Cyclical Neuroactive Steroid Changes, Arousal, and Proximal Suicide Risk: An Experimental Approach

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LIST OF ABBREVIATIONS

COI	Conflict of Interest
DHHS	Department of Health and Human Services
DMC	Data Monitoring Committee
DSMB	Data and Safety Monitoring Board
DSMP	Data and Safety Monitoring Plan
FERPA	Family Educational Rights and Privacy Act
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IBC	Institutional Biosafety Committee
ICD	Informed Consent Document
ICH	International Conference of Harmonization
IDE	Investigational Device Exemption
IDS	Investigational Drug Service
IND	Investigational New Drug
IRB	Institutional Review Board
LAR	Legally Authorized Representative
OHRP	Office of Human Research Protections
OPRS	Office for the Protection of Research Subjects
PHI	Protected Health Information
PI	Principal Investigator
PPRA	Protection of Pupil Rights Amendment
QA/QI	Quality Assurance/Quality Improvement
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
NIMH	National Institute of Mental Health

E2	17-beta-estradiol
P4	progesterone
RCT	Randomized controlled trial
SITBI	Self-Injurious Thoughts and Behaviors Interview
UWRAP/LRAP	University of Washington Risk Assessment and Management Protocol/Linehan Risk Assessment and Management Protocol
EXP	Experimental
PL	Placebo
SCID	Structured Clinical Interview for Diagnosis
IDS	Investigational Drug Services
HC	Healthy Control

1.0 Project Summary/Abstract

Suicide is the second leading cause of death among female of reproductive age, and female suicide attempts occur most frequently around menses (perimenstrually), when estradiol (E2) and progesterone (P4) fall rapidly. A recent prospective study demonstrated that suicidal ideation (SI) and attempts peak perimenstrually in natural cycles, and that this perimenstrual worsening of SI can be prevented by administering stabilizing doses of E2+P4 (relative to placebo). Therefore, while E2+P4 withdrawal is a viable model of proximal suicide risk in females with SI, mechanisms are unclear. GABAergic neuroactive steroid metabolites of ovarian hormones (e.g., allopregnanolone), exert potent sedative and antidepressant effects; we hypothesize that acute perimenstrual withdrawal from these hormone metabolites may increase suicide risk by increasing hyperarousal and hopelessness. The **long-term objectives** of this research are to (1) use the menstrual cycle as a model to probe the proximal mechanisms of suicide, and (2) develop long-term treatments that eliminate hormonal contributions to suicide. The **objective of the current work** is to use a crossover placebo-controlled trial of E2+P4 stabilization (vs. natural E2+P4 withdrawal under placebo) in the perimenstrual weeks to probe behavioral (hopelessness, hyperarousal) and molecular/genetic (neuroactive steroid levels, mRNA expression for neurosteroidogenic enzymes) mediators of perimenstrual suicide risk. **Design**: In this mechanistic trial, 90 female outpatients with past-month SI will complete two counterbalanced conditions: (1) two weeks of placebo during natural perimenstrual E2+P4 withdrawal, and (2) two weeks of perimenstrual E2+P4 stabilization (.1mg/day transdermal estradiol + 200mg/day oral micronized progesterone) to prevent withdrawal. Five labs per condition will capture changes in GC-MS quantified neuroactive steroids and mRNA expression for neurosteroidogenic enzymes, and a wearable device (Oura Ring) will index basal body temperature and physiological arousal (heart rate, heart rate variability). Our app (*BiAffect)* will passively track arousal via movement and typing speed instability. 40 healthy controls (HC) with no SI will be recruited for a two-month long observational substudy, in which five lab visits across one menstrual cycle (timed identically to mirror the observations across one medication phase of the experimental trial) will capture baseline molecular/genetic changes for between-group comparisons. **Specific Aims**. Aim 1 is to evaluate hyperarousal and hopelessness as interacting mechanisms by which perimenstrual E2+P4 withdrawal (vs. experimental E2+P4 stabilization) increases proximal suicide risk. Aim 2 is to evaluate neuroactive steroid withdrawal as a mechanism by which perimenstrual E2+P4 withdrawal (vs. stabilization) increases proximal suicide risk. If appropriate, a multilevel path model will test a path in which E2+P4 withdrawal (vs. stabilization) causes neuroactive steroid withdrawal, which increases in hopelessness and hyperarousal, which in turn increases proximal suicide risk. Aim 3 (substudy) is to characterize GABA-A receptor gene expression plasticity across the cycle in individuals with and without perimenstrual changes in affect and suicide risk. **Relevance.** By conducting a mechanistic experiment to probe the mediators of a known cause of proximal suicide risk, the proposed research responds to public calls from the NIMH-sponsored Suicide Research Prioritization Agenda to identify modifiable causes of *proximal* suicide risk.

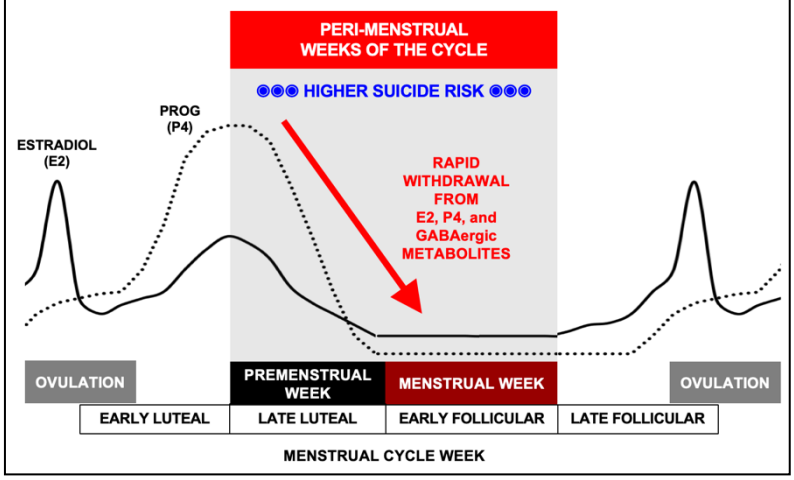
2.0 Background/Scientific Rationale

Among females of reproductive age, suicide is the second leading cause of death, accounting for 13% of all female deaths in America[1]. Although males are more likely to die by suicide (due to higher-lethality methods), females are three times as likely to experience suicidal ideation (SI) or attempt suicide[2]. This sex difference manifests between puberty[3] and menopause[4], implicating fluctuations in ovarian hormones. Despite this, *no published longitudinal or experimental studies have directly examined ovarian hormone effects on suicide risk.*

In cross-sectional studies, suicide attempts occur most frequently peri-menstrually—that is, in the weeks before and during menses—when the ovarian hormones E2 P4 fall rapidly. The perimenstrual weeks have been repeatedly linked with greater risk of suicidal behaviors in cross-sectional hospital and postmortem studies[5–8]. While this further implicates hormone changes in proximal suicide risk, *the impact of this work is limited by a lack of longitudinal or experimental data, and a lack of data regarding mechanisms.*

One possible mechanism of perimenstrual suicide risk is rapid perimenstrual withdrawal from E2 and P4 (Fig 2), as well as withdrawal from the antidepressant[9] and sedative[10,11] effects of their **GABAergic neuroactive steroid metabolites**. The PI's recent longitudinal and experimental studies (K99-MH109667[12]), which form the premise for the proposed work, provide the first (1) longitudinal evidence that daily SI and behavior peak perimenstrually, and (2) experimental evidence that E2+P4 withdrawal causes increased perimenstrual suicide risk that can be prevented by administering stabilizing doses of E2+P4[12]. **The proposed mechanistic trial** responds to the RFA by integrating the PI's experimental paradigm with molecular, genetic, and behavioral measurements to examine the mechanisms by which perimenstrual E2+P4 withdrawal (vs. experimental stabilization) increases proximal suicide risk. We examine mediation across levels of analysis, including changes in neuroactive steroid metabolites (serum neuroactive steroid levels, neuroactive steroid biosynthetic enzyme mRNA expression in peripheral monocytes) as well as hopelessness and hyperarousal.

Fig 2. Hypothesized Role of Perimenstrual Hormone Withdrawal on Suicide Risk



Defining and Operationalizing Behavioral Constructs

We define **hyperarousal** (RDoC Arousal and Regulatory Systems) as (1) self-reported agitation and overwhelm, (2) psychomotor agitation (smartphone accelerometer instability/movement[13,14]), (3) behavioral reactivity to external stimuli (smartphone typing speed instability[13,14], attentional bias toward angry/fearful faces on task[15]), and (4) physiological hyperarousal (reduced resting parasympathetic autonomic function[16], increased sympathetic and decreased parasympathetic autonomic function during an emotionally arousing task).

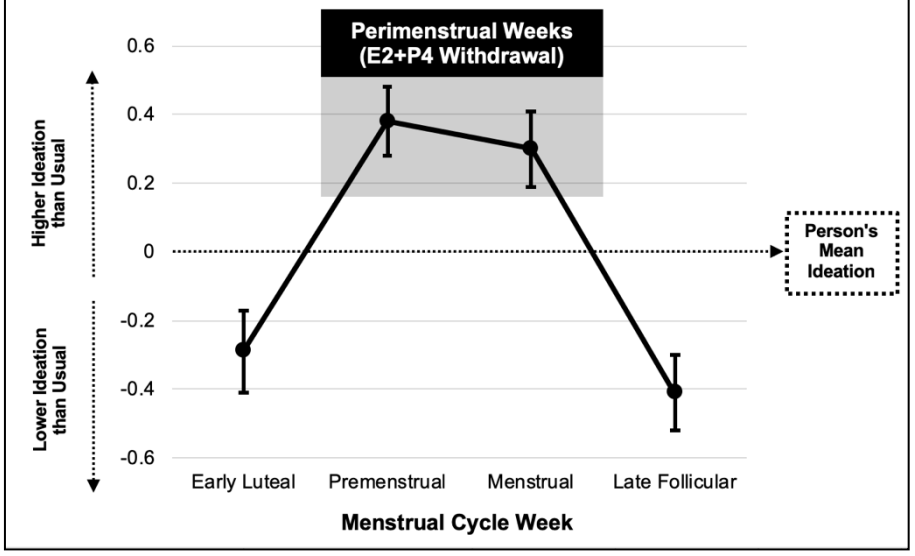
We define **hopelessness** as a state of despair *arising due to repeated experiences of loss or sustained threat* (RDoC Negative Valence Systems). It will be measured primarily as self-reported hopelessness using the Beck Hopelessness Scale (BHS)[17], and we will measure lifetime and daily exposure to loss and threat[18,19].

We define **proximal suicide risk** as the presence and severity of self-injurious thoughts and behaviors as assessed dimensionally using the Adult SI Questionnaire (ASIQ)[20] and categorically using the Self-Injurious Thoughts and Behaviors Interview -Revised (SITBI-R).These instruments assess for passive and active SI; suicide plans; aborted, interrupted, and actual suicide attempts; and suicide death. Although non-suicidal self-injury (NSSI) is a distinct but related phenomenon, we also consider NSSI as an index of proximal risk given its predictive validity for suicide attempt[21].

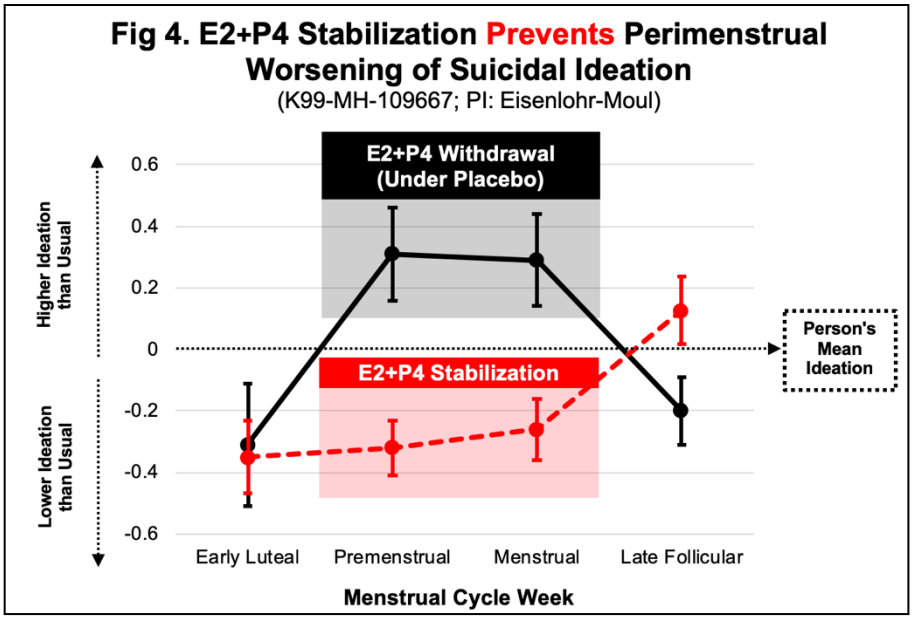
Premise for Aim 1a	**Perimenstrual Hormone Withdrawal Causes Proximal Suicide Risk**

In the first prospective study[12] of daily suicide risk in females with SI (K99MH109667), the PI found that proximal suicide risk peaks perimenstrually, during periods of natural hormone (E2+P4) withdrawal (Fig. 3). 38 females with past-month SI completed daily ratings on the ASIQ[20] across a baseline menstrual cycle. ASIQ scores peaked during the perimenstrual weeks relative to late follicular week, γ=-.88, p<.001 and early luteal week, γ=-.56, p<.001. We also observed greater odds of endorsing suicidal planning items on perimenstrual vs. non-perimenstrual days (OR=4.29, p<.001). 28 of 30 participants reported the highest mean SI perimenstrually. *These data support the premise that suicide risk peaks perimenstrually.*

Fig 3. Suicidal Ideation Worsens Peri-Menstrually
(K99-MH-109667; PI: Eisenlohr-Moul)



