Effects of Pregnenolone on Perceived Social Isolation

PROJECT ABSTRACT

Perceived social isolation (PSI), also known as loneliness, has been shown to increase depression in humans and animal models and to increase the risk of early mortality by 26%. Despite the well-known effects of pregnenolone (PREG) in alleviating depressive behavioral effects of social isolation in mice and post-traumatic disorders and depressive symptomatology in humans, no studies to date have examined the effects of PREG on humans with PSI as well on a specific mechanism of action underlying PSI, such as increased hypervigilance to social threat- a vital first step in the novel use of PREG as an adjunctive therapy for attentional bias to social threats in individuals with PSI. We therefore propose a randomized, double-blind, between-subjects design, where three validated behavioral paradigms on social threats will be combined with high-density electrical neuroimaging to probe the neuro-circuitry of social threat processing in male adults (aged 21-80) high in PSI.

1. BACKGROUND AND SIGNIFICANCE

A growing body of research suggests that the perception of social isolation (PSI, or what has been termed loneliness; Cacioppo and Patrick, 2008) has detectable effects on attention, including increased vigilance for negative social stimuli, such as social threats (Cacioppo & Hawkley, 2009; Cacioppo, Balogh, Cacioppo, 2015, Cacioppo, Bangee, et al., 2015). This focus on self-preservation may promote short-term survival but has been shown to have long-term costs for health and well-being (Cacioppo & Hawkley, 2009). For instance, using a modified emotional Stroop task, we found that lonely subjects, relative to nonlonely subjects, show greater Stroop interference specifically for negative social relative to negative non-social words (Egidi, Shintel, Nusbaum, & Cacioppo, 2008). Stroop interference has been used to gauge the implicit processing of stimuli, so these results suggest that PSI is associated with a heightened accessibility of negative social information. Similarly, in an investigation of the effects of subliminal priming on the detection of painful facial expressions, Yamada and Decety (2009) found that PSI is associated with greater sensitivity to the presence of pain in dislikable faces, as gauged by the sensitivity index, *d*', from signal detection theory. In an eye tracking study, lonely and nonlonely young adults viewed various positive and negative social scenes and exhibited different fixation patterns. Individuals high in PSI (or loneliness) are more likely to first fixate on and to spend a greater proportion of their initial viewing time looking at socially threatening stimuli in a social scene, whereas individuals low in PSI are more likely to first fixate on and spend a greater proportion of their initial view time looking at positive stimuli in a social scene (Bangee, Harris, Bridges, Rotenberg, & Qualter, 2014). Combining high-density electrical neuroimaging with a behavioral task including social and non-social threat (and neutral) IAPS pictures known to probe pre-attentional mechanism for social threats (the pre-attentional social threat task, PAST, Cacioppo, Bangee, et al., 2015), we showed that individuals *high* in PSI (N = 10) differentiate social threat images from non-social threat stimuli more quickly (~116 ms after stimulus onset) than individuals low in PSI (N = 9; ~252 ms after stimulus onset) brains. That speed of threat processing in lonely individuals is in accord with the evolutionary model of loneliness that indicates feeling socially isolated (or on the social perimeter) leads to increased attention and surveillance of the social world and an unwitting focus on self-preservation. Brain source estimates expanded these results by suggesting that *lonely* (but not *nonlonely*) individuals showed early recruitment of brain areas involved in visual attention and self-representation (Cacioppo, Bangee et al., 2015). The next step is to investigate how and when this early hyper-attention for social threats in individuals high in PSI may be reduced.

