continuous variable) and a group by age interaction term as our predictors of interest. Post-infusion scores on each behavioral task will be used as the outcome, and pre-

infusion scores included as covariates when applicable. Additional covariates will include BMI and years of education. For the PRT, computational analyses will be used to derive parameters for learning rate and reward sensitivity; these parameters along with total response bias score will then be used in multiple regression models. For the EEfRT, we will use generalized estimating equations in addition to multiple regression (with proportions of hard trials chosen as the outcome), as is typical for this task. For the emotional dot probe task, attentional bias scores will be calculated by subtracting reaction time when a dot probe replaces a neutral face from reaction time when the probe replaces the emotion face. For the face morphing task, the outcome is reaction time averaged across each emotion type (e.g., happy, sad).

Also consistent with our previous work, we will assess the relationship between changes in IL-6, a key pro-inflammatory cytokine that has previously been linked to reward alterations, and changes in reward task performance. These within-person analyses are more sensitive and may be required given the small sample size. For these analyses, we will conduct multiple regression analyses within the endotoxin group and include age, IL-6, and an age by IL-6 interaction term as the predictors of interest.

C.4.1. Justification of Sample Size

Our previous work with several of the behavioral reward tasks (e.g., emotional dot probe, PRT) has yielded small to moderate effect sizes when assessing the relationship between IL-6 and task performance (e.g., f^2 = .16, .22, .32). Given this range of effect sizes, a multiple regression analysis with three predictors of interest (age, group, agexgroup interaction) and two covariates (bmi, education level) would require a sample size of 38 to 75 to detect effects with .80 power. This work has relied on very mild increases in IL-6, and we expect endotoxin to yield stronger effects. Indeed, prior work with the endotoxin model has shown significant differences on a reward motivation task with a sample of 29 participants (15 saline placebo, 14 endotoxin), and greater feelings of social disconnection following endotoxin vs. placebo were observed in a small sample of women (n=20; age 18-36). Thus, the current study aims to recruit 40 participants with equal randomization between the endotoxin and saline group, and an additional 40 female participants (20 placebo, 20 endotoxin) will be drawn from the associated SHARE-D for a total sample of 80.

Table 1. Categorizing each Behavioral Reward Task by the Dimension and Type of Reward Assessed

Reward Dimension	**Type of Reward**		
	Close Social Reward	General Social Reward	Non-Social Reward
Anticipation	• Affective Forecasting • Daily Diary	• Affective Forecasting • Daily Diary	• Affective Forecasting • Daily Diary
Motivation	• Social Interaction Task Question	• Implicit Motivation Task	• Cartoon Task • EEfRT
Consumption	• Daily Diary • Social Interaction Task Discussion	• Cartoon Task • Daily Diary • Attentional Bias and Emotion Detection (for positive faces)	• Cartoon Task • Daily Diary
Learning		• Probabilistic Reward Task (social version)	• Probabilistic Reward Task (monetary version) • Probabilistic Selection Task

Table 2. List of Questionnaires & Behavioral Tasks at Each Time Point: IRMA Endotoxin Study

	SCREENING AND ELIGIBILITY (VISIT #1)	**PRE CTRC SESSION (VISIT #2)**	**CTRC SESSION (VISIT #3)**
Demographic Screening			
Demographics Questionnaire	**X**		
Substance Use Assessment			
Substance Use History Questionnaire	**X**		
Caffeine Use History Questionnaire	**X**		
Cognitive Screening			
Mini- Mental Status Exam (MMSE)	**X**		
Sleep Assessment			
Duke Structured Sleep Criteria Questionnaire	**X**		
Sleep History Questionnaire	**X**		
Insomnia Severity Index Questionnaire (ISI)	**X**		
Pittsburgh Sleep Quality Index (PSQI)	**X**		
Berlin Sleep Apnea Questionnaire	**X**		
Munich Chrono type Questionnaire (MCTQ)	**X**		
Daily Diary (14 days)	**X**		
Psychiatric Assessment			
Psychiatric Screening Questionnaire	**X**		
Structured Clinical Interview For DSM-5 (SCID)	**X**		
Depression Assessment			
PHQ-9 Questionnaire	**X**		
Beck Depression Inventory (BDI)	**X**		
Beck Anxiety Inventory (BAI)	**X**		
Inventory of Depressive Symptomatology (IDS-SR)	**X**		