Stabilization of E2+P4 Prevents Perimenstrual Worsening of Suicide Risk. In the PI's recently-completed double-blind crossover trial[12] (K99MH109667) in 30 females with past-month SI, SI severity once again peaked during perimenstrual weeks in the placebo condition (black solid line, Fig 4), and perimenstrual administration of E2 (.1mg/day) and P4 (200mg/day) in the stabilization condition prevented this increase in SI (dashed red line, Fig 4; Cond×Phase γ=-.75, *p*<.0001). Further, as predicted, perimenstrual risk was then *shifted* in the active condition, appearing only later during withdrawal from exogenous hormones (γ=.59, *p*<.0001). This further demonstrates the causal effect of E2+P4 withdrawal on proximal suicide risk. When results were person-standardized, the impact of stabilization was roughly a 1 person-standard-deviation condition difference in severity of SI change. Further, more participants reported suicidal behaviors during placebo vs. active (NSSI: 18 vs. 10; preparatory acts: 5 vs. 1; aborted attempts: 2 vs. 0). *This experiment supports the premise that E2+P4 withdrawal causes proximal suicide risk.*



In order to develop safe long-term treatments that eliminate hormonal contributions to suicide, we need information about the mechanisms by which E2+P4 withdrawal cause proximal suicide risk. Unfortunately, recent meta-analytic work demonstrates that poor progress has been made toward predicting attempts among ideators[21], and little is known about reliable time-varying prediction of proximal suicide risk[22]. Therefore, candidate mediators in the present proposal are informed primarily by our general understanding of the most robust mechanisms E2+P4 withdrawal, and preliminary findings from our prior trial of E2+P4 stabilization in females with past-month SI. *Below, we argue that neuroactive steroid withdrawal, hopelessness, and hyperarousal are strong candidate mediators of E2+P4 withdrawal effects on proximal suicide risk.*

Premise for Aim 1b | Hopelessness as a Mediator of E2+P4 Withdrawal Effects on Suicide Risk

Hopelessness features prominently in suicide theories. Although hopelessness has recently been framed as primarily a predictor of SI rather than suicide attempt or death, large prospective studies demonstrate the predictive validity of baseline hopelessness for suicide death in inpatients (n=102)[23] and outpatients (n=1,958)[24], and a meta-analysis of longitudinal studies reports a role for baseline hopelessness as a *prospective* predictor of attempt (OR=1.95), and death (OR=2.15)[25]. Although hopelessness is one of the best-studied constructs in suicide research, little evidence is available about dynamic **changes** in hopelessness and proximal suicide risk. One recent report described the use of daily surveys to evaluate links between risk factors and SI. They found that fluctuations in hopelessness successfully predicted SI severity *at the next time point* across two samples, suggesting that surges in hopelessness increase proximal suicide risk[26].

E2+P4 Withdrawal Causes Hopelessness. E2+P4 withdrawal paradigms increase behavioral despair in animals. While despair can be triggered by withdrawal from E2[27] or P4[28], combined E2+P4 withdrawal is most similar to the perimenstrual phase. Among other likely mechanisms of E2+P4 withdrawal on hopelessness (e.g., serotonergic), we focus on E2+P4 as regulators of GABAergic neuroactive steroids, since prevention of neuroactive steroid formation using finasteride administration (i.e., abrupt neuroactive steroid withdrawal) recapitulates E2+P4-withdrawal-induced despair in animals, even when E2 or P4 remain high[29]. In susceptible females, withdrawal from E2+P4 provokes sadness[27,30,31]; however, a key

limitation of this literature is that few experimental or longitudinal studies specifically examine the impact of hormone withdrawal on hopelessness.

Preliminary Data: Perimenstrual E2+P4 withdrawal (vs. stabilization) increases daily hopelessness. In our recent trial (K99MH109667), we found that ovarian steroid withdrawal (under placebo) was associated with *increased* daily reports of hopelessness in females with past-month SI, and that this was prevented by administering stabilizing doses of E2+P4 (Condition X Phase γ=-.73, p=.023). Further, controlling for daily within-person changes in hopelessness partially mediated the effect of E2+P4 stabilization on SI severity (95% CI for Indirect Effect: -.21 to -.53). *This preliminary work supports the premise that E2+P4 withdrawal causes perimenstrual hopelessness, effects which may explain proximal perimenstrual risk for suicide.*

Premise for Aim 1c	**Hyperarousal as a Mediator of E2+P4 Withdrawal Effects on Suicide Risk**

Hyperarousal, defined as physiological hyperarousal, psychomotor agitation, and agitation or overwhelm[32], is an acute precipitant of suicidal behavior[32–35]. In a meta-analysis, disturbances in arousal were among the strongest predictors of suicidal behavior[25]; however, this is an understudied area. In a meta-analysis focused specifically on *agitation*, agitation predicted suicide attempts[35]. However, little within-person work has been done to understand the time course of risk. Similarly, altered cardiovascular reactivity is linked to SI and attempts[36–38], but no work examines acute changes in physiological arousal and proximal suicide risk.

E2 and P4 withdrawal causes hyperarousal. In animals, E2+P4 withdrawal increases indices of hyperarousal, including autonomic function[39] and heightened behavioral reactivity to stimuli[10]. Withdrawal from neuroactive steroids, which occurs to varying degrees during E2+P4 withdrawal in the perimenstrual phase, causes reactivity-promoting alterations in associated brain circuitry, including the amygdala[40–42], the bed nucleus of the stria terminalis[42,43], and the periaqueductal grey[44,45]. Further, variables linked to suicidal ideation and behavior, including social stress[46] and isolation[47], lifetime abuse[48], impulsivity[49], Borderline Personality Disorder[50,51], and PTSD[52,53], intensify effects of E2+P4 withdrawal on GABAergic neuroactive steroids or hyperarousal.

Preliminary Data: Perimenstrual E2+P4 withdrawal (vs. stabilization) increases daily hyperarousal. In the previous hormone stabilization trial[12], we found increased daily agitation[54] and overwhelm[55] during perimenstrual E2+P4 withdrawal under placebo, which was prevented by administering stabilizing doses of E2+P4 (Cond×Phase γ=-.87, p<.0001; Cond×Phase γ=-.92, p<.0001). Controlling for within-person levels of self-reported agitation on the Brief Agitation Measure partially mediated the effect of E2+P4 stabilization on SI severity (95% CI for Indirect Effect: -.30 to -.55) and probability of endorsing suicide planning[20] (95% CI for Indirect Effect: -.18 to -.34). *This work supports the premise that E2+P4 withdrawal causes hyperarousal in the form of self-reported agitation, which may explain proximal perimenstrual risk for suicide.*

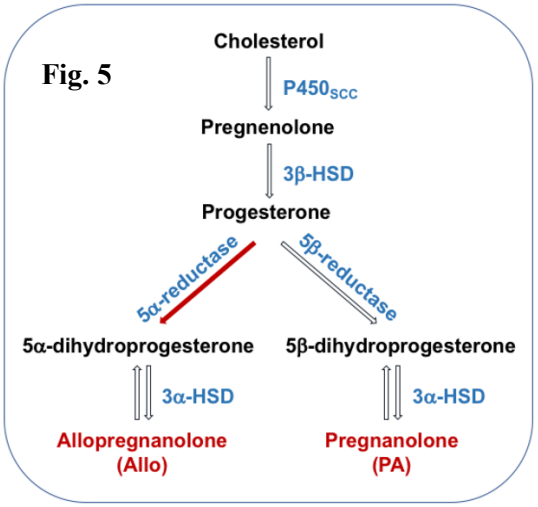
Preliminary Data: Our recent meta-analysis demonstrates that the perimenstrual phase is robustly associated with the lowest parasympathetic nervous system activity, consistent with physiological hyperarousal during perimenstrual E2+P4 withdrawal. Our meta-analysis (under review) aimed to quantify within-person variations in measures of cardiac vagal activity (indices such as heart rate variability, that capture parasympathetic innervation of the heart) across different cycle phases. A literature search following the PRISMA-Statement yielded 38 empirical studies (N=1,018) reporting repeated measures of cardiac vagal activity in at least two cycle phases. We found a significant decrease in parasympathetic activity from the late follicular (stable P4 and neuroactive steroids) to the premenstrual phase (***d*=-1.32**, 95% CI [-2.35, -0.29]) during E2+P4 withdrawal. *This meta-analysis supports the premise that autonomic hyperarousal is heightened during perimenstrual E2+P4 withdrawal. The proposed trial would experimentally test if physiological hyperarousal is caused by E2+P4 withdrawal, and whether these changes mediate effects on suicide risk.*

Premise for Aim 1d — Hyperarousal and Hopelessness as Interactive Mediators of Suicide Risk

Hopelessness and agitation are core features of depression and hyperarousal, respectively. Notably, several studies document that the intersection of depression and hyperarousal signals increased risk of suicide attempt in inpatients and outpatients[33]. Although this has primarily been discussed in the context of "mixed" states related to bipolar disorder, the intersection of depression and hyperarousal is not unique to bipolar disorder, and may increase proximal suicide risk[35]. Thus, for the purposes of the proposed work we hypothesize that hopelessness translates into proximal suicide risk only during hyperaroused states such as psychomotor agitation, physiological hyperarousal, and subjective agitation or overwhelm.

Premise for Aims 2a & 2b — Neuroactive Steroids and Proximal Suicide Risk

Because they rapidly change neuronal excitability, neuroactive steroids are excellent candidate mediators of proximal suicide risk. The neuroactive steroids allopregnanolone (**ALLO**; 3α,5α-THP) and pregnanolone (**PA**; 3α,5β-THP) are cholesterol-derived molecules (see Fig. 5 for biosynthetic pathway) that act as potent positive allosteric modulators at synaptic and extrasynaptic $GABA_A$ receptors, amplifying Cl^- ion flux during GABA binding at $GABA_A$ receptors[56]. Administration of ALLO induces rapid sedative, antidepressant, anxiolytic, anesthetic, analgesic, and anticonvulsant effects[57]. Therefore, ALLO withdrawal can cause mood disturbances (i.e., hopelessness) as well as psychomotor agitation and autonomic hyperarousal[39]. The biosynthetic pathways for these neuroactive steroids are illustrated in Figure 5. ALLO is produced via a two-step process in which P4 is first converted into **5α-DHP** by the enzyme 5α-reductase type 1 (**5α-RI**), which is converted to ALLO by the enzyme 3α-hydroxysteroid dehydrogenase type 2 (**3α-HSDII**). PA is produced via a similar process substituting 5β-reductase. Our review will focus on ALLO, since it is the best studied.



Neuroactive steroid withdrawal mediates many deleterious behavioral effects of E2+P4 withdrawal. Many deleterious behavioral effects of E2+P4 withdrawal are mediated by ALLO withdrawal, as evidenced by the fact that blockade of P4 metabolism to neuroactive steroids (i.e., acute ALLO withdrawal) recapitulates the depressogenic and anxiogenic effects of E2+P4 withdrawal even when E2+P4 remain stable[9]. Further, direct experimental manipulation of ALLO withdrawal also causes these behavioral phenotypes[58].

Sources of neuroactive steroid withdrawal: Changes in Precursor Levels. Changes in ALLO levels can be caused by both changes in the availability of steroid precursors (P4) and by changes in the expression of biosynthetic enzymes that control conversion of the precursor to ALLO. ALLO fluctuates across the menstrual cycle with changes in P4 and E2, showing lower and more stable levels in the follicular phase, increasing during the early luteal week, peaking in the midluteal phase, and falling during the perimenstrual weeks. Since P4 is a precursor, perimenstrual withdrawal from P4 generally causes corresponding withdrawal from ALLO around the onset of menses, *although the trajectory and degree of this withdrawal may be influenced by many additional between- and within-person factors*. For example, because E2 increases synthesis of ALLO by increasing expression of 3α-HSDII in the brain[59], E2 changes also shape perimenstrual ALLO withdrawal.

Sources of neuroactive steroid withdrawal: Changes in central biosynthetic enzyme activity. In addition to effects of neuroactive steroids that have been synthesized from precursors in the periphery, neuroactive steroids can be directly synthesized *de novo* in brain[60], and ALLO's enzymatic pathway (e.g. 5α-RI) is expressed particularly in brain corticolimbic

regions relevant for emotion and stress responses[61–64]. Of note, individual differences and circumstances can affect the degree to which ALLO's enzymatic pathway is expressed in the brain, and these changes in enzyme expression can be reliably measured via peripheral monocyte mRNA expression. Increases or decreases in expression of these biosynthetic enzymes (5α-RI, 3α-HSDII) have been shown to relate to risk for neuropsychiatric illness. Postmortem studies (from co-I Pinna) reported decreased 5α-RI mRNA expression in the PFC of depressed subjects compared with non-depressed controls[65]. Suppressed ALLO levels have been found in the CSF of females with post-traumatic stress disorder (PTSD)[66] and depression[67], and elevated 5α-DHP/ALLO ratios have been observed in the serum of those with PTSD[68], *but not in those with a history of trauma who did not develop PTSD,* suggesting a possible role of disturbed neurosteroidogenic enzyme activity in the etiology of both hyperarousal and hopelessness phenotypes.

In sum, although changes in neuroactive steroids (both serum levels and biosynthetic enzyme mRNA expression) have been strongly implicated behavioral phenotypes associated with suicide, **no studies have examined the roles of state changes in neuroactive steroids on proximal suicide risk.** The proposed study would examine the roles of trait-like differences and day-to-day changes in neuroactive steroids in proximal suicide risk, during a high-risk period in which neuroactive steroids are rapidly changing.