For the past decade, preclinical and clinical data suggest that pregnenolone may be a promising therapeutic in reducing a wide range of emotional responses from anxiety- and depressive-like behaviors. Pregnenolone is neuroprotective, reduces anxiety, and enhances learning, memory, and myelination. Treatment with pregnenolone elevates allopregnanolone (ALLO, a neurosteroid that enhances $GABA_A$ receptor responses) and pregnenolone sulfate (a positive NMDA receptor modulator). In turn, exogenous administration of the neurosteroid allopregnanolone (ALLO) has also been shown to hold significant promises for reducing a wide range of emotional responses in humans with anxiety, depressive-like, and aggressive behaviors and inducing changes in responses to contextual fear conditioning in rodent models of emotional dysfunction in social isolation, and ALLO blockade impairs social and affective functioning. Although studies in humans are less abundant, they are broadly consistent with animal findings. For instance, cerebrospinal fluid levels of ALLO are reduced in women with major depressive disorder (MDD) and post-traumatic stress disorders (PTSD), and increase with successful

pharmacological treatment (Amin et al., 2006; van Broekhoven et al., 2003; Uzunova et al., 1998; Rasmusson et al., 2006; Strohle et al., 1999). Interestingly, various antidepressant agents (including fluoxetine, norfluoxetine, fluvoxamine and paroxetine) elevate ALLO brain levels (e.g., Uzunova et al., 1998; Romeo et al., 1998; Marx et al., 2006), leading to the suggestion that ALLO induction might be an important mechanism for the antidepressant effects of SSRIs (e.g., Pinna et al., 2006, 2009). Based on these observations, it has been suggested that ALLO dysregulation may contribute to the development of neuropsychiatric disorders, and that restoration of ALLO regulation may be a potential pathway for symptomatic improvement (Pinna & Rasmusson, 2011). Because a reduction in the synthesis of ALLO has been observed during social threat processing, potentially by reducing brain-derived neurotrophic factor (BDNF) regulation and increasing hypothalamic–pituitary–adrenal (HPA) activity through the hippocampus, amygdala, and bed nucleus of the stria terminalis (BNST (Cacioppo et al., 2015, Cacioppo & Cacioppo, 2015), we hypothesize that exogenous administration of ALLO precursor, pregnenolone, may help reduce hyperattention to social threats in PSI. Little is known, however, on the effects of oral administration of pregnenolone on hyperattention to social threat.

Historically, treatment with PREG has been found to alleviate the depressive behavioral effects of social isolation in mice and depressive symptomatology in humans by elevating ALLO. Therefore, PREG directed at up-regulating ALLO (neurosteroid deficit target) in individuals high in PSI could reduce PSI and improve its behavioral effects. The doses we propose in the current project have been shown to up-regulate ALLO: A single oral dose of 175 mg PREG approximately doubles ALLO serum levels over the course of 4–8 h, while a single oral dose of 400mg of PREG triples ALLO serum levels over the course of two hours in healthy non-psychiatric subjects with normal neuro-active steroid levels at baseline. One potential mechanism for PREG's ability to directly impact emotion neuro-circuitry is the up-regulation of ALLO in the central nervous system. Sripada and colleagues (2013), for instance, demonstrated that a single oral administration of PREG (400mg) reduces activation of brain areas involved in negative emotions, increases activity in the dorsal medial prefrontal cortex (dMPFC), and enhances connectivity between the amygdala and dMPFC, an effect that was associated with reduced self-reported anxiety. However, the effect of PREG on other negative emotional mechanisms, such as attentional bias to social threats and depressive symptomatology remains unknown. Our proposed novel use of the currently available dietary supplement, PREG, on PSI is based on formative animal work on social isolation by our colleague, Dr. Pinna. In a rodent model, Dr. Pinna demonstrated that: i) the exaggerated contextual fear responses expressed by socially-isolated mice can be normalized by increasing ALLO levels; ii) HPA dysfunction and impairment of hippocampal neurogenesis respectively can be normalized or prevented with the administration of exogenous ALLO either during or following a period of chronic stress (and iii) the establishment of depressive/anxiety-like behaviors in rodents can be precluded also with administration of exogenous ALLO-targeted treatment.

2. Purpose

The goal of the proposed research, which is based on prior human and animal work, is to test a proof-of-concept that acute oral administration of PREG reduces PSI in male adults (aged 21-80) high in PSI. Validated self-report measures (e.g., depression, anxiety, objective social isolation, life satisfaction) will also be used to examine changes in symptom severity and subjective feelings. The long-term objective of this project is to advance the use of PREG at remediating the protracted reduction in the neurosteroid, ALLO in individuals high in PSI and thereby reduce PSI and improve social withdrawal. The proposed project will employ a randomized between-subjects

design, where a validated behavioral paradigm on social threat attention , intention understanding, and perception of others will be combined with validated measures of depressive symptomatology in male adults (aged 21-80) high in PSI.