Rumination Response Scale (RRS)	**X**		
	SCREENING AND ELIGIBILITY (VISIT #1)	**PRE CTRC SESSION (VISIT #2)**	**CTRC SESSION (VISIT #3)**
Depression Assessment (Observer Rating)			
Montgomery- Asberg Depression Rating Scale (MADRS)			**X**
The Hamilton Rating Scale for Depression			**X**
Profile of Mood States			**X**
Fatigue Assessment			
Fatigue Symptom Inventory (FSI)	**X**		
Multi-Fatigue Symptom Inventory (MFSI)	**X**		
Medical Assessment			
SF-36 Questionnaire	**X**		
Charleston Co-Morbidity Index (CCMI)	**X**		
Medical History Questionnaire	**X**		
Medication History Questionnaire	**X**		
Chronic Disease Score Questionnaire (CDS)	**X**		
Physical Activity Assessment			
Godin Leisure Physical Activity Survey	**X**		
Psychosocial Assessment			
Perceived Stress Scale	**X**		
UCLA Loneliness	**x**		**X (pre & post injection)**
Anhedonia (TEPS, MASQ, SHAPS)	**x**	**x**	**X (pre & post injection)**
Social Provision Scale	**x**		
Interpersonal Support Evaluation List (ISEL)	**x**		
Risky Families Questionnaire (RCF) and Adverse Childhood Experiences Questionnaire	**x**		
MRS Rejection Sensitivity	**x**		**X (pre & post injection)**

Social Ladder	**x**		**X (pre & post injection)**
	SCREENING AND ELIGIBILITY (VISIT #1)	**PRE CTRC SESSION (VISIT #2)**	**CTRC SESSION (VISIT #3)**
Interest in Activity Questionnaire	**x**		
EPQ (personality; extroversion and neuroticism)	**x**		**X (pre & post injection)**
ATTS-S	**x**		**X (pre & post injection)**
FES	**x**		**X (pre & post injection)**
Social Support Scale (SSS)	**x**		**X (pre & post injection)**
BSI	**x**		**X (pre & post injection)**
2-Way Social Support Questionnaire	**x**		**X (pre & post injection)**
Social Reward Questionnaire			**X (pre & post injection)**
Stressful Events in the Last Year Checklist	**x**		
Mental Health Continuum		**x**	
Behavioral Avoidance/Inhibition Scales (BIS/BAS)	**X**		
Symptoms Questionnaire			
Physical Symptoms Questionnaire			**X (pre & post injection)**
POMS			**X (pre & post injection)**
Modified Differential Emotions Scale		**X**	**X (pre & post injection)**
How I Feel Right Now			**X (pre & post injection)**
Feelings of Social Disconnection Scale			**X (pre & post injection)**
Behavioral Tasks (Pre and Post)			
Social Reward Task		**x**	**x**
Affective Forecasting		**x**	**x**
Reward Learning (Pizzagalli)		**x**	**x**
Treadway (EEFRT)	**x**		**x**
Cartoon Task			**X post injection)**
Emotion Intensity Task (Face Morphing) (Pollack)			**X (pre & post injection)**

	SCREENING AND ELIGIBILITY (VISIT #1)	**PRE CTRC SESSION (VISIT #2)**	**CTRC SESSION (VISIT #3)**
Implicit Motivation Task			**X (post injection)**
Attentional Bias Task (Dot Probe)			**X (pre & post injection)**
Probabilistic Selection Task			**X (post injection)**
Thematic Apperception Test (TAT) (added: 02/28/18)			**X (photo #1- pre-injection/ Photo #2 and Photo # 3 post-injection**

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