Premise for Aim 3	**GABA-A Receptor Subunit Composition**

Exporatory substudy of healthy controls (HC): The menstrual cycle leads to predictable, cyclical changes in GABAergic neuroactive steroids (e.g., ALLO). The $GABA_AR$ has 19 known genes coding for subunits α1-6, β1-4, γ1-3, δ, ε, θ, π and ρ1-2[69], with the majority of receptors comprised of two α, two β, and one γ or δ subunit[70] (e.g., αβγ or αβδ), although *subunit expression is highly plastic*. Preclinical work shows that the potency of ALLO's positive allosteric modulatory binding to the $GABA_AR$ depends on the subunit composition; for example, γ2-containing receptors are less sensitive to ALLO than α4-containing receptors, and δ subunits augment steroid potency[71]. Further, preclinical models show that P4 and ALLO levels alter gene expression (via mRNA) and/or protein levels (via Western blot) for the α4 subunit[72–74], δ subunit[75–77], and γ2[78–81] subunit, such that plasticity of the receptor is a function of reproductive cycles. However, no human studies have described how subunits change *normatively* across the cycle.

There is further evidence that withdrawal from ALLO (i.e., perimenstrual phase) may also negatively impact affect and behavior[82,83], suggesting that $GABA_AR$ plasticity to ALLO *change* is the trigger for perimenstrual exacerbation of psychiatric symptoms. In animal models, excessive subunit plasticity is associated with concurrent changes in negative affect[73,74,84–88], such as an association between overexpression of α4 and δ with anxiety (e.g., elevated plus maze)[73] and depression (e.g., forced swim test)[89]. Notably, non-hormonal work in humans has also associated $GABA_AR$ subunits with suicidality[90], further underlining the importance of subunit expression on negative affect. Together, research shows that $GABA_AR$ subunit expression is related to negative affect, which is a known predictor of suicidality[91]. The only translational study of hormone-regulated $GABA_AR$ subunit expression to date found that peripheral subunit expression differs by pregnancy status and depression[92], suggesting peripheral $GABA_AR$ subunit expression is a viable biomarker of hormone-related changes in affect. **Given that these outcomes ($GABA_AR$ subunit expression and negative affect/suicidality) are both altered by ovarian steroids, there is an urgent need to translate $GABA_AR$ subunit expression to human females, as this could be a potential biomarker for cyclical affective change and related cyclical suicide risk**.

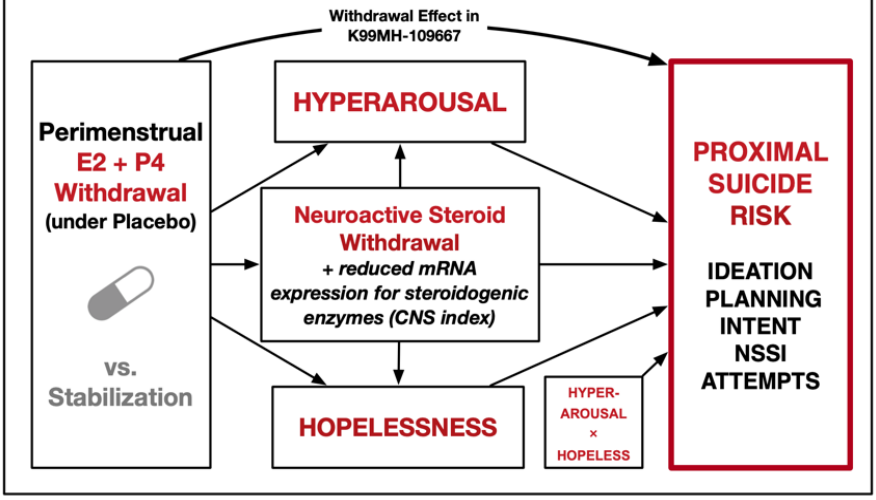
Significance of the Expected Contribution. Upon successful completion of the proposed trial, we expect to contribute a viable, malleable model of proximal suicide risk, complete with information about the molecular, genetic, and behavioral mechanisms that underlie its effects. *This contribution is significant because it offers a novel solution to the field's lack of proximal neurobiological treatment targets[21(p50)] for suicide.* These contributions are **consistent with NIMH objectives** to (1) "*define the mechanisms of complex behaviors"* (i.e., understand the multilevel causal mechanisms of hormone-related changes in suicide)*,* to (2) "*chart mental illness trajectories to determine when, where, and how to intervene"* (see Figure 3; i.e., during periods of hormone flux in females, during periods of neuroactive steroid flux in males and females)*, and* (3)

"to strive for prevention and cures" (i.e., by developing one of the first models of a naturally-occurring, biologically-driven cause of proximal suicide risk that could be prevented to improve outcomes). The **public health relevance** of this work is to **immediately** improve prediction of proximal suicide risk, depression, and arousal-related disorders in females (i.e., given the recurring and highly predictable nature of the cycle); to continue to develop an experimental model for understanding the role of ovarian hormone withdrawal in female-biased risk for psychopathology; and to pave the way for the **long-term** development of stabilization treatments to prevent suicide deaths.

3.0 Objectives/Aims

Our aim is to test the **central hypothesis** is that perimenstrual E2+P4 withdrawal induces rapid withdrawal from calming GABAergic neuroactive steroids, which in turn increases hyperarousal and hopelessness, which then interact to cause proximal suicide risk (Figure 1). P4 is metabolized to GABAergic neuroactive steroids, and E2 promotes expression of enzymes that convert P4 to neuroactive steroids[93]. Perimenstrual E2+P4 withdrawal may increase suicide risk by causing rapid withdrawal from the antidepressant[9] and sedative[11,10] effects of neuroactive steroids[94], as well as withdrawal from the antidepressant effects of E2[95]. Our preliminary experimental work strongly suggests that perimenstrual E2+P4 withdrawal (and neuroactive steroid withdrawal) heightens proximal suicide risk via hopelessness[65] and hyperarousal[12,66].

Figure 1. *Peri-menstrual Hormone Withdrawal and Proximal Suicide Risk*



Design: Using the PI's hormone withdrawal paradigm (K99MH109667[12]), a new sample of 90 completers female outpatients with past-month SI will complete two counterbalanced conditions: (1) natural perimenstrual E2+P4 withdrawal (placebo), and (2) perimenstrual E2+P4 stabilization. Five labs per condition will capture changes in neuroactive steroids, mRNA expression for neurosteroidogenic enzymes, physiological arousal, behavioral indices of arousal, and interview/self-report indices of proximal suicide risk, hopelessness, and hyperarousal. Our app (BiAffect) will passively track indices of arousal including psychomotor agitation (accelerometer) and reactivity (typing speed variability). Additionally, Metricwire will passively track movement using smartphone sensors. 40 healthy controls (HC) will complete five labs across one observational cycle to characterize 1) normative cycle-related variation in neuroactive steroids and mRNA expression, and 2) group-related differences (i.e., past-month SI vs HC) in trajectories of GABAAR subunit change across the cycle.

AIM 1: To evaluate hyperarousal and hopelessness as interacting mechanisms by which perimenstrual E2+P4 withdrawal (vs. experimental stabilization) increases proximal suicide risk. We predict that **(1a)** individuals will demonstrate greater proximal suicide risk during perimenstrual E2+P4 withdrawal (placebo) than during perimenstrual E2+P4 stabilization, an effect mediated by **(1b)** indices of hyperarousal, **(1c)** indices of hopelessness, and **(1d)** their interaction (i.e., with hopelessness predicting suicide risk only during hyperarousal).

AIM 2: To evaluate neuroactive steroid withdrawal as a mechanism by which perimenstrual E2+P4 withdrawal (vs. stabilization) increases proximal suicide risk. We expect that E2+P4 withdrawal (vs. stabilization) will induce neuroactive steroid withdrawal to some degree in all participants. However, we predict that the effect of hormone withdrawal (vs. stabilization) on proximal suicide risk at each lab will be mediated by **(2a)** degree of reduction in serum neuroactive steroid levels and **(2b)** degree of reduction in mRNA expression for neurosteroidogenic enzymes. We will also evaluate

within-person links between neuroactive steroids and all behavioral constructs; if appropriate, a multilevel mediation model will test the full model in Fig. 1.

AIM 3: **To characterize $GABA_AR$ gene expression plasticity across the cycle in individuals with and without perimenstrual changes in affective and suicide risk**. In the HC group, we hypothesize that gene expression for α4 and δ subunits (GABRA4 and GABRD genes, respectively) are upregulated and γ2 (GABRG gene) is downregulated in the midluteal (high ALLO) and perimenstrual (ALLO decline) phases compared to the midfollicular phase (low ALLO). We also hypothesize that clinical group moderates the effect of cycle phase on $GABA_AR$ composition, such that the group with perimenstrual increases in affective symptoms and suicide risk shows higher slope of midluteal-to-perimenstrual change in α4 and δ subunits, and lower γ2 subunit replacement of δ subunit expression than the HC group.

4.0 Eligibility

Recruitment and Screening. We will recruit **170 females (<u>90 completers</u>) with past-month SI** in outpatient treatment as usual with a mental health provider, and 60 healthy controls (HC) (40 completers) with no lifetime history of SI and no DSM-5 diagnoses in the past five years, for a total of 230 participants. Participants will be recruited via fliers, email listservs, online Research "match" websites (Research Match, Research & Me), and social media ads seeking volunteers to participate in a study "on the biology of depression, stress, and suicide" (with no mention of the cycle, hormones, female sex, or women). Participants will complete an online screener and will be scheduled for a phone screen if they meet basic inclusion criteria. Following phone screening, potential participants will be invited to an enrollment visit.

4.1 Inclusion/Exclusion Criteria

Participants will include 170 (90 completers) physically healthy premenopausal females reporting past-month suicidal ideation (but acceptably low imminent suicide risk for outpatient care) receiving treatment as usual at an outpatient mental health clinic in Chicago, **and** 60 (40 completers) physically healthy premenopausal females with no lifetime history of SI and no DSM-5 diagnoses in the past five years (total 230 participants). The HC group inclusion and exclusion criteria are identical *except* where indicated below. Although the HC group will not undergo exogenous hormone stabilization, they will be required to meet identical medical criteria in order to best match the experimental group.

Of note, we will not exclude those without smartphones, and a smartphone will be provided to a subject if they do not have one.

To participate, individuals must meet all of the following inclusion/exclusion criteria:

(1) Must be biologically **female assigned at birth** and between the ages of **18-45 years**.

Justification: Females younger than 18 or older than 45 may have inconsistent menstrual cycles or may be postmenopausal and therefore no longer cycling.

(2) Must self-report **normal menstrual cycles** between 21 and 35 days.

Justification: Abnormal menstrual cycle lengths or variability in lengths could introduce error variance into the experiment and could reduce confidence regarding the timing of the experimental manipulation to cycle events.

(3) Must be under the **current care** of an outpatient mental health provider with whom they are comfortable discussing history of suicidal ideation and are able to visit at least once every 3 months, or they must be willing to begin visiting an outpatient mental health provider at least once every 3 months.

Justification: Because we intend to recruit females with current suicidality, engagement with a mental health provider is required as a protective measure to ensure that participants have access to treatment if needed.

HC group: criterion 3 not applicable; HC participants should *not* be under the care of an outpatient mental health provider for diagnosis or treatment of a psychiatric condition.

(4) If the person has children, they must be at least **1 year postpartum**.

Justification: The postpartum period can be characterized by altered hormone levels and changes; therefore, postpartum females may introduce error variance into the experiment.

(5) Must **not be pregnant, breastfeeding, or trying to become pregnant**. Pregnancy status will self-reported at enrollment, confirmed using urine pregnancy test at the first in-person visit of the experimental condition, and confirmed again at the first visit of the second condition. Participants will be encouraged to use a barrier method of birth control during the study.

Justification: Administration of ovarian hormones to a pregnant person could negatively influence the pregnancy in a variety of ways, including risks to the health of the fetus.

(6) Must not be taking any form of **exogenous hormones** or intrauterine device (IUD) with hormones and must have ended previous use of hormonal preparations at least one month prior to the study. Those who were previously taking oral contraceptives or other hormonal medications must have one normal menstrual cycle (menstrual period) prior to enrollment in the study. **Participants will be required to use licensed barrier methods of (non-hormonal) contraception during study participation; if they are unable to commit to this responsibility, they will not be eligible for participation**.

Justification: The use of additional exogenous hormones poses safety risks and would undermine experimental control of hormones.

(7) Must be of **normal weight** (BMI between 18.000 and 34.999); measured via self-report at screening and confirmed via direct measurement in the laboratory at the Welcome Visit.

Justification: While both obesity (BMI>=30) and use of ovarian steroid medications are each associated with slightly increased risk for thromboembolic events, the best evidence suggests that these risks are additive rather than interactive; further, use of transdermal estradiol (as in the present study) is associated with even smaller increases in thromboembolic risk relative to oral estradiol. Therefore, we have opted to include individuals with a BMI between 18 and 35.

(8) Must report no personal history of any condition that could, in the opinion of the study psychologist or physician, significantly increase physical or behavioral risks to the participant over the course of the study, or which could interfere with the endocrine and immune endpoints of the study. This includes, but is not limited to, metabolic or autoimmune disease, epilepsy, endometriosis, fibroids, PCOS, cancer, diabetes, cardiovascular, gastrointestinal, hepatic, renal, or pulmonary disease, history of thromboembolic events, migraine with aura, or use of a pacemaker or other internal electrical device.

Justification: Administration of exogenous hormones to females with these chronic medical conditions may increase the risk of an adverse health-related response to hormones or the Oura Ring. Further, the purpose of the

present study is to determine the impact of hormonal stabilization (vs. natural withdrawal) in otherwise physically healthy females.

(9) Must not currently use nicotine regularly, and must not regularly smoke any combustible materials (i.e., marijuana cigarettes).

Justification: Although more research is needed to clearly understand these risks, some evidence suggests that administration of ovarian hormones may interact with regular use of nicotine or regular smoking of combustible materials to synergistically increase risk of thromboembolic events. Therefore, any participant endorsing regular use of nicotine or smoking of combustible materials will be excluded.

(10) Must not report substance use, mania, or psychosis symptoms that (in the opinion of the study psychologist or psychiatrist) impair the participant's ability to follow study directions, safely take study medication, or communicate with the study team or their mental health provider.