Reducing PSI, attentional biases, and depressive symptomatology through the use of a pharmacological treatment may, therefore, have potential benefits for health and well-being. Until now, a main challenge for physicians and mental health clinicians has been to become sufficiently informed about the scientific definition of PSI (or loneliness) so that other mental disorders were not mistakenly diagnosed and treated when PSI was either the primary presenting problem or the cause of the depression for which treatment was sought[44]. For decades, many clinicians believed that PSI was simply an aspect of depression with no conceptual distinctions worthy of study[1]. There is now considerable evidence showing that PSI and depression (as well as other factors such as introversion, neuroticism, objective social isolation, & social support) are separable and that PSI increases the risk for (or leads to) depression. For instance, even after controlling for objective social isolation and other potential confounding factors, PSI contributes to a constellation of physical and psychiatric dysfunctions[44-48]. PSI is also a risk factor for alcoholism, suicidal thoughts, aggressive behaviors, social anxiety, and impulsivity. In addition, PSI is a risk factor for cognitive decline and the progression of Alzheimer's Disease, recurrent stroke, obesity, increased vascular resistance and elevated blood pressure, increased hypothalamic pituitary adrenocortical (HPA) activity, diminished immunity, an under-expression of genes bearing anti-inflammatory glucocorticoid response elements and an upregulation of pro-inflammatory gene transcripts, and premature mortality. Moreover, meta-analyses indicate that cognitive/behavioral interventions have proven to be largely ineffective. Thus, a pharmacological treatment for PSI is timely needed. Despite its public health importance, there is no pharmacological treatment for PSI. In addition, a key neurobehavioral characteristic of individuals high in PSI is an increased implicit attentional bias for social threats, which has been linked to an early evoked neurophysiological response and underlie unsuccessful social connections and social withdrawal. This focus on self-preservation reinforces Cacioppo and colleagues' seminal evolutionary model of PSI[8] that emphasizes the role of hyper-vigilance to social threats as a mechanism promoting short-term survival but also as having long-term costs for health and well-being.

3. Innovation

In the present project, 224 PSI subjects will be randomized to a single oral dose of PREG 400mg (n=112), or placebo (PBO, n=112), while behavioral responses and neuro-active steroid levels will be measured *before and after* study drug/placebo administration. This project is innovative is several ways. First, it is the first study of its kind to test the effect of PREG in individuals high in PSI. Second, it strategically combines multiple lines of investigation – behavioral, psychopharmacology, neuroimaging, and advanced statistical techniques (e.g. brain microstate identification via root mean square error modeling; bootstrapping) --- often carried out separately. More precisely, the proposed study aims to test a proof-of-concept that acute oral administration of PREG reduces PSI and its depressive symptomatology and improves attentional bias for social threats in a double-blind randomized research study combining a validated behavioral paradigm on social threat attention, intention understanding, and perception of others in male adults (aged 21-80) high in PSI. Validated self-report measures (e.g., depression, anxiety, objective social

isolation, life satisfaction) will also be used to examine change in symptom severity and subjective feelings. Third, it tests multiple layers of 'target engagement' including ALLO levels (determined by the state-of-the-art gas chromatography-mass spectrometry (GC-MS) quantification technology) in serum and saliva, and behaviors, and proximal and distal clinical outcomes. Importantly, the proposed study is the first to use PREG to engage the target ALLO to improve PSI. Such research has the potential to yield important insights into the neurobehavioral mechanisms of PREG on PSI and its underlying behavioral effects--- a first key step toward the development of adjunctive PREG therapy in individuals with PSI. Moreover, the proposed project is novel as it will attempt to establish PREG, a dietary supplement, as an agent intended to remediate the protracted reduction in the neurosteroid, ALLO, as a treatment for PSI in humans. The overarching objective is to use an acute, daily oral dose of PREG as an "ALLO normalizer" for six weeks to increase the likelihood patients with PSI will benefit from PREG. If successful, the novel use of PREG has the potential to reduce PSI and provide PSI patients with relief from PSI-related symptoms and disorders.

4. Rationale for using pregnenolone (PREG), as opposed to ALLO: The rationale for a PREG approach is that PREG is an ALLO precursor that is lipophilic and readily crosses the blood brain barrier—a characteristic that makes it the first line of pharmacological treatment for PSI. PREG deficiency leads to depression and even suicide and there are also studies showing impairment of cognition and memory and replacement therapy reverses these effects. PREG is the precursor of neurosteroids and also TSPO agonists that increase levels of PREG and downstream steroids, including ALLO have been suggested for anxiety/depression treatment and tested in clinical trials and these molecules show advantages for the treatment of several neuropathologies[63,64]. It is important to emphasize that in this study the dose-response design followed by determining the levels of neurosteroids (e.g., ALLO) aims at a normalization of the PSI-induced downregulation of the serum neuro-active steroid levels rather than increasing them to supraphysiologic levels. Therefore, measuring neuro-active steroids immediately after completion of treatment will guide eventual adjustment of doses and therefore a better utilization of resources during the Present project. The novel application of PREG in the present study is also reinforced by the compelling findings by Sripada and colleagues, who showed an acute, oral dose (400mg) of PREG up-regulate ALLO in young adults (age between 18-32) and reduce activation of brain areas underlying negative emotions processing. An alternative means of increasing ALLO would be to administer it directly. However, such approach would increase the risk of undesirable side effects (e.g., sedation) as it would globally impact ALLO levels even in brain regions where levels are physiologically low. Also, while synthetic neurosteroids (e.g., Ganaxolone) offer another potential approach, they are currently under development or require further testing for potential medical use in humans. Because SSRI, such as fluoxetine (FLX), are also known to elevate ALLO levels, a FLX approach could have been also considered. However, FLX has a low response rate, which varies from 30-50%, and a constellation of undesirable side effects. Thus, pre-clinical work signifying PREG, a generally well-tolerated dietary supplement, up-regulates ALLO suggests it is a viable approach to engage ALLO in humans. Clinical trials that have used a PREG approach on negative emotion processing, schizophrenia, or on inflammatory diseases, consistently show that PREG 25–500 mg/day has minimal side effects, and does not affect weight, heart rate, blood pressure (even in patients with hypertension), menstrual cycle, or glucose levels (either in diabetics or non-diabetics.

Rationale for selected dose range in the present project: Here, 224 subjects high in PSI will be randomized to a single oral dose of PREG 400mg (n=112), or placebo (PBO, n=112) to ascertain dose of administration. The rationale for the selected dose range is the following: First, clinical trials showed that PREG 25–500 mg/day is safe and well tolerated in humans. Second, Sripada and colleagues made the compelling basic science discovery that a single dose (400mg) of PREG up-regulates ALLO in young adults (See Fig. 1) and reduces activation of brain areas underlying negative emotions processing.

Steroid (pg/ml)	Placebo (n=15)		Pregnenolone Group (n=16)		t	p
	Baseline (Mean ± SD)	Endpoint (Mean ± SD)	Baseline (Mean ± SD)	Endpoint (Mean ± SD)		
Pregnenolone	1378.8 ± 579.1	1190.8 ± 468.5	1238.0 ± 378.7	3943.6 ± 696.3	12.3*	<.001
Allopregnanolone	114.3 ± 49.6	118.1 ± 76.5	112.3 ± 40.9	802.2 ± 280.7	10.2	<.001
Pregnanolone	470.6 ± 200.8	490.5 ± 135.6	352.0 ± 106.6	517.4 ± 96.2	3.2	.004

Fig. 1. **Steroid and metabolite levels in Sripada et al.'s 2013.** *T-tests were conducted on change scores (steroid levels at endpoint minus steroid levels at baseline). Pregnenolone group received an acute, oral dose of PREG 400mg.