Justification: These factors may interfere with the individual's ability to complete the study safely. They are also known sources of variability in suicidality and would therefore introduce error into our models as well as increasing the risk that a participant would experience a suicidal crisis during the study. Use of opioids, methamphetamine, and cocaine will be screened at the enrollment visit to validate interview measures of substance use.

(11) Must report **at least some recent suicidal ideation (in the past month)** at enrollment.

Justification: As reviewed in the research strategy, the purpose of the study is to examine the impact of natural perimenstrual hormone withdrawal (vs. prevention of either E2 or P4 withdrawal) on within-person changes in suicide risk. The present sampling strategy **ensures generalizability** of our findings to clinical populations of interest and will also **prevent a floor effect** (i.e., zero-inflation of daily outcome measures and associated limitations in power to detect effects) on primary daily outcome measures (e.g., mediating behavioral constructs, suicidality).

HC group: must NOT report lifetime history of suicidal ideation in order to meet criteria as a control for the experimental study.

(12) Must be categorized as having **acceptably low imminent risk for suicidal crisis/attempt (such that they would be appropriate for outpatient—rather than inpatient—care)** according to established clinical and research guidelines. Specific guidelines were developed during the PI's K99/R00 studies (K99/R00109667) in collaboration with Dr. Mitch Prinstein, a suicide-focused co-mentor on the PI's prior K99 training grant. As Dr. Prinstein notes in his 2014 methodological paper on research-based suicide risk assessment, evaluation of imminent risk of suicidal crisis/attempt is a complex clinical task, requiring integrative consideration of a variety of factors (including: general clinical presentation, ideation severity, frequency, recency, and associated consideration/intent; access to suicidal means; previous attempts and gestures; presence of suicidal planning, recent stressors, frequency of contact with mental health provider; and presence of social support). *Therefore*, our specific criteria for delineating whether a participant is eligible are based on an evidence-based determination, after reviewing all of the above information, that the participant is at **acceptably low imminent risk of suicidal crisis/attempt such that they would be appropriate for ongoing care in an outpatient—rather than inpatient—setting.** These decisions will be based on responses to a variety of questions from the Self-injurious Thoughts and Behaviors Interview- Revised (SITBI-R; clinical interviews at the beginning of an enrollment visit). We will exclude those judged to be at imminent risk of acute suicidal crisis/attempt.

HC group: N/A

Justification: Individuals with current suicidality **are not necessarily at imminent risk for suicidal crises/attempt** (i.e., their risk is manageable on an outpatient basis through frequent clinical contact, such as the daily calls in the present study). Because the proposed hormone protocol simply prolongs the physiologic luteal hormone profile of E2 and P4, it is **not** expected to expose participants to additional risk of suicidal crisis beyond what they would naturally experience during their typical monthly menstrual cycles. However, out of an abundance of caution, we will **exclude females with elevated imminent risk for suicidal crisis/attempt** (determined by structured interview and established guidelines). Individuals with histories of suicidality often have ongoing, habitual thoughts about death or suicide that are highly distinguishable from **imminent risk for suicide attempt**. In further support of this notion and of the feasibility of recruiting this population, it should be mentioned that up to 1/3 of the American population experiences suicidal ideation at some point in their lives, yet less than .01% die by suicide each year**. Data from the preceding similar experiment (K99 experiment) provide clear evidence of the safety of this protocol; no participant was hospitalized for imminent risk or attempted suicide during the study.**

(13) Must not report symptoms consistent with primary dysmenorrhea that indicate immunological involvement (recurrent perimenstrual fever) or significant disruption in daily life (missing >= 2 days of work each month).

Justification: The purpose of the present study is to determine the impact of hormonal stabilization (vs. natural withdrawal) in physically healthy females. Thus, females who present symptoms consistent with severe primary dysmenorrhea will be excluded.

(14) Must not be (1) currently seeking treatment for premenstrual dysphoric disorder (as indicated by (a) treatment-seeking reported at screening AND (b) a PMDD diagnosis confirmed by the study team during baseline ratings using the daily record of severity of problems) and must not be (2) currently experiencing a mood episode that began during pregnancy or within four weeks of giving birth (Note: this criterion only applies if delivery occurred less than 2 years ago).

Justification: Some evidence suggests that the pattern of perimenstrual symptoms that have been linked with these reproductive mood disorders *could* differ meaningfully[27] from that which we be expect in the more general clinical population of females with suicidality[12]. Because these distinctions are not yet clear, and because we do not wish to interfere with the evidence-based treatment of those who are currently diagnosed with these hormone-sensitive disorders, we are excluding these individuals.

(15) Must NOT report family history of >=2 first degree relatives with hormone-related cancers (breast, ovarian, endometrial, uterine, prostate, colon), nor known genetic risk for hormone-related cancers (e.g., BRCA1, BRCA2, PTEN, or Tp53).

Justification: Administration of exogenous hormones to females with increased genetic risk for hormone-related cancers may increase the risk of cancer.

(16) Must NOT report increased risk for blood clots as described by personal history of (or at least 1 first degree relative's) diagnosis of blood clots (VTE, thromboembolism, etc.), nor evidence of genetic risk for clotting disorders (Factor V Leiden).

Justification: Administration of exogenous hormones to females with increased genetic risk for these chronic thromboembolic medical conditions may increase the risk of a blood clot formation in response to hormones.

HC group: Criterion 16 removed for HC group. Increased clotting risk does not apply to the observational group not receiving exogenous hormones, and we have no expectation that menstrual cycle variables, neuroactive steroid, or gene expression outcomes would be altered by heightened risk for blood clot.

(17) Must NOT be allergic to peanuts.

Justification: The facility which processes the study pills indicates that the product is not suitable for those with a peanut allergy, so females with a peanut allergy will be excluded.

HC group: Criterion 17 removed for HC group, who will not receive any study medication.

SPECIFICS OF SUICIDE RISK-RELATED ELIGIBILITY CRITERIA

As in the PI's two prior trials, the proposed study will include only participants with current suicidal ideation (past-month endorsement of suicidal ideation) in order to maximize generalizability to our clinical population of interest. In order to minimize the risk of suicide attempts during the study, we will exclude females who appear to be at high imminent risk of acute suicidal crisis/attempt at the outset of the study. Although we acknowledge that the field's ability to predict imminent risk of suicide attempts remains poor, these criteria are based on the available evidence about which factors sometimes predict imminent risk of suicide attempt or death.

- A person will be excluded if they report more than 1 attempt (total of suicide attempts or interrupted suicide attempts) in the past year.

- A person will be excluded if they report any suicide attempt or any interrupted suicide attempt in the past 3 months, as the majority of repeated attempts occur within the first three months following an initial attempt.

- A person will be excluded if they report any aborted suicide attempt in the past month.

- A person will be excluded (and referred) if they report that they do not currently visit a mental health provider (psychiatrist, therapist) at least once every 3 months AND they are not willing to start visiting a mental health provider at least once every 3 months. If a participant opts to begin treatment prior to starting the study in order to become eligible, eligibility for starting clinical trial conditions will be based on self-report of having had at least one visit with the new provider.

- A person's participation will be delayed for 3 months if they report or expect any extreme external stressors (e.g., death of a loved one, loss of employment) in the past or coming month, as extreme stressors drastically increase the risk of suicidal crisis.

- A person will be excluded if they report a history of manic, psychotic, or substance use disorder symptoms that may reduce the predictability and increase likelihood of suicidal crises/attempt.

- Participants will be counseled in means restriction at the outset of the study; if a person reports access to a firearm or another highly lethal means of suicide and is not willing or able to restrict access to such means, they will not be eligible for the study.

5.0 Subject Enrollment

Recruitment and Screening. We will recruit **170 females (<u>90 completers</u>) with past-month SI** in outpatient treatment as usual with a mental health provider, and 60 healthy controls (HC) (40 completers) with no lifetime history of SI and no DSM-5 diagnoses in the past five years, for a total of 230 participants. Participants will be recruited via fliers, email listservs, online research recruitment websites (Research Match, Research and Me) and social media ads seeking volunteers to participate in a study "on the biology of depression, stress, and suicide" (with no mention of the cycle, hormones, female sex, or women). The social media ad directs participants to complete an online screener. They will be scheduled for a phone screen (via HIPAA-compliant Webex teleconference software) if they meet basic inclusion criteria. Following phone screening, potential participants will be invited to an enrollment visit either in-person or virtually. Virtual enrollments (including digital signatures of the informed consent document hosted on UIC Qualtrics), will occur via UIC's HIPAA-compliant Webex teleconference software. For participants who completed virtual enrollment visits during the covid-19 pandemic, their consent will be re-assessed during their first in-person Welcome Visit, where they will provide written consent and receive a copy of the consent form.

6.0 Study Design and Procedures

Overview of Research Design. The proposed primary experiment (identical to K99MH109667) tests the hypothesis that perimenstrual E2+P4 withdrawal causes varying degrees of neuroactive steroid withdrawal, which produces co-occurring hopelessness and hyperarousal, which interact to cause proximal suicide risk. In a crossover placebo-controlled experiment, 90 completer female outpatients with recent SI (but acceptable suicide risk for outpatient treatment) will complete two counterbalanced perimenstrual conditions: (1) **natural perimenstrual hormone withdrawal** (placebo), and (2) **prevention of perimenstrual hormone withdrawal** (exogenous stabilization of E2+P4). Measures of neuroactive steroids, hopelessness, hyperarousal, and proximal suicide risk will be collected in the laboratory and via smartphone. The basic timeline for participation can be seen in Table 1a. We will utilize scientifically rigorous methods that promote transparency and reproducibility (**NOT-OD-16-012)**: well-defined inclusion/exclusion criteria, validated measures, pre-registration of our protocol, and sharing of data.

Table 1a. Primary study activities and timeline.

STUDY TIMELINE	START	MONTH 1		MONTH 2				MONTH 3	MONTH 4				END
	Enrollment Visit	Welcome Visit	Baseline Menstrual Cycle	STUDY PHASE 1 MENSTRUAL CYCLE				WASHOUT MENSTRUAL CYCLE	STUDY PHASE 2 MENSTRUAL CYCLE				1 Hour Debriefing Visit
				Week 1	Week 2	Week 3	Week 4		Week 1	Week 2	Week 3	Week 4	
Order A (Half of study participants will be randomly selected to do the Active phase first and the Placebo phase second)					Ovulating Testing	ACTIVE E2 + P4				Ovulating Testing	PLACEBO patch and pills		
Order B (Half of study participants will be randomly selected to do the Placebo phase first and the Active phase second)					Ovulation Testing	PLACEBO patch and pills				Ovulating Testing	ACTIVE E2 + P4		
Laboratory Visits (90 minutes)						XXXXX					XXXXX		
Evening Surveys (3-5 minutes)		X	X	X	X	X		X	X	X	X		
Daily Phone Call (1 min per day)						X					X		
Using "Biaffect" App Keyboard		X	X	X	X	X		X	X	X	X		
Using Metricwire App		X	X	X	X	X		X	X	X	X		
Wearing Oura Ring		X	X	X	X	X		X	X	X	X		

SUB-STUDY TIMELINE	START	MONTH 1	MONTH 2				END
	Enrollment Visit	Baseline Menstrual Cycle	OBSERVATIONAL MENSTRUAL CYCLE				
			Week 1	Week 2	Week 3	Week 4	
Ovulation Testing				X			
Laboratory Visits (60 mins)					XXXXX		
Evening Surveys (3-5 mins)		X	X	X	X		
Using "BiAffect" App		X	X	X	X		
Using Metricwire App		X	X	X	X		

Table 1b. substudy activities and timeline.

The proposed substudy characterizes 1) normative cycle-related variation in neuroactive steroids and mRNA expression, and 2) group-related differences (i.e., past-month SI vs HC) in trajectories of GABAAR subunit change across the cycle. 60 healthy controls (HC) with no SI will be recruited to gain a completer number of 40 for a two-month long observational substudy, in which five lab visits across one menstrual cycle (timed identically to mirror the observations across one medication phase of the experimental trial) will capture baseline molecular/genetic changes for between-group comparisons. The basic timeline for participation can be seen in table 1b.

The **double-blind crossover experimental design** is identical to that used during the PI's recently-completed trial (K99MH109667**;** Fig. 6). The TOP (red) panel shows natural perimenstrual hormone withdrawal in the placebo condition; the BOTTOM (black) panel shows prevention of hormone withdrawal in the active E2+P4 stabilization condition. THIS IS NOT A CLINICAL EFFICACY TRIAL, but a RCT design will probe the effect of hormone withdrawal (vs. stabilization) on proximal suicide risk and mediators. Participants will be randomized to condition order A (see Table 1 above; Active E2+P4, washout cycle, then Placebo) or condition order B (Placebo, washout cycle, then Active E2+P4). Investigational Drug Services will manage the double-blind. In each two-week condition, participants will wear a weekly patch (E2 or placebo) and take a daily pill (P4 or placebo). Because this manipulation simply delays natural E2+P4 changes[96], and given our previous finding of benefit[12], this manipulation is *not expected to pose greater risk than a normal menstrual cycle.*

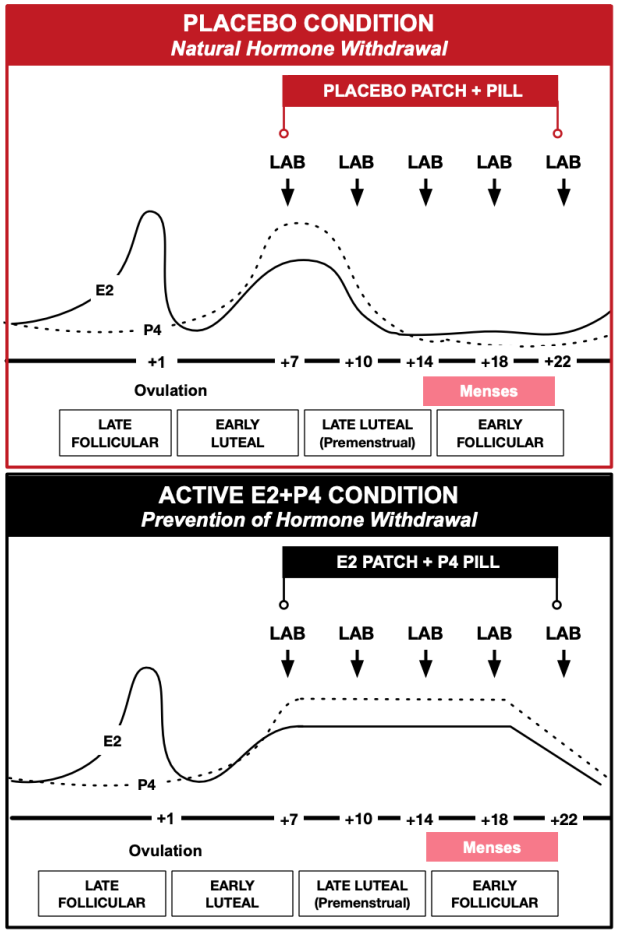


Fig. 6. Experimental Conditions

Timing and Duration. Participants will receive the first seven-day transdermal E2 patch (or placebo patch) and begin daily oral P4 (or oral placebo) at a lab visit scheduled seven days after a positive urine ovulation test (+7 in Fig 6). This coincides with a time in the natural cycle when E2+P4 are already elevated. The weekly patch will be changed at the lab visit on day +14. The goal of this E2+P4 manipulation is to extend and maintain the mid-luteal phase hormone profile beyond the point in the cycle (i.e., around day +10) that would normally be associated with natural hormone withdrawal. Therefore, the active condition effectively prevents perimenstrual E2 and P4 withdrawal.

Formulation and Dose. ***Estradiol***. Peak E2 with transdermal E2 patches (~45 pg/mL) correspond roughly to normative luteal E2 levels[96]. These E2 levels are expected to maintain stable mRNA expression of 3α-HSDII during the experimental period, stabilizing biosynthesis of neuroactive steroids. Transdermal E2 demonstrates reduced thromboembolic risks relative to oral E2. ***Progesterone***. Although transdermal synthetic progestins exist, these molecules have confounding effects at androgen and mineralocorticoid receptors. Thus, oral micronized progesterone 200mg/day will maintain luteal ranges (~13 ng/dL)[96] as in the prior trial[12]; this dose of P4 is expected to buffer neuroactive steroid change by increasing precursors. **Standardized times for evening dosing (7 pm) and lab visits (7-11am)** will minimize variability in neuroactive steroid levels due to timing. Typical within-participant variation in emotional symptoms, physical premenstrual symptoms, and cycle length will maintain participant blind. Demand characteristics will be minimized by telling participants that responses to hormones differ, and they may experience positive or negative moods. Blinding was excellent in our completed trial, with medication-day condition guesses only slightly more accurate than chance (53% accurate). **Manipulation Checks.** In the prior trial, we found that the hormone stabilization condition (E2+P4) was associated with elevated (maintained) levels of E2 and P4 on day +14 (see Figure 6) relative to a drop in E2 and P4 observed in the placebo condition. We will once again measure E2 and P4 in this trial to confirm stabilization.

ASSESSMENTS	Laboratory and smartphone-based measures.

Overview of Assessment Protocols. Laboratory visits (90 minutes; 7-11 am). After a positive home urine ovulation test (day 0), five 90-minute lab visits will be scheduled on days +7, +10, +14, +18, and +22 across each condition to capture the dynamic changes in mediators and outcomes across the perimenstrual window (Figure 6). Consistent with standard practices in the field of reproductive mood disorders[55], participants will complete brief (3 minute) daily evening surveys across the study. During the study participants will undergo passive tracking of physiological arousal, movement, and typing speed instability via the Oura Ring, Metricwire App, and BiAffect App, respectively. **Participant Safety Monitoring.** On medication days and 4 days following, daily phone calls (30-60 seconds) will evaluate physical adverse effects and suicide risk.

Enrollment Laboratory Visit (5 hours; split into 2 visits)

Participants will be asked to attend for a 5 hour enrollment session that is split into 2 visits either in-person or virtually in which participants will be learn more about the purpose of the study and will be assessed for eligibility.

Baseline Interviews: The Structured Clinical Interview for DSM-5 (SCID-5)[97] and Structured Clinical Interview for DSM-5 Personality Disorders[97], as well as a medical eligibility interview. Lifetime suicidal thoughts and behaviors will be assessed using the Self-Injurious Thoughts and Behaviors Interview -Revised (SITBI-R) and categorized with the C-CASA[98]. Participants will complete the Stress and Adversity Interview (STRAIN)[18] interview, which captures lifetime exposure to adverse events (e.g., Negative Valence Systems of Loss, Threat).

Baseline Self-Report Surveys: Participant complete the following measures with respect to the past two weeks: The Adult Suicidal Ideation Questionnaire (ASIQ)[20], a 30-item questionnaire that provides a continuous measure of SI as well as items assessing the intensity of thoughts related to suicidal planning and intent; the Beck Hopelessness Scale (BHS)[17], a widely-used 20-item measure of hopelessness; Arousal will be measured using the Arousal items on the Profile of Mood States (POMS)[99] as well as the Brief Agitation Measure (BAM)[54]. Other measures will be included to capture key transtheoretical constructs, including the Inventory of Depression and Anxiety Symptoms – ii (IDAS-ii)[100], the UPPS-P Impulsivity Scale[101], Interpersonal Needs Questionnaire (INQ; belongingness, burdensomeness)[102], and the Fearlessness About Death Scale[103].

Enrollment Laboratory Visit for HC participants (Aim 3substudy)
HC participants will complete a single enrollment session expected to last 3 hours, either in-person or virtually. Participants will complete all questionnaires and interviews listed above for primary study. Interviews are expected to take less time than the primary study because the HC substudy participants are recruited for a lack of current psychiatric symptoms.

Welcome Visit (1 Hour)

Participants will be asked to attend an in-person introductory session where they will receive ovulation test kits and Oura rings. Participants will also review the study consent form, fill out payment-related paperwork, and form a safety plan with the lab staff in case of elevated suicide risk.

Welcome Visit for HC participants (Aim 3substudy)
HC participants will not be asked to attend an in-person introductory session. Ovulation test kits will be mailed to HC participants after enrollment, paperwork will be signed at the first visit.

Repeated Laboratory Visits (90 minutes; 10 visits total; 5 visits across each condition)

After a positive urine ovulation test (day 0), five 90 - minute lab visits will be scheduled on days +7, +10, +14, +18, and +22. Labs will be standardized to occur between 7am and 11am. Labs will begin with an assessment of side, after which participants will complete tasks, undergo a blood draw and urine pregnancy and standard drug screen (day +7), complete interviews and self-reports, and undergo patch applications (days +7, +14). Health behaviors and illness will be measured during labs and daily survey.

During the study, each participant will also wear an *Oura Ring*, a wearable ring device (that includes a pulse plethysmograph (PPG) and temperature sensors) throughout the study; these rings, which have been validated to index heart rate and heart rate variability (measures of physiological arousal), as well as changes in basal body temperature, will serve the dual purpose of measuring both physiological arousal and progesterone-related temperature changes across the cycle.

NOTE: A prior version of the protocols utilized lab-based measures of physiological arousal using a traditional EKG. However, given ongoing concerns regarding close contact during the COVID-19 pandemic, the NIMH program officer for the grant funding the present study has approved a change from lab-based cardiovascular physiological recording using EKG to ambulatory assessment of physiological arousal using the Oura Ring.

Repeated Laboratory Visits for HC participants (Aim 3 substudy)
HC participants will complete 5 60-minute lab visits across one menstrual cycle. Repeated lab visits will be timed identically in the HC substudy group as in the primary group (i.e., 5 lab visits scheduled on days +7, +10, +14, +18, and +22 relative to a positive urine ovulation test. Participants will undergo a blood draw, urine pregnancy and standard drug screen (day +7), and report health behaviors and illness. Participants will **not receive any experimental patch application,** will not complete cognitive tasks, and will not complete interviews.

Debriefing Visit (1 hour)

After completing both randomized conditions, participants will be asked to attend for a 1 hour debriefing session with Dr. Eisenlohr-Moul either in-person or virtually in which participants will be unblinded, learn more about the purpose of the study, have any concerns or questions participants may have about the study answered and addressed, and evaluate the participant's current risk of suicide upon study completion.

Debriefing Visit for HC participants (Aim 3substudy)
HC participants will not be asked to attend a debriefing session, as they do not complete randomized double-blind experimental conditions, and they do not have suicide risk to assess.

CLINICAL LABORATORY ASSESSMENTS AND SAMPLING:

A point-of-care urine pregnancy test will be completed in our laboratory on site at the first visit of both conditions. A positive pregnancy test will result in termination of participation and will be managed by Dr. Wagner.

We will also collect a urine sample and perform rapid urine toxicology screens for amphetamines, barbiturates, benzodiazepines, cocaine, opioids, cannabis, and methamphetamine on-site through the CRC nursing staff using Alere iCup 12-Panel Drug Test with temperature-sensitive strips. Positive tests will be used primarily for data quality assurance and covariate purposes. Individuals with positive test results will be allowed to continue the study provided that the participant demonstrates an ability to safely complete all study procedures. When there is any positive test that is not

accounted for by an existing prescription, the participant's test results will be reviewed with the study physician and PI at routine team meetings.

At all 10 laboratory assessments during active treatment with placebo or E2/P4, participants will provide blood (35ml at each visit) to be assayed for E2/P4, neuroactive steroids, and from which we will perform RNA gene expression assays, to evaluate the success of the hormone in achieving stable luteal phase levels of E2/P4/ALLO, as well as more stable gene expression markers of risk across various systems (neurosteroidogenic enzymes, GABA-A receptor subunits, and other mechanisms such as inflammatory cytokines). Blood will be collected as follows: (1) 30ml in red-top tubes (no additive), and (2) 5ml in PaxGene RNA tubes, at 2.5ml blood apiece. 30ml of blood (red-top tubes) will be centrifuged and plasma will be stored in cryovials, and the PaxGene tubes will be stored as well; when it is time for assay, samples will be transferred to Dr. Pinna's laboratory. Standard freezer-proof labels will indicate participant ID and study session. Transport from the CRC to Dr. Pinna's laboratory will be managed by Clinical Research Center staff. Samples will be analyzed in small batches with all of one participant's samples being run in the same batch. Following assay, any additional serum will be stored by Dr. Pinna. Following assay, the PaxGene RNA blood samples will be destroyed.

Clinical assessments and laboratory sampling for HC participants (Aim 3 substudy).
Point-of-care pregnancy test, urine toxicology screen, and laboratory samples via blood draw are identical for HC participants as described above.

Self-Reports. Self-report **surveys** from the enrollment visit will be repeated at each laboratory visit, with instructions to report symptoms in the past week or since the last visit. The "since last visit" version of the C-SSRS[104] **interview** will be used to precisely measure discrete self-injurious thoughts and behaviors at each laboratory visit; responses will be coded into suicide outcomes using the C-CASA[98].

We will operationalize behavioral hyperarousal (reactivity) as a longer response time when matching emotional faces relative to shapes. We will utilize these measures as independent predictors of proximal suicide risk, as well as variables with which to further validate *BiAffect* indices (below).

Physiological Arousal. Following the laboratory visit, participants will be mailed an Oura Ring in the appropriate size, along with instructions for charging and use, and asked to wear the ring as much as possible while in the study. This commercially available product is intended for fitness tracking and recreational use. It is worn on the middle finger and also uses PPG technology along with mechanical sensors to measure physical activity, sedentary behaviors, sleep, heart rate, oxygen saturation, and body temperature.

Participants will also be asked to use the Oura app- this commercially available application is required to collect data from supported Oura products. It is publically and free available for both Android and iOS devices in their respective app stores. Data from the Oura Ring will be transferred to the Oura smartphone app, and later to the Oura cloud storage service (where it can be retrieved later by the study team) using an Oura Ring app account created by the study team using the participant's confidential study email address (e.g., clearlabparticipant+3999@gmail.com). Data collected and uploaded to the cloud will not include IP address or any other participant-identifying information.

Self-reports and physiological arousal for HC participants (Aim 3 substudy).
HC participants will not be asked to complete self-report surveys or cognitive tasks at laboratory visits. They will not wear an Oura Ring or use the Oura app.

Neuroactive Steroids (Co-I Pinna). See sample collection, transportation, and preparation information above. In Dr. Pinna's Laboratory, neuroactive steroid biosynthesis will be determined by levels in the plasma using gas chromatography-mass spectrometry (GC-MS of ALLO, PA, 5α-DHP, E2, and P4) and by real time PCR (qPCR) for measuring the peripheral blood mononuclear cells (PBMC) mRNA levels of key enzymes involved in the neuroactive steroid biosynthetic pathway,

3α-HSDII and 5α-RI (the most expressed isoforms in the brain[105]). The methodology to determine neuroactive steroid levels is a 'state-of-the-art' technique developed in co-PI Pinna's laboratory (GC-MS), which allows the simultaneous determination of neuroactive steroid from a single sample due to high sensitivity (fmolar) and unsurpassed structural specificity[66,106–108]. Comparative qPCR determination of 5α-RI and 3α-HSDII expression[65] combined with complementary GC-MS measurement of neuroactive steroid has established a correlation among plasma and CSF neuroactive steroid biosynthesis in neurological disorders[109]. Highly significant correlations were also established among CSF and post-mortem brain[110] and CSF and serum neuroactive steroid levels[66,111,68] Importantly, Co-I Pinna's study of ALLO levels and 5α-RI expression in human prefrontal cortex (BA9) is a further demonstration that down-regulation of neuroactive steroids in CSF[67] and blood[112] *strongly reflects down-regulation of brain neuroactive steroid levels*[65]. Neuroactive steroid levels will be corrected for procedural losses by adding to the samples 1 pmol of deuterium-labeled neuroactive steroid used as internal standards[107,108]. Measuring central mRNAs in peripheral tissues is easy to obtain, reliable, and known to closely reflect changes in neuroactive steroid biosynthesis occurring in the brain[67,112]. The best source of mRNA is represented by isolated PBMC, since hemoglobin from erythrocytes can inhibit PCR[113]. Several neurological/psychiatric clinical studies have correlated brain with lymphocytes findings[114,115]. Various groups confirmed the presence of 5α-DHP and ALLO (GC-MS), 5α-RI and 3α-HSDII expression (PCR) in lymphocytes[116,117]. The qPCR methodology is a suitable method to quantify mRNAs encoding for neurosteroidogenic enzymes expressed in PBMC and is a powerful tool in combination with GC/MS to determine alterations in neuroactive steroid biosynthesis (e.g., 3α-HSDII and 5α-RI, to evaluate altered regulation of ALLO biosynthesis). 3α-HSDI and 5α-RII (peripheral isoform) mRNA will also be measured and used as an internal control.