Based on pharmacokinetic data in humans, we expect the peak serum concentration of ALLO to occur approximately 2 hours after acute, oral administration of PREG 400mg. Saliva levels will be measured at the following 3 time points: -0.5, 2.0, 3.0 to provide various measures and correlations with behavioral tasks and correlations with serum levels.

4. Aims & Hypotheses:

Specific Aim 1: Assess PREG on levels of ALLO in a dose-finding design. Relative to PBO, PREG will dose-dependently increase and normalize ALLO levels in individuals high in PSI such that it will be comparable to Matched Healthy Controls (MHC) (Hypothesis 1A).

Specific Aim 2: Examine PREG on attentional bias to social threats and depressive symptomatology in individuals who are high in PSI. Relative to PBO, PREG will reduce depressive symptomatology and attentional bias to social threats and improve intention understanding and perception of others in individuals high in PSI (Hypothesis 2A). Hypothesis 2B: In PSI and MHC, depressive symptomatology and attention to social threats will correlate negatively with ALLO levels.

5. Duration of the study: The total duration of the entire study on campus is between 4 and 4 ½ hours maximum.

6. Location of the study:
The entire study will be performed at the University of Chicago Department of Psychiatry and the University of Chicago Center for Cognitive and Social Neuroscience Center's High-Performance Electrical Neuroimaging (HPEN) Laboratory (940 E. 57th Street, 4th Floor, Chicago, IL 60637) on a 128-channel EGI system with 1000 HZ sampling rate.

7. RESEARCH METHODS

The Approach

This project will comprise a randomized, placebo- controlled, between-subjects design, where we will couple a validated behavioral paradigm on attention to social threats with an acute pharmacological challenge with oral pregnenolone (PREG) or placebo (PBO) in subjects high in PSI and test behavioral responses and neuro-active steroid levels *prior and after study oral drug administration*. In the present project, 224 PSI subjects will be randomized to a single oral dose of PREG 400mg (n=112), or placebo (PBO, n=112) to ascertain dose of administration. In this project, participants will attend a single laboratory visit. During this single session, behavioral responses and subjective feelings will be tested at two time points (once before and once after). Neuro-active steroids will be measured from blood and saliva at several points to ascertain they correlate. A group of Matched Healthy Controls (MHC) who are low in PSI (n=12) and will not receive PREG but will otherwise complete all questionnaires of the study. Participants will be recruited from the University of Chicago and in the community.

All participants must be 1) Male; 2) Aged 21-80 (inclusive); 3) High in PSI (PSI > 42, except for controls who should have a score ≤ 42); 4) Do not meet Mini-International Neuropsychiatric Interview (M.I.N.I) criteria for psychiatric illness (except untreated major depressive disorder); and 5) BMI <30 (as ALLO levels are higher in obese than non-obese individuals). Key Exclusion Criteria: Female, as ALLO levels vary as a function of the menstrual cycle; 2) Aged < 21 or > 80; 3) Low in PSI (PSI < 42, except for controls)); 4) meet M.I.N. I criteria for psychiatric illness, besides untreated major depressive disorder; 5) Uncorrected vision; 6) Medical history of cancer; 7) Medical history of condition that might be made worse by exposure to estrogen or testosterone; 8) Recent steroid use; 9) Currently taking psychotropic medication; 10) History of seizures, neurological disorders; 11) History of TBI with loss of consciousness and/or with current cognitive impairment related to TBI; 12) Under hormonal therapy (including, but not limited to, testosterone); 13) Any unstable medical illnesses; 14) Lifetime history of bipolar disorder, schizophrenia, or psychotic disorder; 15) Current or recent (past 3 months) substance abuse or dependence; 16) Currently taking any medications that have/may have unfavorable interactions with Pregnenolone; 17) History of irregularities of heart rhythm, or heart palpitations; 18) obesity (defined as BMI>30); 19) illegal drug use as determined by urine drug screen.