In addition to the specific gene expression assays for neurosteroidogenic enzymes performed by Dr. Pinna, We will also perform "whole transcriptome shotgun sequencing" in each sample collected across both conditions, which measures the presence and quantity of RNA (related to the entire genome) in a given sample at a given moment. This will be used to test hypotheses regarding the effects of the manipulation on expression of genes coding for all neurosteroidogenic enzymes (not just those measured by Dr. Pinna using qPCR), GABA-A receptor subunits, and inflammatory cytokines. These analyses will also be performed in-house at the University of Illinois.

Neuroactive steroids for HC participants (Aim 3 substudy).
Neuroactive steroids, mRNA, and genome sequence collection will be identical in the substudy as described above.

Smartphone-Based Assessments

Daily Symptoms Survey (3 minutes each evening throughout the 4-month Study). Each evening throughout the four months of the study, participants will complete the following self-report scales. First, the Daily Rating Form (DRF)[55], capturing hormone-related symptoms listed in the DSM-5[118(p5)] (depression, hopelessness, worthlessness, anxiety, mood swings, rejection sensitivity, anger/irritability, interpersonal conflict, difficulty concentrating, lack of interest, insomnia, hypersomnia, overeating, lack of appetite, emotional overwhelm (hyperarousal), feeling out of control, fatigue, and physical discomfort). In addition to the DRF, participants will complete the Brief Agitation Measure (BAM)[54], a 3-item measure that taps into feelings of agitation (e.g., "I felt so stirred up inside that I wanted to scream"; hyperarousal) as well as dimensional ratings of the three highest-loading items from the Beck Hopelessness Scale[17] (e.g., "The future seems dark to me"). This daily survey will provide an integrated rating of the patient's symptoms across the entire day, consistent with methods used in seminal experiments of hormones and mood[30,119,120]. Medication adherence, stressor exposure[19], medication, drug, and alcohol use, sleep, pain, illness, and exercise will be recorded; any additional probing to clarify these online responses will be take place during the following day's daily safety call.

BiAffect App: Passive Tracking Measures of Hyperarousal (Co-I Leow). BiAffect App. Participants will be asked to type using a custom built virtual keyboard application on their smartphone that will replace the default keyboard. This keyboard will track keyboard dynamics "metadata" but not the actual words. Such metadata may include the start, finish and duration of each keyboard session, the time when a key is pressed and the category of the key. Such information can thus be used to

compute keystroke dynamics such as the mean typing rate. Metadata will be encrypted and transmitted across a secure network to Sage Bionetworks.

The core technology of the **BiAffect** App is a custom-built keyboard that replaces the standard native smartphone keyboard in order to unobtrusively monitor keyboard dynamics metadata (such as typing speed and rhythm, mistakes in texts, and the use of backspace and auto-correct). The app also samples accelerometer displacement (both gross and fine movements of the phone). Together, these typing and movement data will measure:

Psychomotor Changes. Higher accelerometer displacement values arise when participants hold their phones less stably, consistent with psychomotor agitation; therefore, accelerometer displacement will be used in the present study to measure psychomotor agitation. We will also capture and examine quadratic effects of overall physical movement as an index of both psychomotor retardation (infrequent, slow movement) and agitation (frequent, quick movement). Consistent with the intuitive face validity of directly measuring psychomotor activity using the smartphone accelerometer, two of our team's prior studies using these BiAffect variables demonstrate within-person effects linking accelerometer displacement values to concurrent daily reports of disturbed mood[121,122]. These measures will be further validated through other measures of arousal in the study, including daily ratings (e.g., "slowed-down", "sped up") and autonomic measures.

Behavioral Reactivity to External Stimuli*:* Typing speed instability will be calculated as a momentary and daily measure of reactivity to phone-based stimuli. Instability metrics will be calculated as the root mean square successive difference (rMSSD). This metric evaluates variability in consecutively recorded typing speeds and encapsulates both the size and the temporal order of within-subject fluctuations. In a prior study, our team has demonstrated that daily typing instability is able to prospectively predict disturbed mood[122].

A variety of other relevant indices will be available from the BiAffect Keyboard, including but not limited to the number of errors made while typing, diurnal activity patterns and inferred hours of sleep, and amount of phone usage[123].

Physiological Arousal: Oura Ring Devices will collect data on the subject's body temperature, heart rate, interbeat interval) of sufficient quality for heart rate (HR) and heart rate variability (HRV) analysis.

Metricwire is a multi-platform (iOS and Android) smartphone app (*www.metricwire.com*) that has been previously used in many health and behavioral studies. Participants who have a compatible mobile device will be emailed a link and given a lab-generated email and password to sign up for the study. The app is used for a variety of methods in the literature; here, it will be used as an adjunct application to capture accelerometer and movement data from the smartphone's sensors. This is being collected in addition to the movement data from BiAffect since the sampling rate is higher and it can collect data even when the participant is not typing. Metricwire data will be collected up until July 31st, 2023. After this date, participants will no longer be enrolled into the Metricwire app and no data will be collected.

Smartphone-based assessments for HC participants (Aim 3 substudy).
HC participants will complete the following as described above: daily symptoms survey, BiAffect app (psychomotor changes, behavioral reactivity to external stimuli), Metricwire. HC participants will **not** receive Oura rings for physiological arousal.

Participant Compensation.
Participants will receive financial compensation (taxable) for participating in this research study based on the percentage of total possible study visits and surveys completed. We collect social security number or Taxpayer Identification Number (TIN) to issue compensation and for tax reporting purposes to the United States Internal Revenue Service (IRS).

If participants complete all study activities and surveys, compensation is $2000 ($150 total for enrollment visits, and up to $925 for each of the two medication phases). The specific amount of study pay is based on how many of the study visits and surveys are completed as follows:

- **Enrollment visit** payment is $150 total (split into 2 payments of $75) in cash after each in-person enrollment visit, or via digital cash transfer (using private modes in PayPal, Zelle, or Venmo smartphone applications) after each virtual enrollment visit. If participants complete any portion of the enrollment visit and are deemed ineligible, compensated is still provided for the visit(s) completed.
- Pay for each the two **study medication phases** will be up to $925, with the two payments sent following each phase, mailed within approximately 30 days of completing that phase.
    - Within each medication phase, this $925 is broken down as follows:
        - $250 for completing 100% of surveys (prorated if less than 100% complete)
        - $75 for 100% of check-in calls (prorated if less than 100% complete)
        - $120 for each laboratory visit completed
- Participants will also receive the Oura Ring free of charge and will be allowed to keep the ring following the study.

Financial Compensation for HC participants (Aim 3 substudy).
Participants similarly will receive financial compensation (taxable) for participating in the research substudy based on the percentage of total possible study visits and surveys completed. We collect social security number or Taxpayer Identification Number (TIN) to issue compensation and for tax reporting purposes to the United States Internal Revenue Service (IRS).

If HC participants complete all study surveys and visits, compensation is $400.

- **Enrollment visit** payment as $50 in cash after the in-person enrollment visit, or via digital cash transfer (using private modes in PayPal, Zelle, or Venmo smartphone applications). If a participant completes the enrollment visit and is deemed ineligible, compensation of $50 is still provided.
- **Baseline and observational phases:** one payment of $350, mailed within approximately 30 days of completing the study. Study pay will be prorated based on completion of study surveys and activities.
    - Pay for each laboratory visit will be $50 (5 laboratory visits total)
    - Pay for completion of daily surveys will be $100 (prorated based on percent completed, where 100% survey completion = $100).

7.0 Expected Benefits/Risks

EXPECTED RISKS

1) Psychological Symptom Assessment. Clinical interviews and self-report assessments contain questions regarding sensitive personal information, including severe psychological symptoms such as suicidal and other impulsive behaviors (e.g., substance abuse). As a result, participants may become upset or embarrassed when discussing current or past distressing life events and behaviors. On the other hand, recent evidence suggests that research questions pertaining to potentially distressing topics such as psychiatric symptoms, suicidality, and sexual or physical abuse do not significantly increase distress or acute suicide risk in participants who report these issues[124,125]. The chance of increased distress is necessary in order to assess how symptoms change in response to perimenstrual hormone withdrawal (vs. stabilization).

2) Effects of the Ovarian Hormone Stabilization Protocol on Mood/Anxiety and Behavior (for primary experimental group; N/A for HC group).

Mood/Anxiety. As the population being studied in this protocol will have elevated psychiatric symptoms, and as ovarian hormone changes are implicated in depression and anxiety symptoms in some females[126], it is possible that adverse mood reactions may occur (1) during the administration of ovarian hormones in the stabilization condition or (2) naturally during the placebo (natural) perimenstrual withdrawal condition. However, because the proposed hormone manipulation mimics (i.e., stabilizes) the luteal hormone levels that cycling individuals naturally experience[96], the proposed study is not expected to pose any greater risk to participants than what they experience in daily life. Furthermore, although the withdrawal from the experimental hormone condition could elicit negative mood changes, these are not expected to be any more severe than those that would otherwise have arisen from normal perimenstrual hormone withdrawal—that is, we will have simply delayed the normal withdrawal process. This argument is supported by the results of the prior K99 trial, where increases in symptoms observed during withdrawal from exogenous E2/P4 was less severe than that observed in the natural perimenstrual withdrawal condition under placebo.

Suicidality. As detailed above, the population will be recruited to achieve a population of individuals that is simultaneously: (1) experiencing **recent suicidal ideation**, yet (2) at **acceptably low risk for suicidal crisis/attempt** (such that the participant is appropriate for outpatient rather than inpatient care) based on an assessment conducted during the enrollment visit. Because ovarian hormone changes are implicated in psychiatric symptoms in some females , and because such symptoms may increase suicide risk, it is possible that increases in suicidality may occur (1) during the administration of ovarian hormones in stabilization conditions or (2) naturally during the placebo (natural) perimenstrual withdrawal condition. However, because the proposed hormone manipulation mimics (i.e., stabilizes, then shifts) the normal physiologic range of luteal hormone levels and changes that cycling individuals naturally experience, the proposed study is not expected to expose participants to greater risk for suicidality than what they experience in daily life. As above, this argument was supported in the prior K99 trial, where increases in proximal suicide risk observed during withdrawal from exogenous E2/P4 was less severe than that observed in the natural perimenstrual withdrawal condition under placebo.

3) Hormone Side Effects (for primary experimental group; N/A for HC group). We do not expect any adverse side effects associated with the hormonal manipulations outlined in this protocol following reasons: First, we did not observe any side effects occurring significantly more frequently under active hormones than during placebo during the prior K99 trial using the same hormonal manipulation. Second, we will be administering the physiologically relevant steroid hormones (estradiol and/or progesterone) and not the substituted steroids (such as ethinyl estradiol or norethindrone) present in many oral contraceptives, the latter of which have been reported to have potentially more serious side effects[127–129]. Nevertheless, we describe the common side effects of estradiol and progesterone below.

Estradiol. Nausea is the most common side effect of estrogen administration. At conventional hormone doses, this complaint seldom interferes with eating, and no weight loss has been reported as a result of nausea. Breast engorgement and bleeding are also common side effects of estrogen administration. Pre-existing fibroid tumors of the uterus may enlarge under the effects of estrogen; however, at the dosage and for the duration of estrogen administration in this protocol this risk is extremely small. Numerous retrospective case control studies published since 1975 have indicated that post-menopausal exposure to unopposed estrogens for more than one year results in a two to 12-fold increased relative risk for endometrial cancer. A relationship between the dose and duration of estrogen use and the risk for endometrial cancer has also been shown, the risk being increased after one to four years of estrogen use and rising also with the dosage employed. However, the addition of progesterone to estrogen replacement therapy decreases the risk of endometrial hyperplasia and endometrial cancer to equal or below that of females receiving no hormonal treatment, and oral prometrium of 200 mg/day, as is present in the proposed work, is sufficient to prevent endometrial hyperplasia[130]. There is an increase in thromboembolism in females receiving non-contraceptive estrogen therapy[131–133], but this risk is most evident in obese females and in those who smoke (exclusionary factors in the present research).

It may be important to note that there were numerous concerns about increased risk for breast cancer and cardiovascular events related to the use of hormone replacement therapy (estrogen plus a progestin) that stemmed from the initial results of the Women's Health Initiative (WHI), which randomized 27,000 postmenopausal females to HRT or placebo. Critical

reviews subsequent to the initial WHI reports showing an increased incidence of non-fatal breast cancer and cardiovascular events have argued that the discordant findings between the WHI and the observational studies finding benefit of HRT for all cause and cardiovascular mortality reflected problems in the design of the WHI, the most important of which may have been the age and condition of the study subjects[134]. As the risk of breast cancer, heart disease and metabolic dysregulation increase with age; the likelihood of adverse events upon exposure to E2 should also increase with age. Recent reports from the North American Menopause Society[135] and the Endocrine Society[136] confirm that the risk of CVD in the WHI was observed in older but not younger females. Additionally, data suggest that the formulation of E2 (conjugated equine estrogen) used in the WHI is associated with an increased profile of risk compared with transdermal E2, and the progestin used in the WHI (medroxy progesterone acetate) is particularly antagonistic (among progestins) to the beneficial cardiovascular effects of E2[137].

Given our plans to administer transdermal E2 to females: (1) for only 14 days, (2) no older than 45 years of age, (3) who are currently regularly menstruating and medically healthy, and (4) who are at no greater than average risk for CVD, breast cancer or VTE, the **risk of serious adverse events is exceedingly low.**

Progesterone. Progesterone and the synthetic progestins are widely prescribed, with indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea, and precocious puberty[130]. In a recent study, a dose of 300 mg of oral micronized progesterone was given to a sample of females with premenstrual mood disturbance and was well tolerated by this sample[138]. Side effects reported included lightheadedness and fatigue; these were very mild and caused no dropouts.

4) Confidentiality. A breach of confidentiality could indicate to others a participants' history of mental and behavioral health concerns.

5) Venipuncture. Venipuncture (the needle stick during blood draws) may result in syncope (lightheadedness), discomfort, nausea, and bruising. There is a very low risk of infection from venipuncture (less than 1 in 1000).

6) ***Oura Ring.*** The small flashing light on the oura ring could theoretically increase risk of seizure among those with epilepsy (exclusionary in the present study), and could theoretically interfere with pacemakers and other internal electronic devices (also exclusionary in the present study).

PROTECTION AGAINST RISKS

Protections Against Risks Associated with Psychological Assessment. The PI (Dr. Eisenlohr-Moul) is a licensed clinical psychologist with ten years of experience in risk assessment and empirically-supported treatment of chronic suicidality (e.g., Dialectical Behavior Therapy). The PI has personally conducted roughly ten studies assessing daily emotional symptoms, three of which included measures of daily suicidal ideation and depressive symptoms in females with borderline personality disorder or chronic suicidality. Based on these experiences and the available literature, participants are unlikely to become acutely upset in response to the psychological assessments conducted in the present study. If participants do become upset during an assessment, they will be reminded of their right to discontinue participation, suicidal ideation will be assessed and managed using the University of Washington Risk Assessment and Management Procedure (UWRAP) as recommended by NIMH, and appropriate referrals will be made. Participants will be reminded each day that they do not have to answer any question that they do not want to answer.

1) Protections Against Risks Associated with E2 and P4 Administration *(for primary experimental group; N/A for HC group)*. Hormone-related risks are minimized in our protocol by the following: 1) including only young, medically healthy premenopausal females; 2) the use of a very short-term administration of estradiol in combination with oral micronized progesterone will virtually prevent the chance of endometrial hyperplasia; 3) exposure to E2 for less than 3-5 years has not been shown to be associated with an increased risk of breast cancer[133,139]; 4) the exclusion of individuals with a family

history indicative of increased breast cancer risk or thromboembolic disorders; 5) the frequent assessment of side effects at every phone call; 6*)* provision of a drug information sheet with the instruction to call the study doctors immediately if any of the following symptoms are experienced: abnormal or prolonged vaginal bleeding, severe headaches, changes in vision or speech, dizziness or faintness, pains in calves or chest, or sudden shortness of breath; 7) banking a prescription for a back-up, additional 3 pills with the investigational drug service for each participant in an active condition will prevent participants from missing study medication doses on days they are in the lab should they forget to bring their study medication with them to the lab visit; 8) the development of a safety monitoring plan (see below); and 9) monitoring for adverse events by a Data and Safety Monitoring Committee.

2) Effects of the Ovarian Hormone Stabilization Protocol on Mood and Suicidality (for primary experimental group; N/A for HC group).

The following measures will be taken to mitigate any negative impact of the experiment on mood or suicidality:

Depressive symptoms and suicidality will be monitored during a daily evening telephone call during each condition and will also be measured and monitored via smartphone surveys. These daily calls will be carried out by trained research assistants, and the PI will be on-call during these calls. In the case of **(A) severe mood changes** (defined below) or **(B) imminent risk for suicide attempt (requiring inpatient hospitalization),** participation will be discontinued and the individual will be referred for an appropriate level of psychiatric care. Protocols for assessing severity of mood symptom changes, evaluating suicide risk, and discontinuation and referral are described below.

Protocol for Assessing Emergence of Severe Mood Changes. Because they will be recruited for recent suicidality, participants are expected to show elevated baseline levels of mood symptoms. Therefore, the emergence of severe mood symptoms will be defined as three days of daily depression scores (defined as depression, hopelessness, and worthlessness/guilt on the daily symptom record as defined in the research strategy) that are 1) severe or extreme in nature (i.e., above "moderate" on the response scale), 2) at least 50% increased over their self-reported trait levels of depression (as defined by mean scores on these items across the baseline month), 3) maintained for three consecutive days, and 4) a clinical interview must reveal that this worsening of mood is significantly impacting functioning in relationships or ability to engage with work or school. Should such an acute, impairment-related worsening of depression persist for three days, the **Protocol for Discontinuation and Referral** described below will be followed to remove the participant from the study and ensure proper engagement with their mental health provider at the appropriate level of psychiatric care.

Protocol for Assessing the Emergence of Imminent Risk of Suicide Attempt. Participants will be recruited to be at acceptably low current risk for suicide attempt given historical factors as described above. Daily telephone assessments will end with a structured screening for current suicide risk, including suicidal ideation, planning, and intent using the University of Washington Risk Assessment and Management Protocol (or UWRAP; also known as the Linehan Risk Assessment Procedure or LRAP)[125]. The UWRAP is the suicide risk assessment and protocol recommended by the NIMH for use in RCTs when participants are not in an ongoing treatment relationship with the assessor. **The purpose of this daily UWRAP screening is to determine (1) whether the participant's current risk of a suicide attempt has elevated to imminent risk for suicide attempt according to established guidelines[125] and to (2) intervene as appropriate to reduce any emergent imminent suicide risk.** The UWRAP allows the screener to determine the participant's current risk status for a suicide **attempt**, and provides concrete, structured guidelines for appropriate actions at each level of risk specifically in the context of a research study. UWRAP protocols will be followed closely. The UWRAP also includes a template for documenting specific risk classifications based on the participant's responses and the screener's actions. A UWRAP note will be written for each daily contact where nonzero suicidality is reported; notes in which any suicidal ideation is reported will be immediately reviewed with the PI, and will be discussed at weekly and monthly team meetings. Should an individual's responses to the UWRAP indicate imminent current risk for suicide attempt according to established standards, the **Protocol for Discontinuation and Referral** described below will be followed to remove the participant from the study and to ensure the appropriate level of psychiatric care.

More about the use of the UWRAP to manage suicide risk both virtually and in-person. The UWRAP is included as an Appendix to this Document. The UWRAP protocol has been utilized to assess and manage suicide risk in a variety of clinical trials sponsored by NIMH, and was developed by Marsha Linehan, the creator of Dialectical Behavior Therapy (the only evidence-based treatment for chronic self-injury). *It is designed to be carried out either in person or remotely (on a phone or video call).* The protocol includes assessment of risk, and a variety of individualized options for facilitating the participant's use of coping skills to help the participant avoid having to be hospitalized (either voluntarily or via an emergency services call). Ultimately, if the participant's suicide risk cannot be managed appropriately with the brief mood improvement and other risk management protocols included in the UWRAP, they are referred to the emergency department. If the participant is being evaluated in-person for a visit and needs immediate referral to the emergency department, we follow existing clinical protocols for hospitalization in the UIC Department of Psychiatry, which includes calling a security official at UIC who is trained to facilitate a safe walking of the participant from the psychiatry department offices to the emergency department. If the participant is being evaluated over the phone or videoconference (as in the daily phone calls during active conditions, the virtual enrollment option, or the virtual debriefing option), this will occur by either recruiting the help of a family member (often the emergency contact provided at enrollment) or by contacting 911 to facilitate an emergency safety check at the participant's location.

If a participant must be referred for emergency treatment (via emergency services/911 safety check, or walking the patient to the emergency room) at any point in the study, the blind will be broken for the participant and their study participation will be discontinued. If it is revealed that the participant was currently in the placebo condition, they will simply be referred to their mental health provider (or, as necessary, to the emergency department) for treatment. However, if it is revealed that the participant was currently in an active hormone stabilization condition, the supervising physician (Dr. Wagner) will oversee a gradual tapering of the hormonal intervention and the patient will be referred to their mental health provider (or, as necessary, the emergency department) for treatment.

Protocol for Discontinuation and Referral. The protocol for discontinuation and referral is as follows. First, if the patient needs immediate inpatient evaluation or care, we will follow the protocol outlined in the UWRAP (see Appendix) to facilitate referral to emergency department (either via coordination with the emergency contact, by involving emergency services to perform a safety check at the participant's home, or by recruiting the help of UIC security to escort the patient from the outpatient psychiatry offices to the UIC emergency department. While the patient's immediate need for care is being attended to, the study staff will contact the investigational drug pharmacy and the blind will be broken for the study staff, the PI, and the participant. The participant will be notified that their study participation is discontinued and provided with instructions for either tapering medication (if active medication was being taken) or to follow up as needed with outpatient providers. If it is revealed that the participant was currently in an active hormone stabilization condition, Dr. Wagner-Schuman will oversee (or communicate with the participant's physician to recommend) a gradual tapering of the hormonal intervention.

3) Hormone Side Effects (for primary experimental group; N/A for HC group). As discussed above, we do not expect any adverse side effects associated with the hormonal manipulations outlined in this protocol. However, any patient experiencing clinically significant side effects (e.g., nausea, hypertension, vomiting) deemed by Dr. Wagner-Schuman to be due to the hormonal manipulation will be discontinued from the study. The blind will be broken for that participant and their study participation will be discontinued. If it is revealed that the participant was currently completing the placebo condition, they will be informed of this and referred for outpatient medical care. However, if it is revealed that the participant was currently completing the active hormone stabilization condition, Dr. Wagner-Schuman will oversee a gradual tapering of the hormonal intervention and the patient will be referred for outpatient medical care.

4) Confidentiality.

For each of the above measurements, participants will be identified by study ID number on all research documents,

electronic data files, and collected specimens. All paper data will be stored in locked cabinets inside locked offices, and electronic data will be stored only on password-protected file servers only accessible from computers in the Psychiatry Department. The file linking the study ID number to identifying information will be kept separate from any study data on a UIC Psychiatry password protected drive. Only study personnel will have access to these data.

Biaffect Data Confidentiality. Sage Bionetworks, the company who stores the behavioral and EMA response data collected by Biaffect and other Apple HealthKit apps, will provide a business associate agreement to ensure continued HIPAA-compliant handling of data. They will not collect information about location or any other identifying information. Nonetheless, to maintain strict confidentiality of the BiAffect Data linked to this study, the research staff will create an anonymous email address for each study participant and create a linked BiAffect account for the study; this email address (which defines the account) will be linked to the participant's study ID for the trial only on a password-protected spreadsheet in the UIC server. Therefore, Sage Bionetworks will process only deidentified data and will not be able to link study data to any individual.

MetricWire Data Confidentiality. Metricwire is the company that will collect the phone sensor data (movement, acceleration) from participant smartphones as an augment to the use of the Biaffect app. A study-generated email address and password will be used to register each participant's phone to the Metricwire App, and collection of IP address will be turned off; these measures eliminate any identifiers from being coded by the Metricwire app. However, Metricwire is HIPAA-compliant and a Business Associate's Agreement is on file with UIC through our laboratory for data collection and storage through this company.

Oura Ring Data Confidentiality. No identifiable data will be collected from the Oura Ring. The serial number of rings given to participants will not be recorded. A premade study email will be used to create an Oura Ring App account. Therefore, no identifiable information will be collected related to the Oura App or Ring.

5) Venipuncture. The use of a trained phlebotomist who is fully trained in safety protocols will minimize any risk associated with venipuncture.

6) Oura Ring. We will exclude participants with epilepsy, pacemakers, and other internal electronic devices to reduce any possible/theoretical risks associated with use of the Oura Ring.

POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO HUMAN PARTICIPANTS AND OTHERS

This is a mechanistic study to determine the role of ovarian hormone changes in constructs that are relevant to suicidality. There is no direct benefit to the participants other than the benefit of knowing that they are contributing to research on the causes of suicidality.

8.0 Quality Control and Quality Assurance

The project manager Sabina Raja will check over all databases in Qualtrics and generated from tasks for quality assurance monthly. Any missing data or data anomalies will be communicated to the PI for clarification/resolution.

The investigational site will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by NIMH, and inspection by local and regulatory authorities.

9.0 Data and Safety Monitoring

Digital Data Safety. All surveys and tasks, including those that request information about demographics which could theoretically be used to identify an individual, will be completed digitally and immediately stored on UIC's secure, HIPAA compliant server in a password-protected server that can be accessed only by the PI and her designees (i.e., postdoctoral fellows or project manager affiliated with the study). All additional biological assay data will similarly be stored on UIC's secure server.

With respect to data collected using the BiAffect App, all data will be captured digitally and stored on the HIPAA-compliant server at Sage Bionetworks, where all data from apps in the Apple HealthKit (and associated Android app counterparts) are hosted. As noted in the Protection of Human Subjects section, we will generate an anonymous email address and password to correspond to each study identification number, and this email will serve as the link to match *deidentified participant BiAffect data on the Sage Bionetworks server* with their local study data. Given that this unique email "ID" will be used to identify participants, no PHI will be collected using the BiAffect App, since there are no survey responses that contain PHI.