Rationale**:** The recruitment and random assignment of young adults high in PSI to the PREG and PBO conditions allow us to ascertain the effects of PREG on PSI, depressive symptoms, and attentional biases for social threats. In addition, the recruitment of participants between the ages of 21 and 80 years will allow us to compare emotional and neurobiological findings across groups without the confounder of age effects.

Upon receipt of written consent, subjects will fill out self-report questionnaires and undertake practice on our behavioral paradigm.

During the visit, the following will occur:

- The subject will complete several questionnaires, including questions about perceived social isolation and questions about his mood.
- The subject will do a visual task on a computer.
- If the subject has low perceived isolation, the study drug will NOT be given.
- The subject will be asked to provide blood samples throughout the visit so that we

can see how his blood hormones change at different times. The experimenter will draw blood from the subject's arm three times: 1) 30 minutes after your arrival at the high-performance electrical neuroimaging laboratory, 2) one 2 and 30mn later and 3) again an hour later. About 1 teaspoon of blood will be taken each time.

- The subject will also be asked to provide saliva samples during the study at the same three times: 1) 30 mn after your arrival at the high-performance electrical neuroimaging laboratory, 2) one 2 and 30mn later and 3) again one an hour later. About 1 teaspoon of saliva will be taken each time. We ask the subject to provide saliva samples so that we can see how the samples are related to behavioral tasks and how the subject is feeling at different times in the study.
- The subject will watch a 2-hour-long relaxing movie in a quiet and relaxing environment.

Two hours after the subject takes the pill (drug or placebo), the following will occur:

- The subject will do the exact same behavioral visual task on a computer . Control subjects will perform the same tasks at the same time, except they will not take any pills.
- The subject will complete the same questionnaires again.
- The subject will wait for 30 minutes in a relaxing and quiet environment.
- The subject will be given a parking voucher or $5 for public transportation and a $60 monetary compensation for participating in the study.

Estimated Duration of Visit: Between 4 Hours and 4 ½ hours

In the event suicidal ideation or similar events take place during the study, the subject will be removed from the study and referred to the appropriate clinical resources.

C.2.2. Behavioral Paradigms: Subjects will be asked to perform a paradigm our lab has used to investigate social interactions on which PSI-related attentional biases may occur i.e., body language observation, facial expression, and grasping actions: *Body language:* As in S. Cacioppo, Bangee et al. (2014)'s study, participants will be asked to view a total of 2 blocks of pictures of body language with each block containing the 28 pictures. Each picture from each category will be presented once in a block. The order of the blocks will be randomized for each participant, but the order of the pictures within the block will be pre-determined with no more than three consecutive trials of the same picture-type presented. Prior to each trial, participants will view a white fixation cross on a black background that varied between 500 and 1500 ms. Each picture will be presented in color for 1000 ms, followed by a response slide that required a button press to move onto the inter-stimulus interval (ISI) for 500 ms. Participants will view threat and non-threat body language;

C.2.3. Psychological measures: Along with standardized assessments of symptom severity and mood/emotional state, subjects will complete the following measures, at multiple time points (e.g., before and after drug administration): The UCLA-R scale to measure perceived social isolation; Steptoe's questionnaire on objective social isolation; Hospital Anxiety and Depression Scale; Life satisfaction scale; and Demographics.

C.2.4. Study Measures: Along with various psychological measures, subjects will complete: the Edinburgh Handedness Inventory, Big Five Personality trait (short), PSI Scale, Self-esteem scale, Wong-Baker Faces Pain scale, Cacioppo Faces Social Pain scale, Paranoia Scale, Life Satisfaction Scale, Relationship/Marital Satisfaction Scale, Inclusion of the Other Scale, Columbia Suicide Severity Rating Scale, MINI, and the body image scale.

C.2.5. Drug administration: As in Sripada et al.'s 2013, study drug (PREG) and placebo (PBO) identical in appearance will be obtained from Belmar Pharmacy (Lakewood, CO), which provides certificates of analysis. A member of the research team (who is not involved in the data analyses) will randomly assign eligible subjects to the treatment group (PREG 400mg) or to the control group (PBO) through the use of a computerized random number generator. Participants will be blind.