Similarly, with respect to data collected using the Metricwire App, all data will be captured digitally and stored on the HIPAA-compliant server at Metricwire. As noted in the Protection of Human Subjects section, we will generate an anonymous email address and password to correspond to each study identification number, and this email will serve as the link to match *deidentified participant Metricwire data on the Metricwire server* with their local study data. Given that this unique email "ID" will be used to identify participants, no PHI will be collected using the Metricwire App. Metricwire data will be collected up until July 31st, 2023. After this date, participants will no longer be enrolled into the Metricwire app and no data will be collected.

As noted above, the Oura Ring app will not record any identifying information.

Paper Document Safety. Paper documents containing PHI include those used to register patients in the UIC vendor system to be paid for their participation, which include SSN, address, and name, as well as the SCID-5, which includes date of birth and other demographic information. These documents will be digitized (see notes regarding digital data protections above) and shredded. Any paper documents that have not yet been digitized (e.g., SCID-5 interviews awaiting data entry) will be stored in a locked file drawer in the PI's locked office until they are digitized (see above). Only the PI and the project manager will have keys to this file drawer. Once digitized, all paper documents (except consent forms) will be immediately shredded per UIC protocol for protected health information. Consent forms will be kept in a locked file drawer in the PI's office as per UIC ethics protocol.

Data Monitoring and Report Generation. Each month, the project manager will generate a report from the database, including information about participant flow, demographics, and data completeness. This report will be sent to the PI and all Co-Is for review. Reports will also include detailed (but deidentified information about any Adverse Events (AEs) (e.g., suicide risk elevation) and follow-up procedures.

Monitoring and Reporting of Mood Changes, Suicide Risk, and Adverse Events. The PI will supervise the clinical postdoctoral fellow as well as the project manager in monitoring changes in suicidal risk and mood symptoms obtained from laboratory visits, daily online surveys, and daily telephone risk screenings. Research staff will be specifically trained to identify significant changes in mood or suicidality in various assessments (see protocol in Protection of Human Subjects section), which will be reported immediately to the PI and further monitored for the duration and impairment criteria described in the Protection of Human Subjects Section (i.e., symptoms must interfere with either relationships or occupational health, and must remain elevated for three days). As described above, participants who experience significant deterioration in mood or who are judged to be at imminent suicide attempt risk will be discontinued from the

protocol and referred for an appropriate level of psychiatric care.

In addition, specific events to be reported to the data safety and monitoring board (DSMB) will be defined by the DSMB at their first meeting prior to the start of the research.

DATA SAFETY AND MONITORING BOARD

Similar to the PI's ongoing clinical trials, the safety of the study will be monitored by a specially-convened Data and Safety Monitoring Board at UIC. This will ensure the independent oversight of issues related to safety/adverse events. The DSMB will monitor the study for AEs, including a significant worsening of symptom severity, imminent suicide risk, substantial functional impairment, and physical effects (e.g., migraine, thromboembolic events). The DSMB will specify the tables and data it wishes to have presented, including moderate to severe side effects and all serious adverse events. DSMB review of this study will occur every six months. At these meetings the DSMB will also review data from participants who have been discontinued or who have dropped out of the study, and reasons for discontinuation. The DSMB will identify if any study procedures should be altered or stopped in the event of an indication of harm to participants attributable to the study interventions. The DSMB will evaluate issues of participant safety as well as the adequacy and integrity of accumulating data, review of enrollment data, and making recommendations regarding safety.

Members of the DSMB:

Karen Bernstein, MD, MPH (CHAIR), Associate Professor and Director of Division of Adolescent Medicine, expertise with hormonal manipulation in vulnerable populations.

Jamie Haley Kienast, LCSW, Social worker at UIC with professional competence in female mental health and suicide risk assessment among diverse populations such as those seen at UIC.

Deepika Laddu, PhD, Assistant Professor in Department of Physical Therapy, expertise in clinical research with female populations.

Definitions of Adverse Events

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related.

AE SEVERITY

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.
- ***Serious adverse event or serious suspected adverse reaction***. An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, or a persistent or significant incapacity. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate

medical judgment, they may jeopardize the patient or subject and may require medical intervention to prevent one of the outcomes listed in this definition.

- *Notable examples of serious adverse events in the present study include development of a venous thromboembolism or suicide attempt.

AE STUDY-RELATEDNESS

For all AEs, Dr. Eisenlohr-Moul will urgently collaborate with Dr. Wagner to determine the AE's causality based on temporal relationship and clinical judgment. The degree of certainty about causality will be graded using the categories below.

Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Possibly Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.

Unlikely to be related – A clinical event whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Not Related – The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by Dr. Wagner or Dr. Eisenlohr-Moul.

AE EXPECTEDNESS

Dr. Eisenlohr-Moul will collaborate with Dr. Wagner to make determinations about whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

10.0 Data Analysis and Statistical Considerations

ANALYTIC STEPS

For each outcome, we will use model fit indices to select the best-fitting hierarchical model before proceeding with analyses. Based on our prior study (K99MH109667) with a similar experimental design and similar data collection structure for daily surveys and lab visits, we expect that the data structure for primary analyses will be observations nested within participants (two-level regression model). In our prior models, inclusion of a third level of nesting (i.e., observations nested within condition nested within individuals) did not improve model fit and did not substantively alter hypothesis tests. Based on the prior study, we also expect that a combination of a random intercept for participant and an autoregressive (day-1) within-person error structure will be included based on model fit.

Testing Experimental Effects: Aim 1 and 2 require that we first evaluate the impact of the experiment on each of the 3 primary suicide outcomes and 9 (RDoC, neurosteroid) mediators described above. Therefore, we will first examine effects of the within-person hormone stabilization experiment on repeated measures outcomes across the menstrual cycle.

1. **For daily outcomes,** we will utilize multilevel models (with 52 days—26 per condition--nested within each individual; two-level models) to predict each repeated measures outcome from covariates (daily pain, illness, PRN medication use) and the interaction between (1) **experimental condition** (a dichotomous variable coded as 0 for natural hormone withdrawal condition and 1 for hormone stabilization condition) and (2) **condition phase,** a categorical (contrast) variable coded as follows: *midluteal baseline phase*, coded as days +1 to +7 after the positive ovulation test, *perimenstrual experimental phase*, coded as days +11 to +17 after the positive ovulation test, and the *medication withdrawal phase*, coded as days +22 to +26 after positive ovulation test (see Figure 6 in grant for study design and timeline). **Number of daily observations per person**. For the primary contrast between the midluteal and perimenstrual experimental phases, there are 28 observations per person—14 per condition (7 midluteal, 7 perimenstrual). Based on our preliminary data from the K99 trial (identical experimental manipulation), we anticipate receiving an average of 24 observations per participant.

2. **For laboratory visit outcomes,** we will again utilize multilevel models (with 10 labs per person—5 per condition—nested within individuals; two-level models). A two-level model will predict each repeated measures lab outcome from covariates (daily pain, illness, PRN medication use) and the interaction between (1) **experimental condition** (a dichotomous variable coded as 0 for natural hormone withdrawal condition and 1 for hormone stabilization condition) and (2) **condition phase,** a categorical (contrast) variable coded as follows: *midluteal baseline visit*, *perimenstrual experimental visit*, and the *medication withdrawal visit*. **Number of lab observations per person**. For the primary contrast between the midluteal and perimenstrual experimental phases, there are 4 observations per person—2 per condition. Based on our preliminary data from the K99 trial (identical experimental manipulation), we anticipate very minimal missing laboratory data (see grant for details) but have estimated that on average each completer participant will miss .25 visits.

3. **Hypothesized Pattern of Effects for Daily and Lab outcomes**: A significant interaction between experimental condition and condition phase is hypothesized for all twelve specified outcomes such that, in the natural withdrawal condition we expect deleterious changes from the midluteal phase to the premenstrual and menstrual phases (due to corresponding hormone withdrawal), but in the hormone stabilization condition we expect no deleterious changes (or less severe changes) from the midluteal to the premenstrual and menstrual phases (since hormone withdrawal is prevented).

Testing Associations Between Mediators and Suicide Outcomes: Aim 1 and 2 next require that we evaluate the day-to-day or week-to-week associations between single mediators (listed above) and suicide outcome variables on the same day or visit. This will be carried out in similar multilevel models as above, predicting each of the three primary suicide outcomes from person-centered values for each of the 9 mediators (listed above).

Testing Interactive Effects of Hopelessness and Arousal on Suicide Outcomes: Interactions among hopelessness and arousal will be examined as predictors of suicide outcomes and as mediators of the experimental effect. In order to test this, we examine day-to-day interactive effects of daily person-centered hopelessness (noted above) and daily person-centered arousal indices (noted above) predicting same-daily suicide outcomes (noted above). We predict that higher-than-usual hopelessness on a given day will predict greater same-day risk on suicide outcomes, but only when arousal is *also* higher than usual for that individual.

4. Evaluating Mediation Where Appropriate. Finally, each aim indicates that **mediation** will be explored as appropriate. Only when analyses indicate significant effects of the experiment on both the suicide outcome and any mediator, as well as significant associations between the person-centered variable (or interaction variable) and the suicide outcome, we will utilize methods provided by **Bauer, Preacher, and Gil (2006)** to construct a confidence interval for the level 1 indirect effect of experimental condition (condition X phase) on the suicide outcome via the mediator.

Testing for normative cyclical change in the HC group. Aim 3 seeks to characterize how gene expression for GABAAR subunits varies across pre-specified menstrual cycle phase in response to hormonal change. In N=40 HC participants, we will run multilevel growth models to predict subunit gene expression (one model per gene) from specific cycle phase. We will test all pairwise comparisons between midluteal, perimenstrual, and midfollicular phases, controlling for ALLO level, to capture high P4 and ALLO states (midluteal), P4 and ALLO withdrawal (perimenstrual), and low P4 and ALLO (midfollicular).

Testing for group differences between participants with past-month SI and HC. We hypothesize that clinical group moderates the effect of cycle phase on $GABA_AR$ composition, such that the primary experimental group shows higher slope of midluteal-to-perimenstrual change in $\alpha4$ and δ subunits, and lower $\gamma2$ subunit replacement of δ subunit expression than the HC group. In one model of N=80 total participants, we will test the interaction of group and cycle phase on gene expression outcomes. We also hypothesize a correlation with symptoms, such that increases in $\alpha4$ and δ subunit expression will be associated with increases in individuals with perimenstrual increases in SI. We also predict that even in the HC group, higher-than-average $\alpha4$ or δ subunit expression will be associated with higher-than-average negative affect (e.g., depressed mood, anxiety, irritability, or perceived stress). We predict that higher $\gamma2$ expression will be associated with lower negative affect and perceived stress. We will calculate a person-mean for each subunit's expression (mean level across 5 visits) and a deviation score at each visit (visit level minus person mean). We will test overall correlations (e.g., do participants with higher mean $\alpha4$ expression have higher negative affect?) and interaction with cycle phase (e.g., does a perimenstrual increase in $\alpha4$ expression *compared to person-mean* correlate with an increase in negative affect?). To further refine hypotheses, we will generate credible intervals using Bayesian methods in the context of multilevel modeling.

POWER ANALYSIS

80 completers achieves 80% power to detect conventionally small-to-medium sized effects that, in our estimation, are clinically meaningful (i.e., $f^2 = .10$, which would mean that the interaction of condition and phase had uniquely accounted for 10% of the variance in the outcome in the perimenstrual window; Selya et al., 2012, "*A Practical Guide to Calculating Cohen's f^2, a Measure of Local Effect Size*"). We posit that if hormone withdrawal (vs. experimental stabilization) does not account for at least 10% of the variance in the outcome during this critical risky window of the menstrual cycle, then this line of research, which involves significant participant burden, is not worth pursuing further.

11.0 Regulatory Requirements

11.1 Informed Consent

During the SCREENING PROCESS, the screening website and the research staff (or PI) will obtain digital or verbal consent from each participant (see online screener and phone screener, attached). Potential participants will be contacted by phone to assess their interest in participating; although we will note in the phone script that we are affiliated with UIC, we will also explain that they are under no obligation to participate and that their decision to participate has no influence on their clinical care at UIC (if applicable).

In addition, at the ENROLLMENT VISIT, trained members of the research team will obtain full written or digitally-signed informed consent from the participant using the following documented process:

Interested participants will visit the laboratory in-person or have virtual online sessions for their enrollment session where they will each read the consent form.

Research personnel will then ask if the participant has any questions they would like answered.

The participant will sign the consent form, witnessed by research personnel. The original consent for will be maintained in the participant's file, along with documentation that the participant's questions were answered by the informational sheet or that research personnel gave them an opportunity to ask questions. These documents will be secured in Dr. Eisenlohr-Moul's locked laboratory. A copy of the consent form will be provided to the participant. For participants who completed virtual enrollment visits during the covid-19 pandemic, their consent will first be obtained through a secure online survey and then be re-assessed during their welcome visit, where they will provide written consent and given a copy of the consent form.

11.2 Subject Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored on protected servers at UIC. Identifying information will be linked to ID number only in one separate password-protected file on the UIC server. These ID numbers will not contain any identifying information (e.g., initials,

birthdates, SSN) and the file linking the study ID number to identifying information will be kept in a password-protected file, separate from any study data on a UIC Psychiatry password protected drive. De-identified, coded data may be transmitted by hand or scanned and transmitted via email among the research team.

Post-Study Disposition of identifiable data or human biological materials. Once data collection is completed and data are cleaned for analysis (within 1 year after the completion of data collection), data will be de-identified and the dataset including any identifiers will be destroyed. Recordings of study interviews will be deleted immediately following weekly review by the PI. Biological samples will be destroyed according to OSHA procedures once assay is completed.

Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be obtained from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

11.3 Unanticipated Problems

Definition of Unanticipated Problems (UPs)

For the purposes of this study, we will consider unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Reporting of Unanticipated Problems

Incidents or events that meet criteria for UPs require the creation and completion of an UP report form. The PI will report UPs to the IRB and to the study sponsor. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- All UPs will be reported to the IRB, the DSMB, and to the study sponsor within 1 week of the investigator becoming aware of the event.

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