C.2.6. Neurosteroid Analyses: To compare ALLO results with Sripada et al.'s 2013, basal serum levels will also be taken (once prior to drug administration and once two hours after oral administration of the drug study) and analyzed using a gold-standard in the literature: a gas-chromatographic mass-spectrometric (GC-MS) approach. Serum levels of pregnenolone, progesterone, 5alpha-DHP and ALLO and pregnanolone (ALLO's steroisomer and GABA-active) will be measured. As in Majewaska et al (2014), saliva samples will be analyzed using the same gold standard GC-MS approach in ALLO literature i.e., a method that allow for accurate measurement in the femtomolar range of specific neurosteroids. We will ensure that participants will refrain from smoking, eating, drinking alcoholic, caffeinated, or juicy beverages, and brushing teeth at least one hour before the first saliva sample and between saliva samples.

C.2.7. General Statistical approach:

Demographic and self-report psychological measures**:** Statistical analyses for demographic and self-report measures will be computed using SPSS software. Prior to inferential analyses, descriptive statistics, including distributions, means, standard deviations, skewness, and kurtosis will be generated. Data will also be examined for the pattern and mechanism of missing data. Continuous data will be tested for normality and homogeneity of variance. Potential differences between the study groups in terms of demographic and behavioral measures will be explored using multivariate analysis of variance (MANOVA) or appropriate non-parametric tests as appropriate. The overall significance level for these analyses will be at 5% using two-tailed tests and effect sizes and confidence intervals will be reported. In addition, scores at self-report questionnaires will be analyzed via a 2 (Drug Type: PREG 400mg, or PBO) x 2 (time: before, after) repeated measures ANOVA to assess for main effects and interaction effects.

Behavioral paradigms: Statistical analyses will be computed using SPSS software. Reaction times and performance data will be screened and any outliers will be removed based on each participant's trial-by-trial data. A trial will be removed if it differed by 3 standard deviations above or below the participant's mean. Prior to inferential analyses, descriptive statistics, including distributions, means, standard deviations, skewness, and kurtosis will be generated. Data will also be examined for the pattern and mechanism of missing data.

- For the *body language* paradigm: A 2 (Drug Type: PREG 400mg, or PBO) x 2 (type of stimuli: social, non-social) x 2 (nature of the stimuli: threat, non-threat) repeated-measures ANOVA will be performed on the reaction times and valence ratings to assess for main effects and interaction effects. P values, confidence interval, and effect sizes will be reported.

C.2.6. Power Analysis: Using G* power calculation software (Version 3.1.9.2; © Franz Faul, Edgar Erdfelder, Albert-Georg Lang, and Axel Buchner, 2006, 2009) for a within-between interaction in a repeated measures ANOVA, a total sample size of 36 for three groups will yield 95% power to detect a significant difference, with alpha = 0.05 and an effect size Cohen's *d of 0.35* in the present project.

Note: The hormone tested in saliva and blood have the same origin from adrenals glands and gonads. Here we want to establish a correlation within the same subjects among the levels of the same neuro-active steroid determined in the blood and the saliva. We expect the hormone levels measured in saliva and in blood to be different in amount but to correlate.

Saliva samples will be analyzed using the same gold standard GC-MS approach in ALLO literature i.e., a method that allow for accurate measurement in the femtomolar range of specific neurosteroids. We will ensure that participants will refrain from smoking, eating, drinking alcoholic, caffeinated, or juicy beverages, and brushing teeth before each saliva sample.

7. Special Precautions:

Pregnenolone side effects, danger, risk, and the harm it can cause

Overstimulation and insomnia –
Irritability, anger or anxiety –
Acne can occur due to the probable conversion of this hormone into androgens
Headaches are possible with high dosages or low dosages used over several days or weeks.
Possible scalp hair loss if used daily for prolonged periods. Pregnenolone converts into DHEA, which in turn converts into testosterone and possibly on to DHT. It can also be converted into progesterone.
Irregularities of heart rhythm, Heart Palpitations, even on as low a dose as 5 mg. This can be serious in the elderly or those with heart rhythm disturbances.
Unknown effects on the thyroid gland or other organs and tissues.

Special Precautions & Warnings:

Pregnancy and breast-feeding: Although there is not enough known about the use of pregnenolone during pregnancy and breast-feeding, we'll avoid this risk by testing male participants only.

Hormone-sensitive condition such as breast cancer, uterine cancer, ovarian cancer, endometriosis, or uterine fibroids might have an interaction with pregnenolone: We'll exclude participants with any history of condition that might be made worse by exposure to estrogen.

8. Participants:

We will recruit 224 men (between 21 and 80) high in PSI and 12 Matched Healthy Controls (MHC) who are low in PSI.

9. Type and Number of Experimental Subjects:

We expect to collect data from a total of 36 subjects (between 21 and 80).

10. Potential Risks and Benefits:

The study will offer no direct benefits (besides monetary benefits and potential improvement of sexual desire) to participants. The questionnaires and behavioral task also present minimal risks, which may include boredom and fatigue from looking at pictures for a few minutes and anxiety or discomfort or distress related to responding to questionnaires.

11. CONTRAINDICATIONS:

- Estrogens interacts with PREGNENOLONE

  Taking estrogen along with pregnenolone might cause too much estrogen to be in the body.

  Some estrogen pills include conjugated equine estrogens (Premarin), ethinyl estradiol, estradiol, and others.

  Progestin interacts with PREGNENOLONE

  Progestins are a group of hormones. Taking other hormones along with progesterone pills might cause too much hormones in the body. This could increase the effects and side effects of hormone pills.
- Testosterone interacts with PREGNENOLONE

  Taking pregnenolone along with a testosterone pill might cause too much testosterone in the body. This might increase the chance of testosterone side effects.

12. Monitoring of Safety:

A researcher will be present with participants during the duration of the experiment. While participants are in a separate room sheltered, researchers will monitor participants' well-being via cameras and microphones within this room. Participants will have the ability to communicate with researchers through these microphones and may opt out of participation at any time. Participants will also be provided with the IRB's contact information on the consent form, and we will contact IRB should any adverse effects occur.
Regarding pregnenolone's side effects: Subjects will be directed to call a member of the study team if they believe they are experiencing any side effects within the 12 hours following the end of the study.

13. Payment:

Participants will receive the $60 monetary compensation (Internal Fundings/No Fund) for their participation in the entire study. If participants should decide to opt out of participation after the beginning of the study, their participation would be pro-rated ($15 per hour).

14. Informed Consent:

We are asking to waive first consent form to do a brief phone interview and see whether the participants are eligible. Then the participants will come to the University of Chicago and read, understand and sign the written consent form attached in this IRB application. Participants must acknowledge understanding of procedures, risks, and benefits by signing this consent form before the study can proceed.

15. Confidentiality:

De-identified data will be stored in locked drawers or cabinets or on password-protected computers within the HPEN laboratory or on password protected University of Chicago server, such as UChicago Box. Data may be used in publications, but no personally identifiable information will be disseminated outside of researchers included in this protocol.

16. Recruiting Methods:

Subjects will be recruited via Flyers posted on Campus, sona or websites, such as craigslist.

17. Primary Physician Notification:

Dr. Jon Grant, M.D.

18. Anticipated Coordination between Inter-Departmental Faculty:

Stephanie Cacioppo, Ph.D., Jon Grant, M.D., and Royce Lee, M.D. are all members of the Department of Psychiatry and Behavioral Neuroscience. John Cacioppo is the Director of the Center for Cognitive and Social Neuroscience. These researchers will coordinate extensively on this project.

19. Pregnancy Test: N/A. The present research only includes male participants.

20. Drug Test:

Marijuana use may interact with pregnenolone. The drug can antagonize cannabinoid receptors in the brain and if subjects have been exposed to marijuana over a long period of time it may negate the effect of the study drug. Because of this, subjects will be asked to provide a urine sample at the beginning of the visit (after signing the informed consent). This will be used to screen for illegal drugs. The screening must be negative in order for the subject to continue participating in the study.

21. References to Justify Study:

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